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INTERNATIONAL SYMPOSIUM ON ANIMAL SCIENCE 2014

23-25th September 2014, Belgrade, Serbia

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Invited paper

TRANSCRIPTIONAL PROFILING OF GENES INVOLVED IN REPRODUCTIVE IMMUNOLOGY OF FARM ANIMALS

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Abstract

It is now well established that interactions between the immune and reproductive system have important consequences for fertility and reproductive health. Pattern recognition receptors (PRRs) are one of the key components of innate immune recognition in vertebrate species. One of the best studied members of PRRs innate immune system is the family of Toll-like receptors (TLRs), which is a family of conserved transmembrane proteins recognizing conserved molecular motifs in a broad range of bacteria, viruses, fungi and parasites. In this review the role of TLR genes in the reproductive immunology of tract of farm animal species will be discussed.

Key words: *embryos, gametes, gene expression, reproductive organs, toll-like receptors*

Introduction

Many studies have reported on the involvement of TLRs in the interactions between the immune and reproductive system, as well as on the involvement of TLRs in the physiology of reproduction in various vertebrate species (Fazeli et al., 2005; Andersen et al., 2006; Itoh et al., 2006; Soboll et al., 2006; Herath et al., 2007). Furthermore, microbial invasion presents a threat to the health of male reproductive organs that can affect fertility (Dejucq et al., 2001; Com et al., 2003; Palladino et al., 2003). Various pathogens are able to infect the male reproductive tract in vertebrates, resulting in reproductive dysfunction and endocrine disorders. Microbial infection of the testis and epididymis can have as a consequence orchitis or epididymal inflammation, leading to transient or permanent sterility (Dejucq et al., 2001; Hall et al., 2002; Com et al., 2003; Rodrigues et al., 2008). Protection of the male reproductive organs from invading microorganisms is therefore an essential aspect of reproductive physiology.

During the last years, pattern recognition receptors (PRRs) have been identified as one of the key components of innate immune recognition in vertebrate species, providing the first line of defense against potential pathogens (Akira et al., 2001; Brownlie and Allan 2010). One of the members of PRRs, the family of Toll-like receptors (TLRs), has been reported to be expressed in the reproductive organs in various vertebrate female species and thus have been implicated in many aspects of reproductive physiology and pathology, providing evidence to suggest that TLRs may function to affect fertility (Fazeli et al., 2005; Andersen et al., 2006; Shimada et al., 2006; Soboll et al., 2006; Herath et al., 2007; Ozoe et al., 2009;

Michailidis et al., 2010; 2011). However, mechanisms of antimicrobial protection of male reproductive organs mediated by TLRs have not been studied extensively.

Male reproductive tract

It has been reported that rat male reproductive organs, including testis, epididymis and vas deferens express TLRs1-11 (Palladino et al., 2007), while mouse Sertoli cells express TLRs2-5 at relatively high levels and TLR6, 7 and 13 at lower levels and do not express TLR1 and TLR8-12 (Riccioli et al., 2006; Starace et al., 2008). Adult human testis also expresses TLR2 and 4 at high levels and TLR5 and 6 at lower levels (Nishimura and Naito, 2005).

Bacterial and viral infections of the male reproductive organs can cause orchitis, epididymitis, and epididymo-orchitis, as well as obstructions of the reproductive tract, which can result in compromising movement of spermatozoa through the excurrent ductal system, contributing significantly to impaired fertility (Palladino et al., 2003; Bhushan et al., 2009). Protection of male reproductive tissues, such as testis and epididymis from microbial invasion is therefore an emerging aspect of research in reproductive physiology.

In recent years, research on antimicrobial protection of male reproductive organs and spermatozoa have indicated the crucial role of the innate immune system in controlling microbial infections of the male genital tract. As innate immune system competence is of critical importance in preventing microbial penetration, a growing number of known or putative antimicrobial defense systems have been reported to be present in the testis of various vertebrate species. In particular, a number of antimicrobial peptides, such as defensins, eppin, human cationic antimicrobial protein 18, and lipopolysaccharide binding protein (Com at al., 2003; Palladino et al., 2003), have been detected in the male reproductive tract and spermatozoa of various vertebrates. In addition to their roles in innate immunity of male reproductive organs, antimicrobial peptides, such as members of the b-defensin family have been reported to play roles not only in innate immunity but also in male fertility, sperm survival in male reproductive tract, and sperm-egg interaction in mammals (Com et al., 2003; Palladino et al., 2003).

Apart from antimicrobial peptides, during the last years the discovery of Toll-like revealed the significant role of pattern recognition receptors in the innate immune system (Akira et al., 2001). TLRs recognize a range of microbial molecular patterns and generate intra-cellular signals through nuclear factor-kB (NFKB) dependent pathways to induce chemokine and cytokine expression that activate a range of host responses (Zarember and Godowski, 2002). As interactions between the immune and reproductive systems have important consequences for fertility and reproduction, there is increasing evidence that many of these interactions involve the family of Toll-like receptors (Girling and Hedger, 2007). Although their function in the male reproductive tract has not yet been studied extensively, there is increasing evidence that the innate defense system of the testis hinges on recognition of bacterial products by members of the TLR family and TLRs play a role in testicular steroidogenesis and spermatogenesis during normal function, as well as in testicular pathology (Girling and Hedger, 2007; Bhusham et al., 2009). Previous studies have reported that in the testis, TLRs are found in immune cells such as macrophages and dentritic cells, as well as in testicular somatic cells and also in the germ cells (Bhushan et al., 2009). Messenger RNA for TLRs was abundantly expressed in the testis (Palladino et al., 2007), expression of TLR1-9 was detected in human testis (Nishimura and Naito, 2005), while various types of TLRs were expressed in testicular Sertoli cells (Riccioli et al., 2006; Wu et al., 2008).

Bacterial and viral infections of the male reproductive organs are one of the most serious causes of infertility in males as they can cause orchitis, epididymitis, and epididymo-orchitis, as well as obstructions of the reproductive tract, which can result in compromising movement of spermatozoa through the excurrent ductal system, contributing significantly to impaired fertility (Palladino et al., 2003; Bhushan et al., 2009). Protection of male reproductive organs, from microbial invasion is therefore an emerging aspect of research in reproductive physiology.

Although some studies have reported on the testis, there have been relatively few studies on inflammatory signaling in the epididymal epithelium or on the effects of systemic inflammation on epididymal function. However, it is well established that immune responses within the testis, where sperm develop, and immune responses in the epididymis, where mature sperm concentrate and mature, are very different and the immune functions of the epididymis have largely been underestimated (Hedger, 2011; Meinhardt and Hedger, 2011). Intraepithelial lymphocytes and other immunocompetent cells have been observed in the epididymis (Da Silva et al., 2011; Meinhardt and Hedger, 2011), but the precise nature of these cells and their immunological properties remain to be elucidated. Surprisingly, one of the most understudied aspects of epididymal physiology is its interaction with the immune system (Hedger and Hales, 2006; Da Silva et al., 2011). The epididymal mucosal system must protect autoantigen-coated spermatozoa from destruction by the immune system as well as invading pathogens. Because of the role of the epididymis in sperm maturation and storage it is critical that the epithelium of the male reproductive tract be protected from a variety of pathogens that can invade the tract including pathogens that cause sexually transmitted diseases (Palladino et al., 2007). Previous studies have reported on the role of host defense peptides and proteins such as defensins and TLRs in protecting the epididymis and therefore both spermatozoa and the epididymal epithelium from microbes, as well as on motility and fertility in different mammalian species (Frohlich et al., 2001; Avellar et al., 2004; Yenugu et al., 2006; Palladino et al., 2003; 2007; Lin et al., 2008).

Female reproductive tract

TLRs have been reported to be expressed in the reproductive organs of various female species and have been implicated in critical aspects of ovarian, endometrial and placental function (Fazeli et al., 2005; Girling and Hedger, 2007; Michailidis et al., 2010 and 2011). Several studies have reported on the role that TLRs play in the maintenance of the immune system in the reproductive organs in many vertebrate species. Members of the TLR family were expressed in the ovary and granulosa and cumulus cells in mouse, as well as in cattle, pig, chicken, sheep and human ovaries, providing evidence to suggest that they may function to affect fertility (Fazeli et al., 2005; Rodriguez-Martinez et al., 2005; Alvarez et al., 2006; Shimada et al., 2006; Herath et al., 2007; Shimada et al., 2008). A number of studies have also reported the presence of TLR family members in the uterine, vaginal tissues and oviductal stromal fibroblasts in the female reproductive tract (Soboll et al., 2006; Fazeli et al., 2005; Andersen et al., 2006; Itoh et al., 2006).

Conclusion

Collectively, members of the TLR family are abundantly expressed in the ovary, oviduct, testis, epididymis and embryos during embryonic development, their mRNA abundance is developmentally regulated during sexual maturation and that certain TLRs are significantly

induced in the male and female reproductive tract response to various infections. These findings suggest that TLRs are important for innate immunity of the reproductive organs and embryos and suggest that a TLR mediated immune system exists in the reproductive organs and that it probably plays a role in the recognition of pathogens, and in the initiation of immune response to protect the gametes for successful fertilization, and in preventing microbial pathogens from being incorporated into the embryos. Increased knowledge about TLRs may result in the identification of breeds that are more resistant to colonization of pathogens, which will be very important as farm animals are important reservoir of zoonotic pathogens harmful to humans and provide an important food source.

References

1. Akira S, Takeda K, Kaisho T 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat. Immunol. 2, 675-680.
2. Andersen JM, Al-Khairy D, Ingalls RR 2006. Innate immunity at the mucosal surface: role of Toll-like receptor 3 and Toll-like receptor 9 in cervical epithelial cell responses to microbial pathogens. Biol. Reprod. 74, 824-831.
3. Bhushan S, Schuppe HC, Tchatalbachev S, Fijaka M, Weidnerd W, Chakraborty T, Meinhardta A 2009. Testicular innate immune defense against bacteria. Mol. Cell Endocrinol. 306, 37-44.
4. Brownlie R, Allan B 2010. Avian toll-like receptors. Cell Tissue Res. 343, 121-130.
5. Com E, Bourgeon F, Evrard B, Ganz T, Colleu D, Jegou B, Pineau C 2003. Expression of Antimicrobial Defensins in the Male Reproductive Tract of Rats, Mice, and Humans. Biol. Reprod. 68, 95-104.
6. Dejucq N, Jegou B 2001. Viruses in the mammalian male genital tract and their effects on the reproductive system. Microbiol. Mol. Biol. Rev. 65, 208-231.
7. Fazeli A, Bruce C, Anumba DO 2005. Characterization of Toll-like receptors in the female reproductive tract in humans. Hum. Reprod. 20, 1372-1378.
8. Girling JE, Hedger MP 2007. Toll-like receptors in the gonads and reproductive tract: emerging roles in reproductive physiology and pathology. Immunol. Cell Biol. 85, 481-489.
9. Hall SH, Hamil KG, French FS 2002. Host defense proteins of the male reproductive tract. J. Androl. 23, 585-597.
10. Herath S,Williams EJ, Lilly ST, Gilbert RO, Dodson H, Bryant CE, Sheldon IM 2007. Ovarian follicular cells have innate immune capabilities that modulate their endocrine functions. Reproduction. 134, 683-693.
11. Itoh H, Nasu K, Nishida M, Matsumoto H, Yuge A, Narahara H 2006. Human oviductal stromal fibroblasts, but not oviductal epithelial cells, express Toll-like receptor 4: the site-specific mucosal immunity of the human fallopian tube against bacterial infection. Am. J. Reprod. Immunol. 56, 91-101.
12. Soboll G, Schaefer TM, Wira CR 2006. Effect of Toll-like receptor (TLR) agonists on TLR and microbicide expression in uterine and vaginal tissues of the mouse. Am. J. Reprod. Immunol. 55, 434-446.
13. Michailidis G, Theodoridis A, Avdi M 2010. Transcriptional profiling of Toll-like receptors in chicken embryos and in the ovary during sexual maturation and in response to *Salmonella enteritidis* infection. Anim. Reprod. Sci. 122, 294-302.

14. Michailidis G, Theodoridis A, Avdi M 2011. Effects of sexual maturation and *Salmonella* infection on the expression of Toll-like receptors in the chicken vagina. Anim. Reprod. Sci. 123, 234-241.

15. Ozoe A, Isobe N, Yoshimura Y 2009. Expression of Toll-like receptors (TLRs) and TLR4 response to lipopolysaccharide in hen oviduct. Vet. Immunol. Immunopathol. 127, 259-268.

16. Palladino MA, Mallonga TA, Mishra MS 2003. Messenger RNA (mRNA) expression for the antimicrobial peptides b-defensin-1 and b-defensin-2 in the male rat reproductive tract: b-defensin-1 mRNA in initial segment and caput epididymidis is regulated by androgens and not bacterial lipopolysaccharides. Biol. Reprod. 68, 509-515.

17. Palladino MA, Johnson TA, Gupta R, Chapman JL and Ojha P 2007. Members of the Toll-Like Receptor Family of Innate Immunity Pattern-Recognition Receptors Are Abundant in the Male Rat Reproductive Tract. Biol. Reprod. 76, 958-964.

18. Riccioli A, Starace D, Galli R, Fuso A, Scarpa A, Palombi F, De Cesaris P, Ziparo E, Filippini A 2006. Sertoli Cells Initiate Testicular Innate Immune Responses through TLR Activation. J. Immunol. 177, 7122-7130.

19. Rodrigues A, Queiroz DBC, Honda L, Silva EJR, Hall SH, Avellar MCW 2008. Activation of Toll-Like receptor 4 (TLR4) by in vivo and in vitro exposure of rat epididymis to lipopolysaccharide from escherichia coli. Biol. Reprod. 79, 1135-1147.

20. Shimada M, Hernandez-Gonzalez I, Gonzalez-Robanya I, Richards JS 2006. Induced expression of pattern recognition receptors (PRRs) in cumulus oocyte complexes (COCs): novel evidence for innate immune-like cells functions during ovulation. Mol. Endocrinol. 20, 3228-3239.

21. Soboll G, Schaefer TM, Wira CR 2006. Effect of Toll-like receptor (TLR) agonists on TLR and microbicide expression in uterine and vaginal tissues of the mouse. Am. J. Reprod. Immunol. 55, 434-446.

22. Starace D, Galli R, Paone A, De Cesaris P, Filippini A, Ziparo E, Riccioli A 2008. Toll-like receptor 3 activation induces antiviral immune responses in mouse Sertoli cells. Biol. Reprod. 79, 766-775.

23. Zarember KA, Godowski PJ 2002. Tissue expression of human toll-like receptors and differential regulation of toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. J. Immunol. 168, 554-561.

Invited paper

ENDOCRINE DISRUPTION BY HEAVY METALS ON STEROIDOGENESIS IN MODEL SYSTEMS

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Abstract

In this study human adrenocortical carcinoma cell line NCI-H295R was used as an *in vitro* biological model to study the effect of heavy metals on steroidogenesis. The cell cultures were exposed to different concentrations of cadmium (1.90; 3.90; 7.80; 15.60; 31.20; 62.50 μM of $CdCl_2$), mercury (1.0; 5.0; 25; 50; 100 μM of $HgCl_2$), nickel (3.90; 7.80; 15.60; 31.20; 62.50; 125; 250; 500 μM of $NiCl_2$) and compared to control. Cell viability was measured by the metabolic activity (MTT) assay for estimation of mitochondria structural integrity. Quantification of sexual steroid production directly from aliquots of the medium was performed by enzyme linked immunosorbent assay (ELISA). Cadmium decreased the release of progesterone and testosterone already at a very low concentration (1.90 μM) of $CdCl_2$, while the cell viability remained relatively high (> 75%) up to 7.80 μM of $CdCl_2$ and significantly (P<0.01) decreased at 15.60 μM and higher concentrations of $CdCl_2$. Concentration-dependent depression in testosterone production was detected particularly for higher concentration of $HgCl_2$. Progesterone production was also decreased, but at the lower concentrations (1.0 and 5.0 μM) of $HgCl_2$ this decline was lower compared to depression of testosterone. The cell viability significantly decreased at 25 μM and higher concentration of $HgCl_2$. Results of the our study indicate dose dependent decreases in both sexual steroid hormones by NCI-H295R cell culture following a 48 h *in vitro* $NiCl_2$ exposure. The lowest concentration of progesterone was significantly (P<0.01) detected in groups with the higher doses (≥ 500 μM) of $NiCl_2$, which elicited significant cytotoxic effect. The testosterone production was decreased as well, but this decline was more pronounced compared to depression of progesterone. These results suggest that heavy metals have detrimental effects on steroid hormone synthesis even at very low concentrations and consecutively on reproductive physiology.

Key words: *cell viability, endocrine disruption, heavy metals, NCI-H295R cell line, steroid hormones*

Introduction

Currently, there is increased evidence that various chemicals introduced to the environment have the potential to disrupt the endocrine system, which may result in adverse effects on differentiation, growth and development. It is possible for certain environmental

contaminants (including metals) to cause or contribute to a hormonal disruption and interfere with functions of key enzymes involved in steroidogenesis (Sanderson, 2006). Heavy metals such as cadmium, mercury and nickel are also reported to have an endocrine disruptive potential (Zhu et al., 2000). Cadmium (Cd) is an industrial and environmental contaminant unique among metals because of its non biodegradable nature, long environmental persistence, extremely protracted biological half-life, low rate of excretion from the body and predominant storage in soft tissue (primarily liver and kidney) (Massanyi et al., 2002). As a well-known endocrine disrupting chemical, Cd is not only a regulator of hypothalamus and pituitary hormone secretion (Lafuente et al., 2003), but also disrupts steroidogenesis including the syntheses of androgen, progesterone and estrogen, leading to suppression of reproductive functions (Takuguchi and Yoshihara, 2006). Mercury (Hg) is one of the oldest toxicants known and is considered to be a risk factor of the environment and food chain (Tazisong and Senwo, 2009). Exposure to a high concentration of Hg causes an increase in reproductive problems (Schuurs, 1998), which can be reflected in the process of steroidogenesis. The endocrine disruptive effects of Hg have recently become one of the major public concerns. There in sufficient evidence from animal studies supporting the disruptive effects of Hg on the functions of the thyroid, adrenal gland, ovary and testis, although several factors make it difficult to extrapolate the animal data to humans (Zhu et al., 2000). Nickel (Ni) is considered to be an essential micronutrient (Eisler, 1998) that is only required in very small amounts (Das, 2009). Nickel serves as a cofactor or a structural component of several metalloenzymes (Przybyla et al., 1992). There is sufficient evidence that nickel ions (Ni^{2+}) have potent toxic effects on the reproductive system (Das and Dasgupta, 2000). Nickel salts are capable of morphological changes such as degeneration of testicular germinal epithelium (Pandey et al., 1999), testicular sarcomas and functional disorders such as inhibition of spermatogenesis (Yokio et al., 2003) or disruption of steroidogenesis (Krockova et al., 2011).

In the present study we investigated the effects of heavy metals on steroidogenesis in the human adrenocortical carcinoma cell line (NCI-H295R), which serves as a model system for screening endocrine-disruptive chemicals (Ding et al., 2007). This cell line was derived from H295 cells, which were established from a primary hormonally active adrenocortical carcinoma (Rainey et al., 2004). The NCI-H295R cells have physiological characteristics of zonally undifferentiated human fetal adrenal cells (Staels et al., 1993) and represent an unique *in vitro* model system which has the ability to produce all of the steroid hormones found in the adult adrenal cortex and gonads, allowing to test the effects on both corticosteroid synthesis together with the production of sexual steroid hormones (Gazdar et al., 1990) as well as permit the measurement of hormone production. The objective of our study was to determine the effects of heavy metals (Cd, Hg and Ni) on steroidogenesis of human adrenocortical carcinoma cell line (NCI-H295R). Specifically, we examined the dose-dependent changes of heavy metals as endocrine disruptors in relation to release of progesterone and testosterone by adrenocortical carcinoma cells *in vitro*.

Material and methods

Cell Culture

The human adrenocortical carcinoma cell line (NCI-H295R) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in a Good Laboratory Practice (GLP) certified laboratory (National Institute of Chemical Safety, Budapest; OGYI/31762-9/2010) according to previously established and validated

protocols (Hilscherova et al., 2004; OECD, 2011). The adrenocortical carcinoma cells were grown (37ºC, with a 5% CO_2 atmosphere) in a 1:1 mixture of Dulbecco´s Modified Eagle´s Medium and Ham´s F-12 Nutrient mixture (DMEM/F12) (Sigma-Aldrich, St. Louis, MO, USA) (1:1) supplemented with 1.20 g/L $NaHCO_3$ (Sigma-Aldrich, St. Louis, MO, USA), 5.00 mL/L of ITS+Premix (BD Biosciences, San Jose, CA, USA) and 12.50 mL/L of BD Nu-Serum (BD Biosciences, San Jose, CA, USA). The medium was changed 2-3 times per week and cells were detached from flasks for sub-culturing using sterile 0.25% trypsin-EDTA (Sigma-Aldrich, St. Louis, MO, USA).Cell density was determined using a hemocytometer and adjusted with culture medium to a final concentration of 300 000 cells/mL. The cell suspensions were plated (with final volume of 1.00 mL/well) into sterile plastic 24-well plates (TPP, Grainer, Germany) for estimation of steroid hormones. For cytotoxicity evaluation the cells (100 μL/well) were seeded into 96-well plates (MTP, Grainer, Germany). The seeded plates were incubated at 37ºC with a 5% CO_2 atmosphere for 24 h to allow the cells to attach to the wells (Knazicka et al., 2013).

In vitro exposure

After 24 h attachment period the cell culture medium was removed from the plates and replaced with a new medium supplemented with 1.90; 3.90; 7.80; 15.60; 31.20; 62.50 μM cadmium chloride ($CdCl_2$; Sigma-Aldrich, St. Louis, MO, USA), 1.0; 5.0; 25; 50; 100 μM mercury chloride ($HgCl_2$; Sigma-Aldrich, St. Louis, MO, USA) and 3.90; 7.80; 15.60; 31.20; 62.50; 125; 250 or 500 μM nickel chloride ($NiCl_2$; Sigma-Aldrich, St. Louis, MO, USA). Cell cultures were maintained for 48 h.

Cytotoxicity evaluation

The viability of the cells exposed to heavy metals was evaluated by the metabolic activity (MTT) assay (Mosmann, 1983). This colorimetric assay measures the conversion of a yellow tetrazolium salt [3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT), to blue formazan particles by mitochondrial succinate dehydrogenase of intact mitochondria of living cells. Formazan was measured spectrophotometrically. Following the termination of heavy metals ($HgCl_2$, $CdCl_2$ and $NiCl_2$) exposure, the cells were stained with MTT (Sigma-Aldrich, St. Louis, MO, USA) at a final concentration of 0.20 mg/mL. After 2 h incubation (37ºC, with a 5% CO_2 atmosphere), the cells and the formazan crystals were dissolved in 150 μL of acidified (0.08 M HCl) isopropanol (CentralChem, Bratislava, Slovak Republic). The optical density was determined at a measuring wavelength of 570 nm against 620 nm as reference by a microplate reader (Anthos MultiRead 400, Austria). The data were expressed in percentage of the control (i.e. optical density of formazan from cells not exposed to heavy metals).

Hormone measurement

Enzyme linked immunosorbent assay (ELISA) was used for the quantification of steroid hormones (progesterone, testosterone) directly from the aliquots of the medium. The ELISA kits were purchased from Dialab GmbH (Wiener Neudorf, Austria). According to the manufacturer's data the sensitivity of testosterone assay was 0.075 ng/mL, and the intra-and inter-assay coefficients of variation were 4.60% and 7.50%, respectively. Cross-reactivity with 5α-dihydroxytestosterone was 16.00%. The sensitivity of progesterone assay was 0.05 ng/mL, and the intra- and inter-assay coefficients of variation were ≤

4.00% and ≤ 9.30%, respectively. The absorbance was determined at a wavelength 450 nm using an Anthos MultiRead 400 (Anthos MultiRead 400, Austria) microplate reader and the data were evaluated by WinRead 2.30 computer software. Values were expressed in percentage of the untreated controls.

Statistical analysis

Obtained data were statistically analyzed by the PC program GraphPad Prism 6.00 (GraphPad Software Incorporated, San Diego, California, USA). Descriptive statistical characteristics (arithmetic mean, minimum, maximum, standard deviation and coefficient of variation) were evaluated. One-way analysis of variance (ANOVA) and the Dunnett´s multiple comparison test were used for statistical evaluations. The level of significance was set at * $P<0.05$; ** $P<0.01$ and *** $P<0.001$.

Results and discussion

Release of steroid hormones by adrenocortical carcinoma cells after $CdCl_2$ exposure

Recently, the effects of Cd on steroidogenesis have been described, but results vary depending on the experimental model, time-duration of exposure and the dose used. Our results suggest a direct toxic action of Cd on the steroid-producing cells and subsequent changes in hormonal release. Cadmium decreased the release of progesterone and testosterone in the whole applied range even at a very low concentration (1.90 μM) of $CdCl_2$ (Table 1), while the cell viability remained relatively high (> 75%) up to 7.80 μM of $CdCl_2$ and significantly ($P<0.01$) decreased from 15.60 μM and higher concentrations of $CdCl_2$ (Figure 1). These results are in agreement with a previous report by Tchounwou et al. (2001) indicating the high degree of $CdCl_2$ toxicity to human liver carcinoma cells (HepG2).

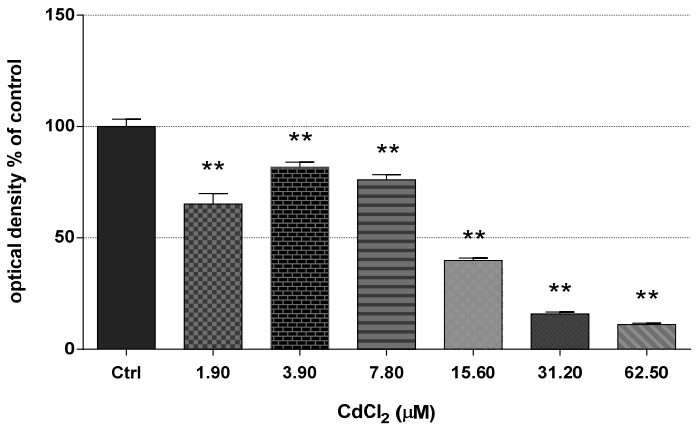

Figure 1. *The viability of NCI-H295R cells in culture after 48 h of $CdCl_2$ exposure*

Legend: Cytotoxicity was assessed using the MTT assay following $CdCl_2$ exposure. Bar values represent the arithmetic mean (±S.D.) optical density in % of (untreated) controls. The number of replicate wells was 21-32 at each point. A decline in optical density reflects a decline in cell viability. Statistical difference between the values of control and treated cells was indicated by asterisks * $P<0.05$; ** $P<0.01$; *** $P<0.001$ (One-way ANOVA with Dunnett´s multiple comparison test).

Cadmium disrupts steroid biosynthesis in a variety of cells (Paksy et al., 1992). The studies conducted using cultured human placental trophoblastic cells suggest that Cd reduces progesterone synthesis by inhibiting the gene expression of the low-density lipoprotein (LDL) receptor, which controls the internalization of cholesterol into steroidogenic cells (Jolibois et al., 1999), cytochrome $P450_{SCC}$, which coverts pregnenolone to progesterone (Kawai et al., 2002). The results of our present study indicate dose-dependent decrease in progesterone release by NCI-H295R cell line in culture following a 48 h *in vitro* $CdCl_2$ exposure. The lowest amount of progesterone was significantly detected in groups with the highest doses (≥ 31.20 μM) of $CdCl_2$. The authors Paksy et al. (1992 and 1997) reported a decrease of progesterone production by human granulosa cells after Cd treatment *in vitro*. Cadmium did not cause a significant alteration in progesterone accumulation during 4 h incubation period, however, progesterone production decreased after 24 h. The testosterone production was decreased as well, but this decline was more evident at 7.80 μM of $CdCl_2$ in comparison to the decrease in progesterone release. Laskey and Phelps (1991) examined effect of Cd^{2+} and other metal cations (Co^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+} and Zn^{2+}) on *in vitro* Leydig cell testosterone production. Testicular cells were incubated with metal cations (1.0 to 5000 μM) for 3 h absence and presence of maximally stimulating concentrations of human chorionic gonadotropin (hCG), dibutyl cyclic adenosine monophosphate (db-cAMP), 20α-hydroxycholesterol (HCHOL) or pregnenolone (PREG). The results showed no change in Leydig cell viability with any metal cation treatment during 3 h incubation. Dose-response depletion in both hCG- and db-cAMP-stimulated testosterone production were noted with Cd^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+} and Zn^{2+} treatment. Surprisingly, Cd^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+}, which caused depletion in hCG- and db-cAMP-stimulated testosterone production, caused significant increases in HCHOL- and PREG-stimulated testosterone production over untreated and similarly stimulated cultures. This indicated that these cations may act at multiple sites within the Leydig cell.

Table 1. *Effect of 48 h $CdCl_2$ exposure on release of sexual steroid hormones (ng/mL) by adrenocortical carcinoma (NCI-H295R) cells*

Groups	**Control**	**1.90**	**3.90**	**7.80**	**15.60**	**31.20**	**62.50**
	Ctrl	**F**	**E**	**D**	**C**	**B**	**A**
$CdCl_2$ (μM)							
Progesterone (ng/mL)							
x	26.72	19.03	20.97	22.93	9.85	5.34*	2.49**
Minimum	15.24	11.77	10.89	8.15	4.88	2.44	1.25
Maximum	42.88	34.24	35.07	32.53	12.25	6.88	3.88
S.D.	9.68	13.84	9.17	10.60	2.70	1.58	0.90
CV (%)	36.24	43.05	43.71	46.25	27.37	29.55	36.15
%	100.00	71.20	78.47	85.80	36.86	19.99	9.31
Testosterone (ng/mL)							
x	14.02	9.56	13.31	8.82*	4.10**	2.65**	2.21**
Minimum	9.22	4.03	8.28	4.97	2.13	1.08	1.02
Maximum	18.50	14.66	22.24	12.28	7.12	4.88	3.88
S.D.	3.15	4.26	4.65	2.26	1.78	1.37	0.87
CV (%)	22.49	44.51	34.96	25.66	43.37	51.77	39.36
%	100.00	68.13	94.83	46.57	19.84	18.88	15.74

x – arithmetic mean; S.D. – standard deviation; CV (%) – coefficient of variation; *P<0.05; **P<0.01; ***P<0.001

Release of steroid hormones by adrenocortical carcinoma cells after $HgCl_2$ exposure

The results of our present study indicate dose-dependent decreases in both progesterone and testosterone release by NCI-H295R cell line in culture following a 48 h *in vitro* $HgCl_2$ exposure detected at low concentration (Table 2), which does not elicit significant (P>0.05) cytotoxic action. The viability of cells significantly decreased at 25 μM and higher concentration of $HgCl_2$ (Figure 2). However, at 25 μM $HgCl_2$ exposure the cell viability remained relatively high (> 80%). The measurement of cell viability and *in vitro* sexual steroid production proved to be sensitive for assessing a direct action of environmental chemical factors. Our presented data showed that progesterone was decreased, but at the lower concentrations (1.0 and 5.0 μM) of $HgCl_2$ this decline was less pronounced comparing to depression of testosterone. Burton and Meikle (1980) found that mitochondrial conversion of cholesterol to pregnenolone was inhibited in testicular tissue following mercury (MeHg) exposure and this could inhibit steroidogenesis. Veltman and Maines (1986) studied the synthesis of corticosterone in rats following exposure to $HgCl_2$, and determined that there was an increase in specific cytochrome P450 enzymes (CYPs) in the mitochondrial fraction of the adrenal glands, which in turn caused an increase in side-chain cleavage of cholesterol and a seven fold increase in the rate of production of pregnenolone.

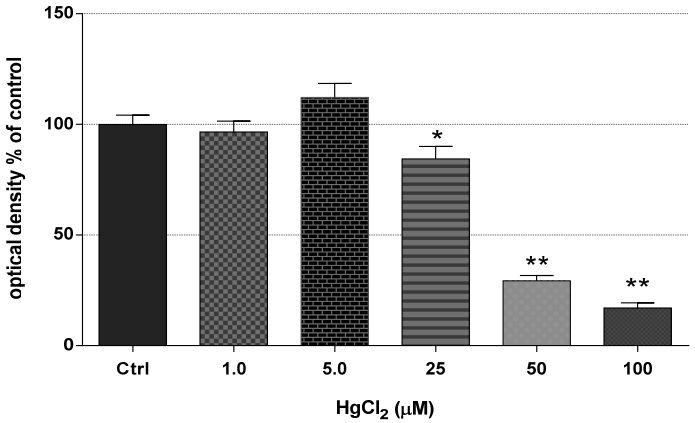

Figure 2. *The viability of NCI-H295R cells in culture after 48 h of $HgCl_2$ exposure*

Legend: Cytotoxicity was assessed using the MTT assay following $HgCl_2$ exposure. Bar values represent the arithmetic mean (±S.D.) optical density in % of (untreated) controls. The number of replicate wells was 20-32 at each point. A decline in optical density reflects a decline in cell viability. Statistical difference between the values of control and treated cells was indicated by asterisks * P<0.05; ** P<0.01; *** P<0.001 (One-way ANOVA with Dunnett´s multiple comparison test).

The testosterone seems to be more vulnerable than progesterone to $HgCl_2$ exposure suggesting multiple sites of action of this metal in steroidogenesis. Disorders of the testosterone synthesis could result in a reduction of the activity of the key enzymes implied in the biosynthesis of testosterone (McVey et al., 2008). Vachhrajani and Chowdhury (1990) examined the testicular steroidogenesis after an intraperitoneal administration of $HgCl_2$ (5.0; 10 μg/kg) and methylmercury (50; 100 μg/kg of MeHg) for 90 days. Both ($HgCl_2$, MeHg) inhibited the activity of 3β-hydroxysteroid dehydrogenase in the rat, leading to a significant decrease in serum testosterone levels and an induced cellular disintegration of Leydig cells.

Table 2. *Effect of 48h $HgCl_2$ exposure on release of sexual steroid hormones (ng/mL) by adrenocortical carcinoma (NCI-H295R) cells*

Groups	**Control**	**1.0**	**5.0**	**25**	**50**	**100**
	Ctrl	**E**	**D**	**C**	**B**	**A**
			$HgCl_2$ (μM)			
Progesterone (ng/mL)						
x	18.53	11.79*	17.31	1.43**	0.007**	0.01**
Minimum	12.14	7.92	11.88	1.09	0.00	0.00
Maximum	23.58	15.21	22.24	2.02	0.02	0.02
S.D.	4.80	2.99	4.37	0.41	0.009	0.01
CV (%)	25.89	25.40	25.26	28.65	127.66	115.47
%	100.00	63.63	93.42	7.72	0.38	0.05
Testosterone (ng/mL)						
x	12.21	4.85**	5.51**	1.06**	0.36**	0.88**
Minimum	6.69	2.59	2.84	0.89	0.10	0.05
Maximum	18.50	8.29	8.24	1.38	0.78	2.05
S.D.	4.90	2.45	2.59	0.22	0.30	0.92
CV (%)	40.16	50.51	47.04	20.27	83.40	104.53
%	100.00	39.72	45.13	8.68	3.00	7.20

x – arithmetic mean; S.D. – standard deviation; CV (%) – coefficient of variation; *P<0.05; **P<0.01; ***P<0.001

Release of steroid hormones by adrenocortical carcinoma cells after $NiCl_2$ exposure

Following 48 h culture of the cells in the presence of $NiCl_2$ a concentration-dependent depletion of steroid hormones release was observed in the whole applied range of concentrations (1.90 to 500 μM), including the lower concentrations of $NiCl_2$. The lowest amount of progesterone was significantly (P□0.01) detected in groups with the higher doses (≥ 500 μM) of $NiCl_2$ (Table 3), which elicited significant cytotoxic effect. However, the cell viability remained relatively high up to 62.50 μM of $NiCl_2$ (Figure 3). The testosterone production was decreased as well, but this decline was more pronounced compared to depression of progesterone. These results clearly confirm previous reports by Forgacs et al. (2011) and Ocztos et al. (2011), who observed the effect of Ni^{2+}, Hg^{2+} and Cd^{2+} on the progesterone and testosterone production of H295R cells.

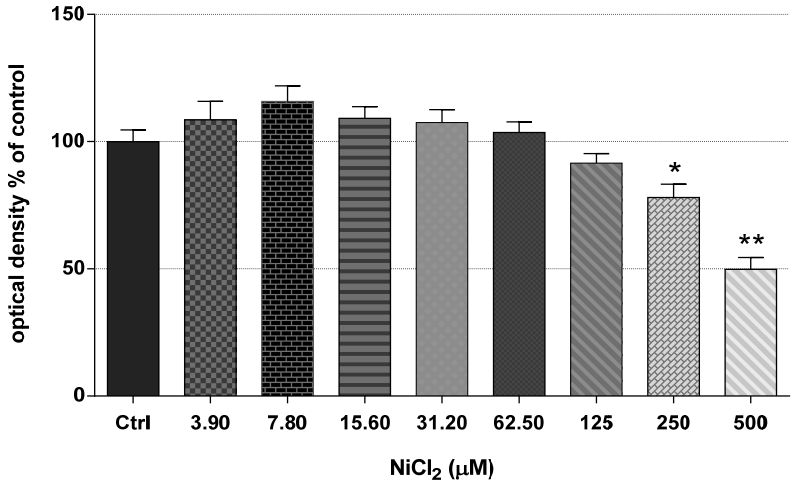

Figure 3. *The viability of NCI-H295R cells in culture after 48 h of $NiCl_2$ exposure*

Legend: Cytotoxicity was assessed using the MTT assay following $NiCl_2$ exposure. Bar values represent the arithmetic mean (±S.D.) optical density in % of (untreated) controls. The number of replicate wells was 22-32 at each point. A decline in optical density reflects a decline in cell viability. Statistical difference between the values of control and treated cells was indicated by asterisks * $P<0.05$; ** $P<0.01$; *** $P<0.001$ (One-way ANOVA with Dunnett´s multiple comparison test).

Table 3. *Effect of 48 h $NiCl_2$ exposure on release of sexual steroid hormones (ng/mL) by adrenocortical carcinoma (NCI-H295R) cells*

Groups	**Control**	**3.90**	**7.80**	**15.60**	**31.20**	**62.50**	**125**	**250**	**500**
	Ctrl	**H**	**G**	**F**	**E**	**D**	**C**	**B**	**A**
$NiCl_2$ (μM)									
Progesterone (ng/mL)									
x	21.05	19.56**	11.07**	10.59**	11.70**	9.93**	7.33**	6.18**	5.79**
minimum	15.24	15.64	6.24	6.84	8.27	6.88	5.27	4.25	3.55
maximum	28.25	24.12	14.25	15.26	14.85	14.49	10.58	8.18	7.58
S.D.	4.40	4.00	3.26	3.06	2.65	3.00	1.99	1.62	1.35
CV (%)	20.91	20.46	29.48	28.96	22.96	30.24	27.15	26.13	23.41
%	100.00	68.39	52.59	50.28	55.50	47.19	34.83	29.37	27.48
Testosterone (ng/mL)									
x	10.75	4.42**	3.18**	1.98**	4.96**	1.46**	1.22**	2.18**	1.86**
minimum	6.54	2.12	1.84	0.48	3.02	0.27	0.25	0.88	0.57
maximum	16.44	7.28	5.87	3.54	7.12	2.71	2.33	3.19	3.21
S.D.	3.45	2.02	1.47	1.18	1.71	0.90	0.74	0.89	1.07
CV (%)	32.12	45.66	46.33	59.52	34.45	61.85	60.43	41.05	57.51
%	100.00	41.10	29.55	18.41	46.14	13.53	11.30	20.23	17.26

x – arithmetic mean; S.D. – standard deviation; CV (%) – coefficient of variation; *P<0.05; **P<0.01; ***P<0.001

Krockova et al. (2011) examined the effects of $NiCl_2$ on the testosterone secretion, cell viability and apoptosis in mouse Leydig cells *in vitro*. Their study demonstrated that $NiCl_2$ decreased the testosterone production already at a low dose (15.67 μM) and subsequently confirmed structural and functional alterations in the Leydig cells. Ng and Liu (1990) reported that $NiCl_2$ had no deleterious effects on cell viability and hormone-induced

steroidogenesis in the adrenal gland and Leydig cells when tested up to a concentration of 100 μM.

Conclusions

Data obtained from this *in vitro* study indicate that the release of sexual steroid hormones by adrenocortical carcinoma cells can be associated with the dose of heavy metals administration. Testosterone release seemed more vulnerable than progesterone to heavy metals exposure. Probably the effect of enzymatic action of 17β-hydroxysteroid dehydrogenase is more expressive, which results in decreased release of testosterone in comparison with progesterone and thereby the effect of enzymatic action of 3β-hydroxysteriod dehydrogenase. In conclusion, the present study suggests the endocrine disruptive and reproductive toxicological effects of these selected heavy metals.

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References

1. Burton GV and Meikle AW 1980. Acute and chronic methyl mercury poisoning impairs rat adrenal and testicular function. J. Toxicol. Environ. Health. 6, 3, 597–606.
2. Das KK 2009. A comprehensive review on nickel (II) and chromium (VI) toxicities – possible antioxidant (*Allium Sativum Linn*) defences. Al. Ameen J. Med. Sci. 2, 43-50.
3. Das KK and Dasgupta S 2000. Effect of nickel on testicular nucleic acid concentrations of rats on protein restriction. Biol. Trace. Elem. Res. 73, 175-180.
4. Ding L, Murphy MB, He Y, Xu Y, Yeung LWY, Wang J, Zhou B, Lam PKS, Wu RSS and Giesy JP 2007. Effects of brominated flame retardants and brominated dioxins on steroidogenesis in H295R human adrenocortical carcinoma cell line. Environ. Toxicol. Chem. 26, 764-772.
5. Eisler R 1998. Nickel hazards to fish, wildlife, and invertebrates: A synoptic review. Biological Science Report USGS/BRD/BSR-1998-0001. Contaminant Hazard Reviews Report 34, 98 pp.
6. Forgacs Z, Szivos-Racz M and Ocztos G 2011. Study the effects of hormone disruptors on H295R cell line. XVIII. Primer Prevencios Forum, Budapest, May 19, 2011. Magyar Onkológia, 2011, 55, 3, 220–221, from http://huon.hu/2011/55/3/0213/0213a.pdf.
7. Gazdar AF, Oie HK, Shackleton CH, Chen TR, Triche TJ, Myers CE, Chrousos GP, Brennan MF, Stein CA and La Rocca RV 1990. Establishment and characterization of a human adrenocortical carcinoma cell line that expresses multiple pathways of steroid biosynthesis. Cancer Research 50, 5488-5496.
8. Hilscherova K, Jones PD, Gracia T, Newsted JL, Zhang X, Sanderson JT, Yu RMK, Wu RSS and Giesy JP 2004. Assessment of the effects of chemicals on the expression of ten steroidogenic genes in the H295R cell line using real-time PCR. Toxicol. Sci. 81, 1, 78–89.
9. Jolibois LSJr, Burow ME, Swan KF, George WJ, Anderson MB and Henson MC 1999. Effects of cadmium cell viability, trophoblastic development, and expression of low density lipoprotein receptor transcripts in cultured human placental cells. Reprod. Toxicol. 13, 473-480.

10. Kawai M, Swan KF, Green AE, Edwards DE, Anderson MB and Henson MC 2002. Placental endocrine disruption induced by cadmium: effects on P450 cholesterol side-chain cleavage and 3beta-hydroxysteroid dehydrogenase enzymes in cultured human trophoblasts. Biol. Reprod. 67, 178-183.

11. Knazicka Z, Lukac N, Forgacs Z, Tvrda E, Lukacova J, Slivkova J, Binkowski Ł and Massanyi P 2013. Effects of mercury on the steroidogenesis of human adrenocarcinoma (NCI-H295R) cell line. J. Environ. Sci. Health. Pt. A. 48, 348-353.

12. Krockova J, Massanyi P, Sirotkin AV, Pivko J, Makarevic AV, Lukac N, Capcarova M, Toman R and Polakova Z 2011. Nickel induced structural and functional alterations in mouse Leydig cells *in vitro*. J. Tr. Elem. Med. Biol. 25, 1, 15-18.

13. Lafuente A, Cano P and Esquifino AI 2003. Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent? Biometals 16, 243–250.

14. Laskey JW and Phelps PV 1991. Effect of cadmium and other metal cations on *in vitro* Leydig cell testosterone production. Toxicol. Appl. Pharmacol. 108, 296-306.

15. Massanyi P, Bardos L and Toman R 2002. Effects of cadmium on the testicular structure and the level of retinoids and β-carotene in testis in mice after an experimental administration. Polish Journal of Natural Sciences 10, 133-139.

16. McVey MJ, Cooke GM, Curran IH, Chan HM, Kubow S, Lok E and Mehta R 2008. An investigation of the effects of methylmercury in rats fed different dietary fats and proteins: testicular steroidogenic enzymes and serum testosterone levels. Food Chem. Toxicol. 46, 270-279.

17. Mosmann T 1983. Rapid colorimetric assay for cellular growth and surrival: application to proliferation and cytotoxicity assays. J. Immunol. Meth. 65, 1-2, 55–63.

18. Ng TB and Liu WK 1990. Toxic effect of heavy metals on cells isolated from the rat adrenal and testis. *In Vitro* Cell Dev. Biol. 26, 1, 24-28.

19. Ocztos G, Forgacs Z and Szivos-Racz M 2011. H295R Steroidogenesis Assay for study endocrine disruptors and reproductive toxicants. Fiatal Higi´enikusok VII. Fóruma, Esztergom, May 26–28, 2011, 55, 2, 132–133, from http://egeszsegtudomany.higienikus.hu/cikk/2011-2/2011-2.pdf.

20. Organization for Economic Cooperation and Development (OECD) 2011. H295R Steroidogenesis Assay, OECD Guideline for the Testing of Chemicals No. 456, Paris, 2011, from http://www.oecdilibrary.org/environment/test-no-456-h295r-steroidogenesis-assay 9789264122642-en.

21. Paksy K, Varga B and Lazar P 1992. Cadmium interferes with steroid biosynthesis in rat granulose and luteal cells *in vitro*. BioMetals 5, 245-250.

22. Paksy K, Rajczy K, Forgacs Z, Lazar P, Bernard A, Gati I and Kaali GS 1997. Effect of cadmium on morphology and steroidogenesis of cultured human ovarian granulosa cells. J. Appl. Toxicol. 17, 321-327.

23. Pandey R, Kumar R, Singh SP, Saxena DK and Srivastava SP 1999. Male reproductive effect of nickel sulphate in mice. Biometals 12, 339-346.

24. Przybyla AE, Robbins J, Menton N and Peck HD 1992. Structure-function relationship among the nickel-containing hydrogenases. Microbiol. Rev. 8, 109-135.

25. Rainey WE, Saner K and Schimmer BP 2004. Adrenocortical cell lines. Mol. Cell. Endocrinol. 228, 23-28.

26. Sanderson JT 2006. The steroid hormone biosynthesis pathway as a target for endocrine-disrupting chemicals: Review. Toxicol. Sci. 94, 3-21.

27. Schuurs AH 1998. Working with mercury: cause of infertility in women? Ned Tjidschr Tandheelkd 105, 11, 401-403.

28. Staels B, Hum DW and Miller WL 1993. Regulation of Steroidogenesis in Ncl-H295 Cells – a Cellular-Model of the Human Fetal Adrenal. Mol. Endocrinol. 7, 3, 423-433.

29. Takiguchi M and Yoshihara S 2006. New Aspects of Cadmium as Endocrine Disruptor. Environmental Sciences 13, 2, 107-116.

30. Tazisong I and Senwo Z 2009. Mercury concentration and distribution in soils impacted by long-term applied broiler litter. Bull. Environ. Contam. Toxicol. 83, 291-294.

31. Tchounwou PB, Ishaque AB and Schneider J 2001. Cytotoxicity and transcriptional activation of stress genes in human liver carcinoma cells (HepG2) exposed to cadmium chloride. Mol. Cell. Biochem. 222, 21-28.

32. Vachhrajani KD and Chowdhury AR 1990. Distribution of mercury and evaluation of testicular steroidogenesis in mercuric chloride and methylmercury administered rats. Ind. J. Exp. Biol. 28, 8, 746-751.

33. Veltman JC and Maines MD 1986. Alterations of heme, cytochrome P-450, and steroid metabolism by mercury in rat adrenal. Arch. Biochem. Biophys. 248, 2, 467-478.

34. Yokio K, Uthus EO and Nielsen FH 2003. Nickel deficiency diminishes sperm quantity and movement in rats. Biol. Trace. Elem. Res. 93, 2, 141-154.

35. Zhu X, Kusaka Y, Sato K and Zhang Q 2000. The endocrine disruptive effects of mercury. Environ. Health. Prev. Med. 4, 174-183.

Invited paper

PHENOTYPIC RELATION GROWTH RATE IN PERFORMANCE TEST AND LITTER SIZE OF SOWS

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Abstract

In this study we investigated influence of weight gain level of gilts reached during performance test on the expression of important reproductive traits. For the purpose of research, data on the litter size from 2nd to 9th parity of 700 tested gilts were monitored. Characteristics that were examined are: growth at the end of the test, the number of piglets born alive, stillborn and weaned piglets per parity and a total from 2nd to 9th parity, and the percentage of sows entering the next farrowing. Results obtained in this study indicate that gilts with a lower weight gain in performance test have smaller number of live born (8.8:9.23 $p<0.01$) and reared piglets (8.46:8.55, $p<0.01$) in regard to the higher weight gain gilts, but a larger number of stillborn piglets (0.35:0.29). Also, gilts with the lowest weight gain during the performance test had smaller average litter size from 2nd to 9th parity. Even greater difference is determined if the average litter size from 3rd to 7th parity (9.8:10.0 and 10.4) is observed. The largest percentage of sows farrowing for the second time was in gilts with the highest weight gain during the performance test (39.5%: 14.8% and 15.5%) which expressed statistically significant difference ($p<0.01$). Gilts with a lower gain weight in performance test had a higher percentage of sows included in the next farrowing. Data showed a large drop in the number of sows with the second and third parity in the total sample, where the percentages of earned second and third litters were 16.5 and 45.3%, while the percentages of actual parity from the 4th to 7th parity were: 79.2; 94.7; 83.3; 96.7; 72.4 and 90.5%.

Key words: *gilts, litter size, live births, parity, sows, weight gain*

Introduction

Pig production, in addition to poultry, is one of the most intensive type of production, which requires the perfect operation of all technological processes. Great emphasis is placed on the production of breeding material, mainly gilts (Stančić et al., 2006), which after proper selection are to be engaged in production (Hughes et al., 2010), with the assumption that, as Prunier et al. (2003) and Hoving et al. (2010) stand out, they will remain in production long enough to cover the costs of purchasing and breeding. In this case, it is primarily related to gilts/sows longevity with achieving maximal reproductive performance (Lucia and March, 1999; Lucia et al., 2000a; Serenius et al., 2006; Filha et al., 2010). For this reason, it is necessary to correct the traditional breeding technology (Foxcroft et al., 2005), even if it is, in addition to the impact of growth, difficult to define effects of age, weight and thickness of a side of bacon on reproductive efficiency (Bortolozzo et al., 2009).

A current problem in production is high percentage of culled sows which, according to Linda Engblom et al. (2007) reaches an average of 49.5% on commercial farms in Sweden, which is also similar to the results obtained in other EU countries (Boyle et al., 1998; Lucia et al., 1999; Lucia et al., 2000a and 2000b). For the purpose of ensuring a sufficient number of animals for herd repair, it is necessary to pay special attention to gilt raising, in order to align the appropriate body weight and age at the first possible moment of fertilization so they could effectively be involved in breeding (Radović, 2002; Stančić et al., 2003; Radović et al., 2007). Factors influencing the moment of reaching puberty and reproductive efficiency are numerous, from the influence of sow genotype and boar impact (Langendijk et al., 2000; Kemp et al., 2005) up to factor of accommodation (Stančić and Šahinović, 1998; Stančić et al., 2003; Akos and Bilkei, 2004). Recently, emphasis has been placed on defining the criteria for identification and exclusion of sows with low productivity (Gordon, 1997; Takai and Koketsu, 2007).

In addition to these factors, an essential factor is nutrition (Kirkwood et al., 1998*).* Feeding during the growth period has to provide a high daily gain, efficient food conversion and good carcass quality, as they are main criteria for assessing the performance test in the gilts and their involvement in reproduction *(Main Pig Breeding Program, 2010).* However, according to Rydhmer et al. (1995) and Chen et al. (2003), high meatiness caused by high weight gain affects reproductive parameters. Gerasimov et al. (1997) and Imboonta et al. (2007) indicate that, because of different degree of heritability of reproductive and fattening characteristics, it is not possible to apply the same methods of selection in order to obtain corresponding phenotype with a good reproductive characteristics, which according to Radović et al. (2006) and Petrović et al. (2006) represent the secure key for the future successful production.

In our production conditions only data from performance test are used for gilts selection. Obtained data favor growth characteristics, which later represent the basis for a decision on the gilt use in breeding (*Main Pig Breeding Program*, 2010; Radojković et al., 2012). Takai and Koketsu (2007) and Filha et al. (2010) pointed out that for maximum utilization of genetic potential of gilts first fertile insemination should be done with the body mass of 120 kg and a minimum backfat thickness of 16 mm.

Due to the foregoing, there is always an interest to find the links between growth of gilts in performance test and their subsequent reproductive potential, with the aim to determine optimal selection strategy and management of gilts breeding technology.

Material and methods

Study was carried out on the farm with capacity of 2,500 sows. For the purpose of research data on litter size from 2nd to 9th parity of 700 gilts that finished performance test were monitored. Performance test lasted from 25 to 100 kg body weight. Animals were placed in a box in a group of three and were fed with a standard meal for gilts in up-growth. Daily gain for each one of gilts has been calculated at the end of the test. Groups were defined on the basis of growth in performance test: first group (I) with a growth rate of 295-418 g; second group (II) with a growth rate of 419-542 g and third group (III) with a growth rate of 543-665 g. Within the defined groups the number of live births, stillbirths and weaned piglets was calculated. Calculations were performed in the descriptive statistics, analysis of variance, using the general linear model and phenotypic correlations between growth in test performance and litter size of parity. Due to a large number of first litter sows to be excluded from breeding, as a result of applied technology and set of selection criteria, the results of the total number of live births, stillbirths and weaned piglets were analyzed and

monitored for each sow, ranging from 2nd to 9th parity, respectively, only in those animals that have begun to be actively used in the reproduction. In particular, an average litter size from 3rd to 9th parity was calculated regarding the period that potentially includes sows that, based on reproductive performance and according to the selection criteria on the farm, could enter the nucleus level. The percentage of sows included in the next parity has been calculated in relation to the number of sows that made prior farrow.

Results and discussion

Table 1 shows results of the number of live births, stillbirths and weaned piglets from 2nd to 9th parity, based on the weight gain in performance test. Results show that gilts with lower gain in performance test had less live births (8.8:9.23, $p < 0.05$) and reared piglets (8.46:8.55, $p < 0.05$) compared to gilts with higher gain. Serenius et al. (2004) and Serenius et al. (2005) reported that a small gain, as a result of low food intake, causes great loss of backfat during lactation, which is directly related to the longevity of sows and its reproductive performance. The same authors (Serenius et al., 2006) state that there is insufficient information in the literature about the influence of genetic factors on food intake and backfat loss during lactation. Number of stillborn piglets (Table 1) gives us information that gilts with lower gain had more stillborn piglets (0.35:0.29) than gilts with higher weight gain, which is inconsistent with the results of Filha et al. (2010), where gilts with weight gain over 770g had a higher number of stillborn piglets. The same authors suggest that taking into account the cost of food, number of non-productive days, number of piglets born alive and uniformity of weight of piglets the production should be focused on obtaining gilts with less gain in the moment of first fertilization.

Table 1. *Number of liveborn, stillborn and weaned piglets in the first parity in relation to the daily gain in performance test*

Liveborn					
Gain, g	**Valid N**	**Mean**	**Variance**	**Std.Dev.**	**Stand. Error**
295-418 (I)	147	8.8^{A}	5.58	2.36	0.19
418.4-542 (II)	454	8.99	5.21	2.28	0.11
543-665 (III)	38	9.23^{A}	7.02	2.65	0.43
Stillborn					
Gain, g	**Valid N**	**Mean**	**Variance**	**Std.Dev.**	**Stand. Error**
295-418 (I)	147	0.35	0.036	0.061	0.05
418.4-542 (II)	454	0.34	0.04	0.065	0.03
543-665 (III)	38	0.29	0.03	0.056	0.09
Weaned					
Gain, g	**Valid N**	**Mean**	**Variance**	**Std.Dev.**	**Stand. Error**
295-418 (I)	147	8.46	2.31	1.52	0.13
418.4-542 (II)	454	8.5	1.81	1.34	0.06
543-665 (III)	38	8.55	1.66	1.29	0.21

Values with the same superscript are significantly different ($p<0.05$)

In order to determine the reproductive efficiency of sows by parity, Table 2 shows the number of sows, average number of live-born piglets from 2nd to 9th parity, and the average number of live-born piglets from 3rd to 7th parity depending on the gain that gilts achieved during the performance test. It also shows the percentage of sows that made next farrowing.

Table 2. *Total number of liveborn piglets and percentage of included sows from 2nd to 9th parity in relation to the daily gain in test*

Gain		Parity										
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**3.-7.**	**2.-9.**
295-418 (I)	**No. of sows**	148	23	22	16	16	12	12	6	5		
	% of included sows	100.0%	15.5%^{B}	14.9%	10.8%	10.8%	8.1%	8.1%	4.1%	3.4%		
	liveborn		9.0	9.4	9.3	9.4	10.7	10.3	10.3	10.4	9.8	9.8
419-542 (II)	**No. of sows**	458	68	22	19	18	16	15	13	13		
	% of included sows	100.0%	14.8%^{A}	4.8%	4.1%	3.9%	3.5%	3.3%	2.8%	2.8%		
	liveborn		8.9	9.7	9.9	9.9	10.0	10.2	10.0	9.6	10.0	9.8
543-665 (III)	**No. of sows**	38	15	4	3	2	2	2	2	1		
	% of included sows	100.0%	39.5%^{AB}	10.5%	7.9%	5.3%	5.3%	5.3%	5.3%	2.6%		
	liveborn		7.3	9.5	11.6	9.0	9.9	11.7	10.8	10.0	10.4	10.0
Total of sows	644	106	48	38	36	30	29	21	19			
% of included sows	100	16.5%	45.3%	79.2%	94.7%	83.3%	96.7%	72.4%	90.5%			

Values with the same superscript are significantly different ($p<0.01$)

According to Takai and Koketsu (2007), the objective of piglet production is to provide a sufficient number of sows in parities from 2nd to 9th because of the great potential of sows in litter size in those parities. In our study, the results obtained by analyzing the reproductive efficiency of sows by parity (Table 2) show that gilts with the smallest gain (group I) during the performance test had a lower average number of live-born piglets from 2nd to 9th parity compared to gilts of group III. Even greater difference appears if one considers the average litter size from 3rd to 7th parity (9.8:10.4). According to Lucia et al. (2000a) this is explained by higher selection pressure on sows in these parities and high weight gain in performance test, which are potential nucleus animals, i.e. producing animals. Takai and Koketsu (2007) point out that the percentage of gilts that do not satisfy the selection criteria in the commercial farms is approximately 20%. The same authors state that those are the sows with low reproductive index which causes reduction of farrowing percentage by 10%. Moreover, low reproductive index later causes extended service intervals with an increase of non-productive days and a reduction in herd productivity. Among the abovementioned values statistical significance was not manifested because of the small percentage of sows involved in the next farrowing and a small number of recorded litters. Highest percentage of second farrowing sows was in gilts with the greatest weight gain during performance test (39.5%: 14.8% and 15.5%), where a statistically significant difference was expressed ($p<0.01$) compared to gain of gilts in the first and second group. This demonstrates the efficient translation to the second farrowing

sows category of those animals that as gilts had the largest gain in performance test. Todd (2000) states that in order to ensure quality production on commercial farms, optimal parity structure should not have more than 18 - 22% of first farrowing sows related to total number of sows on farm. According to Brisbane and Chesnais (1997), reduction of longevity of sows increases the cost of their replacement, ie.with the reduction in parity from 6 to 5.8 price of replacement of sows increases by 1.2%, while the reduction in parity from 3 to 2.8 increases the price of sows replacement up to 5%. Radović et al. (2008) state that the optimal period of use of sows in order to recover the investment in gilt production is from 3rd to 8th parity, and in the case of poor production they were excluded from breeding after 8th parity. For more precise way of making decisions about the culling of sows Kim et. al. (2014) recommended AHP - technique (Analytical Hierarchy Process) to make decisions based on multiple criteria. Percentage of sows involved in farrowing from 2nd to 9th parity is shown in Chart 1 and Table 2.

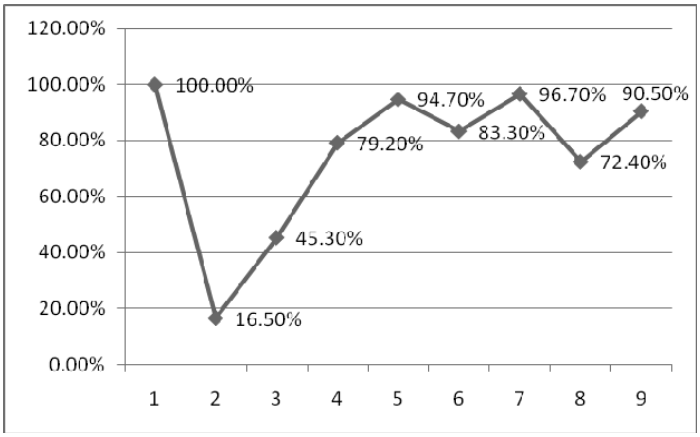

Chart 1. *Number of farrowing sows in relation to the number of sows in previous farrowing*

A large decline in the number of second and third farrowing sows in total sample was manifested, where percentage of realized second and third litters is only 16.5 and 45.3% (Table 2, Chart 1). According to Linda et al. (2007) and Hughes et al. (2010) most common reasons for exclusion of lower parity sows are reproductive problems, locomotory problems, a small number of piglets born alive and increased number of non productive days. The same authors state that there is a high percentage of unplanned culling exactly in this category of young sows.

In addition, the results show (Table 2, Chart 1) that the number of realized litters from parities 4 to 7 decreased in a much lesser extent and the percentage of actual farrowing was 79.2; 94.7; 83.3; 96.7; 72.4 and 90.5%. Observed by the groups, gilts with less weight gain in performance test had a higher percentage of sows included in the next farrowing. Reasons for exclusion of sows in subsequent parities, according to Linda et al. (2007) are: reproductive disorders (26.9%), age (18.7%), udder problems (18.1%), low productivity (9.5), problems with the locomotor apparatus (8.6%) and traumatic injuries (7.1%). These authors state that the most common reasons for exclusion of sows from parities 4 to 6 are problems with udder, while in sows after 7th parity the most common reason is age.

Conclusion

The results obtained in this study show a positive impact of the weight gain in performance test of gilts to the total number of live births and reared piglets, as well as the percentage of the realized next farrowing. Further research on the impact of characteristics of performance test on subsequent reproductive efficiency of sows can provide guidance in defining the criteria for characteristics in the aggregate genotype, which would give a more accurate estimation of breeding values of animals.

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References

1. Amaral Filha WS, Bernardi ML, Wentz I and Bortolozzo FP 2010. Reproductive performance of gilts according to growth rate and backfat thickness at mating. Animal Reproduction Science, Volume 121, Issues 1–2, August, 139–144.
2. Akos K and Bilkei G 2004. Comparison of the reproductive performance of sows kept outdoors in Croatia with that of sows kept indoors, Livest. Prod. Sci. 85, 293–298.
3. Hughes PE, Smits RJ and Xie Y 2010. Relationships among gilt and sow live weight, P2 backfat depth, and culling rates. J Swine Health Prod. 2010;18(6), 301–305.
4. Bortolozzo PF, Bernardi LM, Kummer R and Wentz I 2009. Growth, body state and breeding performance in gilts and primiparous sows. Soc. Reprod. Fertil. Suppl., 66, 281–291.
5. Brisbane JR and Chesnais JP 1997. Relationship between backfat and sow longevity in Canadian Yorkshire and landrace pigs, Ottawa: Canadian Center for Swine Improvement.
6. Chen P, BaasJT, Mabry WJ and Koehler JK 2003. Genetic correlations between lean growth and litter traits in U.S. Yorkshire, Duroc, Hampshire, and Landrace pigs[1], J. Anim. Sci. 81, 1700-1705.
7. Foxcroft G, BeltranenaE, Patterson J and Williams N 2005. The biological basis for implementing effective replacement gilt management. Proceedings of the Leman Pre-Conference Reproduction Workshop, Saint Paul, MN, 5–25.
8. Gerasimov V, Pron E and Danilova T 1997. The effect of genotype and final live weight after finishing on economic traits of pigs. Pig News and Information, 18(1), 35.
9. The main breeding program in swine R Serbia, 2010. https://sites.google.com/site/odeljenjezaselekcijusvinja/odgajivacki-program.
10. Gordon I, 1997, Controled Reproduction in Pigs**.** CAB Int.Oxon, UK,.
11. Hoving LL, Soede NM, Graat EAM, Feitsma H and Kemp B 2010. Effect of live weight development and reproduction in first parity onreproductive performance of second parity sows.Animal Reproduction Science 122, 82–89.
12. Imboontaa N, Rydhmerb L and Tumwasorn S 2007. Genetic parameters and trends for production and reproduction traits in Thai Landrace sows, Livestock Science, Volume 111(1), 70-79.
13. Kemp B, Soede MN and Langendijk P 2005. Effects of boar contact and housing conditions on estrus expression in sows, Theriogenology 63, 643–656.

14. Kim S, Park H and Yoe H 2014. Study on the Sow's Culling Decision Method Using AHP Technique. Advanced Science and Technology Letters Vol.49 (SoftTech 2014), 197-201.

15. Kirkwood RN, Mitaru BN, Gooneratne AD, Blair R and Thacker PA 1998. The influence of premating feed intake on the reproductive perfomance of gilts. Can. J. Vet. Res. 52(1), 134-136.

16. Langendijk P, Soede MN and Kemp B 2000. Effects of boar contact and housing conditions on estrus expression in weaned sows, J. Anim. Sci. 78, 871–878.

17. Engbloma Linda, Lundeheima N, Anne-MarieDalin and Andersson K 2007. Sow removal in Swedish commercial herds, Livestock Science, 106, 76–86.

18. Lucia, T, Dial DG and March EM 1999. Estimation of lifetime productivity of female swine, J. Am. Vet. Med. Assoc. 214, 1056–1059.

19. Lucia T, Dial DG and Marsh EW 2000a. Lifetime reproductive and financial performance of female swine, J. Am. Vet. Med. Assoc. 216(11), 1802-1809.

20. Lucia T, Dial DG and Marsh EW 2000b. Lifetime reproductive performance in female pigs having distinct reasons for removal, Livest. Prod. Sci. 63, 213–222.

21. Petrović M, Živković B, Mijatović M, Radojković D and Radovic Č 2006. Phenotypic variability of carcass side traits of pigs fattened to higher body mass. The 35th International Scientific Communications Session of the Faculty of Animal Science, Bucharest, November, 15-17.The scientific papers of the Faculty of Animal Science, 51-56.

22. Prunier A, Soede N, Quesnel H and Kemp B 2003. Productivity and longevity of weaned sows. Weaning the Pig. Wageningen Academic Publishers, Wageningen, 385–419.

23. Radojković D, Petrović M and Radović Č 2012. Current And Future Challenges Of Selection Of Pigs. Proceedings Of The First International Symposium On Animal Science, November, Belgrade. Book I, 195-206.

24. Radović I 2002. Morphometric parameters of reproductive organs and reaching puberty in various genotypes of pigs. Master's thesis, Faculty of Agriculture, University of Novi Sad.

25. Radović I, Stančić B, Popov, Radmila, Trivunović Snežana and Teodorović M 2006. Reproductive performance of gilts and sows of higher parity. Faculty of Agriculture, vol. 55, no. 1-2, 83-90.

26. Radovic I, Uzelac Z, Stancic B, Trivunović Snežana, Popov Radmila and Teodorović M 2007a. Influence of body weight on ovarian activity and the occurrence of puberty in gilts. Contemporary Agriculture 56 (3-4), 1-7.

27. Radovic I, Stancic B, Popov Radmila and Vasiljevic V 2008. Association between fattening and reproductive traits krmača.1.Povezanost body weight at the end of the test and litter size. Contemporary Agriculture, 57, No.1-2, 33-37.

28. Rydhmer L, Lundeheim N and Johansson K 1995. Genetic parameters for reproduction traits in sows and relations to performance-test measurements. Journal of animal breeding and genetics, vol.112, no1, 33-42 (1 p.1/4).

29. Serenius T and Stalder JK 2006.Selection for sow longevity J. Anim. Sci. 84, 166-171.

30. Serenius, T., and Stalder JK 2004. Genetics of length of productive life and lifetime prolificacy in the Finnish Landrace and Large White pig populations. J. Anim. Sci.82, 3111–3117.

31. Serenius T, Stalder JK, Baas JT, Mabry WJ and Goodwin NR 2005. A comparison of six maternal genetic lines for sow longevity. J. Anim. Sci. 83 (Suppl. 2), 53. (Abstr.).

32. Stancic LB and Šahinović HR 1998.Biotechnology in the reproduction of pigs (monographs) University of Novi Sad, Faculty of Agriculture, Institute for Animal Husbandry. Novi Sad, Serbia.

33. Stancic B, Kovčin S and M Gagrčin 2003. Physiology and technology of reproduction (monograph). University of Novi Sad, Faculty of Agriculture. Novi Sad, Serbia.

34. Stancic B, Radovic I, Gagrčin M, S and Kovčin Trivunović Snow 2006. Effect of paragenetic factors on the age of the gilts in reaching sexual maturity - an overview. Faculty of Agriculture, vol. 55, no. 1-2, 95-105.

35. Takai Y and Koketsu Y2007. Identification of a female-pig profile associated with lower productivity on commercial farms. Theriogenology 68, 87–92.

36. Todd S 2000. Managing the sow for optimum productivity. North Carolina State University, Department of Animal Science Raleigh, 27695-7621.

Review paper

SIMMENTAL BREED PRODUCTION CHARACTERISTICS AND BREEDING GOALS

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Abstract

This paper presents the most important production results obtained for the population of Simmetal breed raised in majority of the European countries which with a total population of about 9 million animals rates as the second biggest important cattle breed in Europe. Breeding goals from previous period were analysed along with newly defined breeding goals in some Simmental breed populations. Current mean values for milk yield of Simmental cows in standard lactation in the European countries range from 5500 to 7500 kg. Depending on the Simmental breed population in most countries a breeding goal set for first lactation cows is milk yield of 5500 or 6000 kg with 4.0% milk fat and 3.5% protein, while in cows who finished their third lactation required milk production is more than 7000 kg for Simmental breed, over 8000 kg for Simmental breed with share of Red and White Holstein genes and over 8500 kg for Montbeliard breed. A special emphasis is given on the prolonging of the duration of life and production span. During life span the demand is to produce minimum 30 000 kg milk/cow. This demand can be realised if functional traits in breeding goal participate with over 40 % with proper implementation of improvement programme. As for the traits that are significant for meat production, depending on the population, defined breeding goal for daily liveweight gain are values above 1500 g/day (i.e. 1400 g /day) for Simmental male bullcalves.

Key words*: breeding goals, production traits, Simmental breed*

Introduction

Since the second half of the XX century the Simmental breed has developed into a high producing breed with pronounced milk yield. In some European countries (Switzerland, Hungary, Czech Republic, Slovakia), a Simmental type for pronounced milk yield is developed and improved by Red Holstein breed. In the other European countries (Scandinavian countries, Great Britain) and almost all non-European countries Simmental breed is well-known and raised as fatenning breed in system cow-calf, both in pure breed and in the programmes for dual and three breeds crossing with other cattle breeds. It is raised in various climate conditions and production systems and therefore represented in a significant degree in the Alpine region of Europe, where it originated, but it is also present in a significant degree in southeast Europe, Great Britain, Russia, China, North and South America, Southern Africa and other worldwide countries. Different sub-populations of Simmental breed have been created with different breeding goals. Nowadays in majority of the European countries the Simmental breed is mostly

raised in pure breed so that it possesses a high potential for expressing heterosis efect if crossed with dairy and specialised fatenning breeds.

Total world population of Simmental breed in the last two years numbers about 41 million heads. Simmental breed in Europe with about 9 million heads ranks second in population size after the population of Holstein Friesian cattle. According to the data of some national associations of breeders of Simmetnal breed (www.asr-rind.de) number of Simmental breed cows in Europe is about 6 million. As for the European countries the most significant populations of Simmental breed are raised in Germany and Austria. According to the Simmental breeders societies and associations an absolutely largest population of Simmental cattle is in Germany of about 3450000 heads out of which 1130000 are cows. In Austria the share of Simmental breed is 76% in total cattle fund of the country what makes about 1500000 heads. Austria and Germany are main exporters of Simmental breeding animals. The important export category for European countries is mostly the pregnant heifers category. Besides this export category Austria and Germany were in the past and still are major exporters of Simmental bulls semen into all parts of the world. Besides good genetic potential for milk yield it is desirable that the animals have well developed body shape. For this reason the process of holstenisation of Simmental breed in Germany and especially in Austria was not of a larger scale.

In France, Montbeliard breed is the most important among all spotted cattle created on a basis of Simmental breed from Switzerland. This breed belongs to the dual production breeds of pronounced milk yield. Red and White Holstein Friesian breed participated in its creation. Montbeliard breed is today used as an improving breed for poorer performance populations of Simmental breed such as that of our country.

In Serbian cattle breed structure Simmental breed makes about 80 % total fund of cattle, Holstein-Friesian breed and Black and White improved by Holstein Friesian breed 12 to 13 % and crosses of Simmental with other breeds (Busha in the first place) make about 6 to 7%, while pure breeds of native breeds (Busha and Podolska breed) have in total less than 1000 heads. In milk production in Serbia Simmental breed plays a major role due to its abundance – size of population, while as regards the individual production performance per animal Holstein Friesian breed is by far higher dairy producing breed compared to Simmental breed. General trend in Serbia is to reduce the number of milk producers, particularly producers with smaller number of cows (1-3 cows), so that in the forthcoming period we can expect significant reduction in the number of farmers-households producing milk (Popović, 2008; Perišić et al., 2012).

Simmental breed milk yield

Milk yield of Simmetnal cows in some European countries is at the level of bovine milk yield in specialised dairy breeds. Besides high level achieved in previous period the milk yield is constantly increasing. About less than 10 years ago in controlled populations of Simmental breed, in the countries which had more siginificant share of Simmental genes, milk yield ranged from 5000 to 7000 kg (Table 1).

According to the data produced by Simmental breeders national associations (www.asr-rind.de; www.fleckvieh.at; http://zar.at), bovine milk yield in two most important populations of Simmental cattle (German Simmental, Austrian Simmental) is increasing and milk production mean values both in control animals and the animals in registered breeding stock are higher than 7000 kg in standard lactation.

Table 1. - *Simmental cows milk yield traits in some European countries 10 years ago*

Country	Year	Duration of lactation, days	Milk yield, kg	Fat content %	Yield of fat, kg	Protein content, %	Yield of protein, kg
Hungary	2004	293	5 023	3.99	201	3.43	175
Czech Republic	2006	294	6 162	4.08	252	3.46	213
Slovenia	2006	305	5023	4.17	209	3.29	165
Slovakia	2004	297	4 919	4.09	201	3.30	162
Switzerland (Fleckvieh)	2006	299	6 847	3.99	273	3.24	222
Switzerland (Simmental)	2006	297	5 681	3.88	220	3.30	187
Austria	2006	305	6 483	4.18	271	3.43	222
Norway	2004	266	5 229	4.15	217	3.35	175
Croatia	2006	305	4 459	4.07	181	3.35	149
Italy	2006	293	6 528	3.92	256	3.41	223
France (Montbeliard)	2006	296	6 907	3.91	270	3.46	239
Germany	2006	316	6 854	4.14	284	3.48	239
Poland	2006	-	4785	4.01	192	3.36	161

(*www.Cattlenetwork.net*)

Austrian Simmental cows milk yield is at the level of mean values for bovine milk yield of all breeds. These mean values include milk yield of Holstein Friesian cows represented in Austria by about 10 % of total number of cows under milk yield control, i.e. about 12.5% of total registered cows.

In Switzerland the milk yield of spotted cattle populations in the type of Simmental breed had no significantly increase compared to the milk yield 10 years ago. However, milk yields of bovine populations of Simmental section (5819 kg), Fleckvieh section (6741 kg) and Montbeliarde breed cows in Switzerland in 2012/2013 were still placed among the European highest dairy producers when speaking of Simmental breed.

Table 2. - *Simmental cows milk yield traits in 2012 in Germany*

Population		Number of lactations	Milk yield (305 days), kg	Fat content, %	Fat yield, kg	Protein content, %	Yield of protein, kg
Fleckvieh, Control cows		885086	7107	4.11	292	3.48	247
Fleckvieh, Cows in breeding stock		693 599	7439	4.14	308	3.50	260
Fleckvieh, firstcalved	2012	245940	6481	4.13	267	3.46	225
	2002	239 900	5654	4.16	235	3.47	196

(www.asr-rind.de)

In Serbia in the regions of more intensive cattle production the bovine populations with pronounced milk yield are being raised and crossbreeding of Simmental breed with dairy breeds is quite common. In hilly-mountainous regions lower producing animals are being raised for the meat-milk production. According to selection reports milk yield of Simmental registered cows in Serbia is in the range of 4000 kg to 5500 kg milk in standard lactation depending on the lactation on order and breeding region.

Table 3. - *Simmental cows milk yield traits in 2013 and in preceding years in Austria*

Population	Number of lactations	Milk yield (305 days), kg	Fat content, %	Fat yield, kg	Protein content, %	Yield of protein, kg	Number of animals
Fleckvieh, Control cows, 2013	249030	7103	4.15	294	3.41	242	536
Average milk yield in all control bovine breeds in Austria per years							
2013	**339032**	**7200**	**4.14**	**298**	**3.40**	**245**	**543**
2012	337988	7148	4.14	296	3.41	244	540
2011	333191	6942	4.13	287	3.39	235	522
2010	328035	6841	4.12	282	3.38	231	513
Fleckvieh, Cows in breeding stock, 2013	240.745	7.141	4.15	296	3.41	244	540
Average milk yield in all bovine breeds in Austria per years							
2013	**326176**	**7234**	**4.14**	**299**	**3,40**	**246**	**545**
2012	326190	7177	4.14	297	3.41	245	542
2011	321700	6970	4.13	288	3.39	237	525
2010	316865	6867	4.12	283	3.38	232	515
Fleckvieh, Firstcalved of breeding stock, 2013	65766	6479	4.12	267	3.38	219	486
Average milk yield of firstcalved in breeding stock of all breeds in Austria per years							
2013	**91290**	**6575**	**4.11**	**270**	**3.37**	**221**	**491**
2012	91082	6516	4.11	268	3.39	221	489
2011	94928	6331	4.12	261	3.38	214	475
2010	92736	6235	4.11	256	3.36	209	465

(http://zar.at)

For the purpose of improving Simmental milk yield traits and fatenning traits in our country the improvement was implemented mostly by selection in pure breed. In addition there was an import of Simmental breeding animals from the countries that have high genetic potential individuals for milk production. Thus the animals were most often imported from Austria and Germany in which the mean milk production per cow for overall population in the last decade was from 6500 – 7000 kg milk with over 4 % milk fat. Since the milk yield traits are more rapidly enhanced by means of crossing our country followed the example of some European countries and in a considerable degree implemented the crossing of Simmental with Red Holstein and Montbeliard breeds. This

way enhances also the milking traits in a significant degree what is particularly important in intensive milk production and machine milking.

Table 4. - *Milk yield traits in Simmental cows in 2012/2013 in Switzerland*

Population	Number of lactations	Milk yield (305 days), kg	Fat content, %	Protein content, %
Simmental	15312	5819	3.93	3.32
Fleckvieh	41818	6741	4.05	3.26
Montbeliarde	5381	7388	3.77	3.32
Mean value for all control bovine breeds	167763	7389	4.05	3.26

(www.swissherdbook.ch)

In Serbia in the last three decades the crossbreeding of Simmental with higher dairy breeds (Montbeliard, Red and White Holstein Friesian breed) of different scope was implemented. The results thereof can be found in the research papers by Medić et al. (1983), Zečević (1986), Perišić (1998) and Perišić et al. (2002). The effects of crossing were monitored by Knežević et al. (1991) for populations in Croatia. These results indicate that cross-breeds compared to Simmental breed show somewhat earlier maturity, have higher milk production with lower content of milk fat and longer duration of lactation and service-period. The share of genes in Simmental dairy breeds should comply with breeding conditions in which improved animals realise their production, as well as with the market demands for milk production. Since in the European countries there is a trend of reducing the number of cows for milk production and increasing the population of bovine fattening breeds we should not, in our conditions, expect that holstenisation of Simmental breed will be of a larger scale in the forthcoming period, primarily due to current uncertain placement of milk and dairy products on the European market what reflects the situation in our dairy sector as well (Popović, 2008; Perišić et al., 2012).

Simmental as fattening breed

In Simmental breed, from its beginning until today, the improvement directions changed frequently although the endeavours to keep dual production could often be perceived with differences regarding the expressiveness of production traits (milk yield or fattening) all in accordance with the needs of the countries in which this same breed was bred for combined production. In the last several decades in non-European and some European countries Simmental breed is being raised exclusively for the purposes of specialised meat production by breeding in pure breed or by crossing with other breeds.

Simmental fattening breed is represented in Great Britain where this breed is crossed with dairy breeds in order to obtain crosses for fattening purposes. Simmental cattle in pure breed and for crossing with dairy breeds for the purpose of meat production is being bred in Ireland, Denmark and Sweden.

Due to its production traits and good acclimatisation abilities Simmental breed has spread to all continents and became one of the most significant world cattle breeds.

Besides spreading to the European Mediterranean countries Simmental has from the Alpine regions spread successfully to all continents. It is raised in a considerable degree also in South America where it is being crossed with American zebu (humped) cattle for the purpose of meat production by pasture fattening lasting for about two to three years. It is mostly crossed with larger format zebu cattle. Similar situation is in North America,

Australia and New Zealand, where Simmental breed is raised as specialised fattening breed. It is frequently crossed with fattening breeds of smaller format such as British fattening breeds with the aim to obtain cross-breeds which can be fattened to higher finishing weights compared to British breeds. The types of breeding used there are pasture cow-calf system and extensive fattening in fenced stables by use of forage feeds. It is raised also in southern and southwestern Africa, less in pure breed and more as hybrid breed Simbrah (Simmental x Braman).

Table 5. - *Performance test results on Simmental bullcalves fattening traits*

Country	Year	Mass at birth, kg	Mass with 200 days, kg	Mass with 365 days, kg	Liveweight gain in fattening, gr
Czech Republic	2006	39	284	531	1348
Slovenia	2006	45	276	-	-
Slovakia	2004	32	202	-	-
Serbia	2004	45	251	508	1254
Switzerland (Simmental)	2006	43	311	536	1469
Sweden	2006	48	342	610	1470
Austria	2006	44	285	445	1103
Norway	2004	44	340	568	1492
Ireland	2004	40	400	-	-
Great Britain	2004	41	422	600	1530
Germany	2006	41	286	525	1326
Denmark	2006	47	355	636	1614

(www.cattlenetwork.net)

The results of Simmental young bulls performance test from earlier period (shown in Table 5), as well as more recent results of the test in the animals up to the one year of age are at the level of the results realised by specialised fattening breeds which belong to the group of terminal breeds.

In Germany in 2010 (www.asr-rind.de) in performance test which lasted from 112 to 350 day of age young Simmental bulls realised liveweight gain of 1365 g/day and an average liveweight daily gain of 1208 g/day. According to the same source, in the test conducted in 2011 which lasted up to the 423 day of age young bulls realised daily liveweight gain of 1325 g/ day and achieved body mass of 601 kg at the end of test. In 2012, young bulls had body weight of 593 kg at the end of test which lasted 418 days and realised daily liveweight gain of 1322 g/day.

The results of test regarding fattening in Austria in 2013 (http://zar.at) confirm that Simmental breed in relation to all examined breeds had best results in the test which lasted up to the 365 days of age. Average daily liveweight gain in male animals was 1174.5 and in females 986.5 g/day. In specialised fattenning breeds Sharolais realised liveweight gain of 1165 g/day in males and 987.5 g/day in females while Limousine realised daily liveweight gain of 1092.5 g/day in males and 950.9 g/day in females.

As a maternal basis, Simmental breed is used in specialised systems of meat production (system "cow-calf"), where the cross breedings with terminal fattening breeds (Sharolais, Limousine, Belgian Blue and White) are most oftenly carried out. Better milk yield of Simmental breed compared to cows of specialised fattening breeds has a positive effect on weight gains of calves in suckling period which lasts 6 months. Thus it often happens that

calves crosses have considerably greater body mass at weaning compared to pure breed calves when those pure breeds were crossed with Simmental.

In Serbia the crossings of Simmental and fattening breeds, primarily with Sharolais and Limousine (Perišić et al., 2006; Perišić, 2007; Perišić et al., 2008; Perišić et al., 2009) were conducted. The effects of crossing were in general positive in fattening and slaughter breeds what made possible for crossed bullcalves (Simmental x Sharolais; Simmental x Limousine) to be fatenned to greater body masses in relation to the bullcalves of pure Simmental breed without compromising the quality of carcass or meat. Production was organised according to the cow-calf system and on most farms the production was conducted in stable conditions throughout the whole year. Such way of production and keeping (stable keeping, cow-calf system) could not be fully efficient primarily due to the transport of forage feeds to the stables throughout a whole year so that the farms gave up this kind of production. Better effects would certainly be achieved by organising this kind of production with use of pastures what would considerably reduce the costs of feeding.

Breeding goals for Simmental breed

Improving the production traits of Simmental breed can be realised by selection in pure breed and by use of crossing methods with other breeds. When breeding goals and programmes for Simmental breed in the European countries are in question we can observe that they differ from one another. It is certain that they must be reconciled with the needs of each country to meet the requirements of their markets for milk and meat. Selection goals depend in a great degree on the size of population of Simmental breed and its share in total cattle fund in a given country. The important factor which can influence the defining of production goals for Simmental cattle is also the participation of cattle dairy breeds in total cattle fund and their productivity. The higher the participation of specialised dairy breeds in some country, especially if their milk yield is high or showing the tendency of further rise can directly influence the reduction in Simmental cattle populations raised for combined production and influence the increase in populations grown in the ”cow-calf” system.

Defined goals in previous period (2006) are also of the interest today, i.e. they are not fully realised yet. The importance of some traits in selection goals for Simmental breed in certain European countries is shown in Table 6. Functional traits have a great importance in selection goals and breeders programmes for Simmental breed populations which achieved a high average lactation milk production (Simmental breed in Germany, Austria, Switzerland...), so in the forthcoming period we can expect more improvement in functional traits (longevity, regular fertility, resistance to mastitis), and not striving to have further increase in milk yield.

In the countries that have highly producing populations of Simmental breed the attention is particularly being paid to the improvement of functional traits what might result in longer life span and higher life production (minimum 30000 kg per individual during life). For the sake of comparison the breeding goal for Holstein Friesian breed is life milk production of 40000 kg.

On the basis of many trials and analyses of performace results it has been determined that the increase of milk yield per cow is always connected with longer lactations. It has also been found out that a significant increase in profit per farm is the result of cows increased longevity.

Table 6. - *The importance of some traits (in %) in breeding goal for Simmental breed*

Country	Milk, %	Meat, %	Functional traits, %	Exterior, %
Hungary	60	40	0	0
Czech Republic	40	24	0	36
Slovenia	45	10	23	22
Slovakia	60	40	0	0
Serbia	50	50	0	0
Switzerland (Fleckvieh)	40	10	30	20
Switzerland (Simmental)	35	20	25	20
Romania	60	35	5	0
Poland	50	50	0	0
Austria	38	16	46	0
Croatia	50	50	0	0
Italy	44	24	12.5	19.5
France (Simmental)	51	-	31	18
France (Monbeliard)	50	-	37.5	12.5
Germany	38	16	46	0

(*www.Cattlenetwork.net*)

Breeding goals for Simmental breed in some European countries

Breeding goals for Simmental breed in most of the European countries which raise Simmental as dual production breed are generaly defined in accordance with concrete situation in each state (as regards cattle breed structure, population milk yield) and by following the example of leading countries which raise Simmental as breed of combined production traits (Germany, Austria). Therefore in Austria the breeding goals (according to ZAR - Central working association of Austrian cattle breeders), defined in 2010 for Simmental breed directed for milk –meat production are:

- the importance of some groups of traits in breeding goal

  (38 % milk, 16 % meat, 46 % functional traits),

- milk yield of cows in first lactation 6000kg,

- milk yield of cows in later lactations from 7000 kg to 9000 kg with 4.2% fat and 3.7% protein,

- cow body mass from 650 to 850 kg,

- body mass of bulls with finished growth from 1100 to 1300 kg,

- withers height 140-150 cm,

- bulls withers height 150 to 165 cm,

- first calving at 30 months,

- calving interval 365 days,

- cow production life longer than 3.8 years,

- daily liveweight gain of male bullcalves 1400g,

- daily liveweight gain of female bullcalves 1150 g,
- carcass quality: > 80% E class + U class,
- optimal marbling.

Similar demands regarding production and exterior traits are for German Simmental as well. A special emphasis is given to the increase of the duration of life and production span. During life span the requirement is to realise minimum 30000 kg milk/cow. By this requirement the emphasis is being given to functional traits which must participate with more than 40% in breeding goal with duly implementation of the improvement programme.

Along with the traits important for milk production in Germany a significant attention is being paid to improving the traits important in meat production when Simmental is bred in the cow-calf system. General preconditions required for the successfull and economic meat production in cow-calf system, besides pronounced fattening traits of Simmental breed is regular fertility as well.

Breeding goals for Simmental populations directed at specialised meat production differ from breeding goals for the populations directed at combined production. Thus in Germany (www.asr-rind.de) the efforts are made to have animal age at first calving of 24 to 28 months, calving interval 365 days and calf mass at birth adequate for cow-calf system (females weight 39 kg, males weight 41 kg).

In Switzerland separate breeding goals have been defined for the sections of Simmental and Montbeliard breed.

Table 7. - *Breeding goals for some traits in cattle population in Switzerland*

Trait		Simmental (stand. 2014.)	Fleckvieh (stand. 2009.)	Montbeliarde (stand. 2009.)
Cow body height, cm		138-146	140 - 150	140 – 150
Cow body mass, kg		650 – 800	650 - 800	650 - 850
Bulls body height, cm		150 - 160	154 - 164	152 - 162
Bulls body mass, kg		>1200	>1200	>1200
Calving interval, days		365	365	365
Milk yield, kg	I lactation	5500	6000	6500
	II lactation	6500	7000	7500
	III lactation	7500	8000	8500
Milk fat content, %		4.00	4.00	4.00
Protein content, %		3.50	3.50	3.50
Weight gain of male bullcalves in fatenning, g/day		>1500	>1400	>1500

(www.swissherdbook.ch)

Breeding goal for Simmental breed in Serbia

On the basis of analysis of the size of Simmental breed in our country the conditions prevailing on the market have imposed that Simmental cattle breeding in forthcoming period will be predominantly in pure breed with less application of improvement crossing (lower share of genes of Red and White Holstein or Montbeliard breed).

For the purpose of improving the milk yield and fattening traits of Simmental breed in a greater part of Serbia the selection is conducted in pure breed. By application of artificial insemination, i.e. by wider use of insemination in overall population and by introducing the genes of more productive Simmental populations from some European countries (import of semen of elite bulls, import of breeding offspring), genetic potential of Simmental breed is being improved as a breed of combined purpose milk-meat production.

According to the Breeding Programme for Simmental breed in Serbia of 2010 breeding goal was defined in relation to up to then reached levels in production in majority of Simmental cattle populations in the European countries:

- Average milk production in standard lactation (305 days) of over 6 000 kg with 4.10% milk fat and 3.6% protein;
- Average milk flow of 2.0 kg/min minimum;
- Average daily liveweight gain higher than 1 300 g;
- Hot carcass percentage over 58.00%;
- Ridge and withers height in adult cows over 140 cm;
- Adult cow body mass over 650 kg;
- Age at first insemination from 16-17 months, and body mass over 400 kg;
- Age at first calving from 25-26 months;
- Calving interval 370 days maximum;
- Cow production life 7-8 years;
- Functional traits (udders, extremities);
- Milk quality (somatic cell count less than 250 000);
- Ridge and withers height in adult bulls over 155 cm;
- Adult bulls body mass over 1200 kg;
- Improved udders.

When the animals from breeding stocks are in question the crossing with other breeds is less applied. There are some limitations regarding the share of genes of other breeds in Simmental breed. For instance in Serbia in previous period it was allowed to have up to 12.5% of share of genes of Red and White Holstein Friesian breed while today this share of genes is up to 25% in accordance with the Breeding Programme for Simmental breed from 2010. The gene share of Simmental dairy cows should correspond to the breeding conditions in which improved animals realise their production as well as to the market demands for milk production.

The effects of Simmental cattle crossing, with the exception of the increase in milk production, are reflected also on the milking traits improvement. The results of numerous studies show that crosses compared with Simmental breed attain earlier maturity, have higher milk production with lower content of milk fat and only slightly poorer reproductive traits. The most important improvement in the appearance and structure of udders which can be perceived in the crosses of Simmental breed and Red and White Holstein is improved uniformity of the udders quarters and index, enlarged udder spaciousness, improved udders tightness and better tits shape and size.

Conclusion

Milk yield in Simmental cows in some European countires is at the level of milk yield of cows of specialised dairy breeds. Mean values for milk yield 10 years ago for Simmental breed population in the European countries were in the interval of 5000 to 7000 kg for standard lactation, while today they are in the interval of 5500 to 7500 kg. Milk yield, besides high level reached in previous period, is still rising.

Depending on the population of Simmental breed in most countries the production goal according to breeding goal for first lactation Simmental cows is 5500 or 6000 kg milk with 4.0% milk fat and 3.5% protein, while in cows after third lactation the required milk yield is higher than 7000 kg for Simmental breed, over 8000 kg for Simmental breed with the share of Red and White Holstein and over 8500 kg for Montbeliard cows. Special emphasis is given to increase of life and production cycle. During life span the required minimum realised is 30000 kg milk/cow. This requirement can be achieved by emphasis placed on functional traits which must participate with over 40% in breeding goal with proper implementation of improvement programme.

The traits important in meat production must be highlighted so that in higher producing populations of Simmental breed, daily liveweight gain of 1500 g/day for male bullcalves of Simmental breed and 1400 g/day for male bullcalves of Simmental breed with the share of genes of Red and White Holstein is set as breeding goal.

In Serbia the methods of improvement of Simmental breed must be designed in accordance with already existing genetic potential of each specific population, its purpose (registered breeding stocks, production breeding stocks), as well as market demands for bovine milk, dairy products and meat.

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References

1. Knežević L, Matić L, Barišić A, Rastija T 1991. Utjecaj križanja simentalske i crvene holštajn-frizijske pasmine goveda na njene osobine mlečnosti, reprodukcije i tjelesnih mjera. Stočarstvo 5-6,141-145.
2. Medić D,Veselinović S, Petković Danica., Bodulić S 1991. Ispitivanje tovnih i klaničnih osobina meleza dobijenih ukrštanjem krava kombinovanog i mlečnog tipa sa bikovima tovnih rasa. Biotehnologija u stičarstvu 7, 1-2, 5-24.
3. Perišić P 1998. Reproduktivne i proizvodne osobine različitih genotipova krava simentalske rase. Magistarska teza, Poljoprivredni fakultet, Beograd.
4. Perišić P, Skalicki Z, Latinović D, Trifunović G, Bogdanović V 2002. Uticaj udela gena crvenog holštajna na reproduktivne i proizvodne osobine krava simentalske rase. Biotehnologija u stočarstvu 18, 5-6, 37-43.
5. Perišić P, Skalicki Z, Petrović MM, Bogdanović V 2006. Simentalska rasa i pravci njenog razvoja. XVII Inovacije u stočarstvu, Biotehnologija u stočarstvu, 22 (poseban broj), 231-244.

6. Perišić P 2007. "Reproduktivne i proizvodne osobine simentalske rase pri kombinovanom smeru proizvodnje i sistemu krava-tele", Doktorska disertacija, Poljoprivredni fakultet, Beograd-Zemun.
7. Perišić P, Skalicki Z, Petrović MM, Bogdanović V, Trifunović G 2008. Simentalska rasa u kombinovanoj i specijalizovanoj proizvodnji. Biotehnologija u stočarstvu, 24 (Posebno izdanje), 25-38.
8. Perišić P, Skalicki Z, Petrović MM, Bogdanović V, Ružić–Muslić Dragana 2009. Simmental cattle breed in different production systems. 9th Interantional Symposium "Modern Trends in Livestock Production"Belgrade, 7-9 October 2009, Biotechnology in Animal Husbandry, 25 (5-6), Book 1, 315-326.
9. Perišić P, Skalicki Z, Bogdanović V 2012. Changes in the cattle sector in EU with possibile effect on dairy and beef production in Serbia. Proceedings of The first International Syimpozium on animal science (8-10th November, 2012), pp. 1-14.
10. Popovic I R 2008. Trendovi na svetskom tržištu mleka i uticaj na tržište Srbije. Prehrambena industrija- mleko i proizvodi od mleka. Vol 19, 1-2, 38-43.
11. Zečević B 1986. Ispitivanje uticaja ukrštanja domaćeg šarenog govečeta sa crvenim holštajnom na osobine mlečnosti i plodnosti. Magistarski rad, Poljoprivredni fakultet,Beograd.
12. Major breeding programme in cattle breeding – for Simmental breed (2010): Institute for Animal Husbandry, Belgrade- Zemun.
13. Reports on selection measures conducted in the territory of the Republic of Serbia for Simmental breed - Institute for Animal Husbandry – Belgrade-Zemun (2010).
14. www.Cattlenetwork.net (Cattle encyclopedia): date of visit: April 2014.
15. (www.asr-rind.de): date of visit: August 2014.
16. www.fleckvieh.at; date of visit: August 2014.
17. http://zar.at date of visit: August 2014.
18. (www.swissherdbook.ch) : date of visit: August 2014

Original paper

ESTRUS INDUCTION IN SEXUALLY MATURE GILTS WITH DIFFERENT HORMONAL TREATMENTS

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Abstract

The aim of this study was to determine the effectiveness of the synchronized estrus induction in sexually mature gilts, after treatment with different hormonal treatments, given in the unknown phase of a spontaneous estrus cycle. Sexually mature gilts were used, with at least one spontaneous estrous cycle. A total of 90 gilts were treated with single i/m injection of 1,000 IU eCG (Folligon®), 40 gilts were treated with two separate i/m injection of 1 ml PGF2α (Dinolytic®), at an interval of 11 days, and 40 gilts were treated orally (within diet) with 20 mg of synthetic progestagen preparation Altrenogest (Regumate®), during 18 days. Estrus was detected twice daily, in the 10h to 12h intervals, by direct contact with the teaser boar. The best induction of synchronized estrus (in 90% of gilts), which appeared within the first 7 days (mean 5.3 days) after treatment, was found in gilts treated with progestagen preparations. In the three repetitions of treatment with eCG, within the first 7 days (mean 4.2 days) after the treatment, estrus was detected in 65%, 40% or 33% of the treated gilts. After treatment with luteolytic preparation $PGF_{2\alpha}$ (Dynolitic®), estrus was detected in 40% of gilts, on average 5.5 days after treatment. The obtained results clearly show that the successful induction of synchronized estrus in sexually mature gilts can be done only by the method for prolongation of the luteal phase of the spontaneous estrous cycles, using progestagen preparations.

Key words: *estrus, gilt, hormones, induction, treatment*

Introduction

In the intensive pig production, it is often necessary to induce synchronized estrus, for a number of sexually mature gilts (Gordon, 2005; Stančić, 2005). The goal is to get all the treated gilts at the beginning of the follicular phase (proestrus) of the induced estrous cycle, after cessation of treatment. In general, the induction of synchronized estrus in sexually mature gilts can be done by the control of spontaneous cycles luteal phase duration, using different hormone preparations (Stančić, 2002; Coffey et al., 2002; Cassar, 2009; Brüssow and Wähner, 2011). Luteal phase of the spontaneous estrous cycles can be either shortened by

using luteolytic preparations (native or synthetic $PGF_{2\alpha}$ preparations) or prolonged by using synthetic progestagen preparations, as well as by regression accessory corpora lutea (with a single $PGF_{2\alpha}$ injection), induced by a single injection of gonadotropin eCG (Stančić et al., 1998; Estill, 2000; Flowers, 2001; Marić et al., 2003; Estienne, 2003; Stančić et al., 2007; Bošnjak, 2007; Davis, 2008; Stančić, 2010; De Rensis et al., 2012; Stančić et al., 2013). However, the success of the degree of estrus synchronization depends on the phase of the spontaneous estrous cycle of the gilts at the treatment beginning, as well as on the applied hormonal preparations. In the practical conditions, most often, the phase of spontaneous estrous cycle in gilts at the start of hormonal treatment is unknown. This results in a highly variable success of synchronized estrus induction, depending on the applied hormone preparations (Stančić, 2005; Gordon, 2005; Stančić et al., 2013).

Therefore, the aim of this study was to determine the degree of synchronized estrous response in sexually mature gilts treated with various hormonal preparations (gonadotropins, prostaglandin $F_{2\alpha}$ and progestagens) in an unknown stage of spontaneous estrous cycle.

Materials and methods

Farm. Investigations were carried out on an intensive pig production farm in AP Vojvodina, Serbia. For the experiment we used gilts of 7 to 8 months of age, 125 to 140 kg body weight, in which at least one spontaneous estrous cycle was detected. Detection of estrus was performed twice daily at an interval from 10h to 12h, by full contact with the teaser boar. The experimental gilts were housed in group pens, with the possibility of individual nutrition. Gilts were treated in an unknown stage of spontaneous estrous cycle, using gonadotropin (eCG - equine chorionic gonadotropin), luteolytic ($PGF_{2\alpha}$) or synthetic progestagen (Altrenogest) hormonal preparations.

Gonadotropin treatment. A total of 90 gilts (30 gilts per group, for three treatment replications) was treated with gonadotropin preparation eCG (Folligon®, Intervet - Boxmer, Holland). The treatment was performed by a single i/m injection of 1,000IU eCG.

Luteolytic treatment. Prostaglandin $F_{2\alpha}$ preparation ($PGF_{2\alpha}$) Dynolitic® (Phizer) was used as a luteolytic hormone for gilts treatment. A total of 40 gilts was treated by two separate i/m injections of 1ml Dynolitic solution (containing 5mg Dinoprost), within an interval of 11 days.

Progestogen treatment. Preparation Regumate® (Roussel Uclaf, Bernburg, Germany) was used. The active substance of the preparation is Altrenogest, highly potent synthetic analogue of progesterone. Each gilt (n=40) received 5ml Regumate daily, containing 20mg Altrenogest, for 18 days. The preparation was applied by the original spray bottle, directly into the individual gilts morning part of daily meal, just before consumption, so each gilt consumed the entire daily dose of preparations.

Induced estrus detection was performed as described for the detection of spontaneous estrus, starting about 24h after the cessation of the hormonal treatments. Estrus which appeared within the first 7 days after treatment was considered to be induced by hormonal preparations (Gordon, 2005; Stančić, 2005).

Results and discussion

The experimental results are shown in Table 1. The degree of synchronized induced estrous reaction in gilts, within the first 7 days after treatment with gonadotropin preparation eCG, was highly variable among the three treatment groups (63%, 40% and 33%). The average interval from the end of treatment to the occurrence of induced estrus was 4.2 days (3 to 6 days). Total estrous response, in this group of gilts, was high (93%, 83% and 97%), but the average interval from the end of treatment to estrus, was much longer (lasting 24 to 27 days).

On average 5.5 days (4 to 7 days) after second luteolytic ($PGF_{2\alpha}$) injection, synchronized estrus was induced in only 40% of treated gilts. Total estrus reaction was high (90%), with an average interval of 11.2 days (8 to 15 days) from the second $PGF_{2\alpha}$ injection to estrus detection.

Progestagen treatment resulted in high proportion of synchronized estrus induction (90% of treated gilts). On average, induced estrus was detected 5.3 days (4 to 7 days) after cessation of treatment. In the remaining four gilts (10%) estrus was not detected within 30 days after cessation of treatment with progestagen.

Table 1. *Estrus reaction in sexually mature gilts after different hormonal treatments*

		Hormonal treatments		
		eCG	$PGF_{2\alpha}$	Progestagen
Gilts treated, n		30[1], 30[2], 30[3]	40	40
Average gilts age at the start of treatment, days		218 (205-234)	231 (212-240)	223 (210-238)
Gilts with detected estrus within 7 days after treatment*	n	19[1], 12[2], 10[3]	16	36
	%	63[1], 40[2], 33[3]	40.0	90.0
Average interval from the end of treatment to induced estrus, days		4.2 (3-6)	5.5 (4-7)	5.3 (4-7)
Total gilts with detected estrus	n	28[1], 25[2], 29[3]	36	36
	%	93[1], 83[2], 97[3]	90.0	90.0
Average interval from the end of treatment to estrus in the total gilts with detected estrus, days		24[1], 26[2], 25[3] (24-27)	11.2 (8-15)	5.3

* Estrus induced by hormonal treatment. [1, 2, 3] First, second and third treatments.
Minimal and maximal values in parenthesis.

The results of our study clearly show that there is a considerable variation in the degree of synchronized estrous response after treatment performed with various hormonal preparations, in the unknown phase of a spontaneous estrous cycle of gilts. Thus, within 7 days after treatment with placental gonadotropin eCG, in the three groups of gilts, estrus was detected in 63%, 40% or 33% of the treated gilts. In the same interval after treatment with luteolytic preparation $PGF_{2\alpha}$, estrus was detected in 40% treated gilts. Higher degree of synchronized estrous reaction was found after treatment with progestagen preparations. Namely, within 7 days after cessation of treatment, estrus was detected in 90% gilts. The interval from the end of treatment to the appearance of induced estrus was similar (average 4.2 to 5.5 days), regardless of the used hormonal preparations.

Synchronized ovulation can be induced by placental (eCG and hCG) or pituitary (FSH and LH) gonadotropic hormone preparations in sexually immature (prepubertal, acyclic) and sexually mature (pubertal, cyclic) gilts, as well as in weaned sows (Flowers, 2001; Stančić,

2005; Tummaruk et al., 2011; Brüssow and Wähner, 2011). Prepubertal gilts and weaned sows respond with very high proportion of synchronized estrus, within 4 to 6 days after a single injection of 500 to 1,500 IU eCG. This effect is achieved due to the fact that, at the moment of treatment, only follicles with non-functional corpora lutea (CL) are present at ovaries of both females category (Stančić et al., 1998; Coffey et al., 2002; Gordon, 2005; Stančić et al., 2007; Cassar, 2009). In 80 % to 100 % of sexually mature gilts, eCG injection given at any stage of spontaneous estrous cycle causes a synchronized ovulation and formation of functional accessory corpora lutea. However, the synchronized estrus, within 4 to 6 days after the eCG, occurs only in the gilts treated during the follicular phase or at the end of the luteal phase of spontaneous cycles. This is due to the inhibitory action of the induced (accessory) and spontaneous estrus cycles corpora lutea. Therefore, the degree of synchronization of estrus is highly variable in sexually mature gilts treated at an unknown stage of spontaneous estrous cycle, depending on the proportion of gilts in certain phases of spontaneous estrous cycle at the moment of eCG treatment (Ramapacek et al., 1992; Heavenly et al., 1997; Haff et al., 2002; Stančić, 2005; Bošnjak et al., 2007; Stančić et al., 2012; Stančić et al., 2013).

The injection of $PGF_{2\alpha}$, within the first 12 days of the luteal phase of the spontaneous estrous cycle does not cause regression of corpora lutea (CL). This is due to the fact that the pigs CL, unlike cows CL, are responsive to the luteolytic action of $PGF_{2\alpha}$ only within the last 4 days of the luteal phase, ie. after day 12 of diestrus (Guthrie and Polge, 1976; Guthrie, 1979; Puglisi et al., 1979; Stančić, 1979; Stančić and Vuković, 1995; Stančić et al., 1998; Stančić et al., 2007). Therefore, there is a high probability that a large number of gilts, at the moment of $PGF_{2\alpha}$ treatment, are not in the reactive phase of spontaneous estrus cycle (follicular and first 12 days of luteal phase). Such gilts will not react with CL regression, and with synchronized estrus manifestation. The result is low degree of estrus synchronization after $PGF_{2\alpha}$ treatment of gilts in the unknown stage of spontaneous estrous cycle (Stančić et al., 1995; Almond, 1997; Nebesni et al., 1997; Stančić et al., 1998; Stančić, 2010; De Rensis et al., 2012).

The highest level of synchronized estrus in sexually mature gilts can be achieved by peroral treatment with synthetic progestagen preparations (Davis et al., 1979; Almond, 1997; Stančić et al., 2005). Recently, the most commonly used preparation has been Altrenogest (Regumate®) for 16 to 18 days peroral treatment. After such treatment, synchronized estrus occurs 4 to 7 days after cessation of treatment in 80% to 100% of gilts (Estienne et al., 2001; Estienne and Harper, 2002; Marić et al., 2003; Gordon, 2005; Stančić, 2005; Stančić et al., 2007; Bošnjak et al., 2007; Stančić, 2010).

The results of the present study and the results of other authors clearly demonstrate that a high degree of estrus synchronization in sexually mature gilts can only be achieved by applying treatment with progestagen preparations. Treatment of these gilts with gonadotropin or luteolytic hormonal preparations is not effective and has no relevance to the practical application for estrus synchronization in sexually mature gilts.

Conclusion

Based on the obtained results, we can conclude the following:

1. The degree of synchronized estrous response in sexually mature gilts significantly varies depending on the applied hormonal preparations (gonadotropins, luteolytics or

progestagens) and the phase of the spontaneous estrous cycle in which the treatment is carried out.

2. Within the first 7 days after treatment, estrus was detected in 33% to 63% gilts treated with gonadotropin eCG (Folligon®), in 40% gilts treated with luteolytic $PGF_{2\alpha}$ (Dynolitic®), and in 90% gilts treated with progestagen preparation (Regumate®).
3. Synchronized estrus in high proportion of sexually mature gilts can only be achieved by progestagen treatment. Treatment with gonadotropin or luteolytic hormonal preparations is not effective and has no relevance to the practical application for estrus synchronization in sexually mature gilts.

References

1. Almond WG 1997. Synchronization of estrus in gilts 1997. Proc. North Carolina Hweakthy Hogs Seminar, UK, 1-3 pp.
2. Bošnjak D, Stančić B, Pejin B, Đurđević I and Timotijević M 2007. Estrusno reagovanje dugotrajno anestričnih nazimica, posle tretmana preparatom «Regumate» ili PMSG. Simpozijum «Veterinarska medicina, stočarstvo i ekonomika u proizvodnji zdravstveno bezbedne hrane». Herceg Novi, Serbia, 119 pp.
3. Brüssow K-P and Wähner M 2011. Biological and technological background of estrus synchronization and fixed-time ovulation induction in the pig. Biotechnology in Animal Husbandry, 27, 533-545.
4. Cassar G 2009. Hormonal Control of Pig Reproduction. London Swine Conference – Tools of the Trade, UK, 1-9 pp.
5. Coffey DR, Parker RG and Laurent MK 2002. Manipulation of the Estrus Cycle in Swine. Agric Educ ASC-152, 1-7.
6. Davis LD, Knight WJ, Killian BD and Day NB 1979. Control of estrus in gilts with a progestogen. J Anim Sci 49,1506-1509.
7. Davis LD 2008. Using Regumate to Control Estrus in Swine. Swine Day Report of Progress 940, Kansas State University, USA, 11-15 pp.
8. De Rensis F, Saleri R, Tummaruk P, Techakumphu M and Kirkwood RN 2012. Prostaglandin $F_{2\alpha}$ and control of reproduction in female swine: a review. Theriogenology 77, 1-11.
9. Estienne JM and Harper FA 2002. Synchronization of Estrus and Fertility in Gilts Administered P.G. 600® After Treatment with Regu-mate® for 14 or 18 Days. The Professional Animal Scientist 18,158–161.
10. Estienne JM, Harper FA, Horsley RB, Estienne EC and Knight WJ 2001. Effect of P.G.600 on the onset of estrus and ovulation rate in gilts treated with Regumate. J Anim Sci 79, 2757-2761.
11. Estienne M 2003. A Newly Available Tool for Synchronization of Estrus in Swine. Virginia Cooperative Extension, Livestock, USA, 1-6 pp.
12. Estill TC 2000. Current concepts in estrus synchronization in swine. J Anim Sci 77, 1-9.
13. Flowers LW 2001. Synchronization of estrus in swine. North Carolina State University, Pork Information Gateway, USA, 1-8 pp.
14. Gordon I 2005. Reproductive Technologies in Farm Animals. CAB Int. Publ. Oxfordshire, UK.

15. Guthrie DH and Polge C 1976. Luteal function and oestrus in gilts treated with a synthetic analogue of prostaglandin $F_{2\alpha}$ (ICI 79,939) at various times during the oestrous cycle. J Reprod Fert 48, 423-425.

16. Guthrie DH 1979. Fertility after estrous cycle control using gonadotropin and prostaglandin $F_{2\alpha}$ treatment of sows. J Anim Sci 49, 158-162.

17. Haff ten W, Thacker AP and Kirkwood NR 2002. Effect of injecting gonadotrophins during the luteal phase of the estrus cycle on the inter-estrus interval of gilts. Can J Anim Sci 82, 457-459.

18. Marić Z, Stančić B and Gagrčin M 2003. Sinhronizacija estrusa u polno zrelih nazimica primenom preparata Regumate. Savremena poljoprivreda 52, 219-224.

19. Nebesni A, Stančić B and Šahinović R 1997. Ovarian reaction in gilts treated with PMSG at different time after standing oestrus. Proc. 3rd Symp. Anim. Reprod. Macedonia, Ohrid, 92 pp.

20. Puglisi TA, Rampacek GB, Kraeling RR and Kiser TE 1979. Corpus luteum susceptibility to prostaglandin F2-alpha (PGF2-alpha) luteolysis in hysterectomized prepubertal and mature gilts. Prostaglandins, 18, 257–264.

21. Rampacek GB, Kraeling RR, Barb CR, Estienne CE and Estienne MJ 1992. Regression of induced corpora lutea in mature cyclic gilts by human chorionic gonadotropin. J Anim Sci 70, 3144-3148.

22. Stančić LB 2005. Reprodukcija svinja (monografija). Univrezitet u Novom Sadu, Poljoprivredni fakultet.

23. Stančić B, Gagrčin M, Stanković A and Budinčević A 1998. Primena egzogenih hormona u sinhronizaciji estrusa nazimica. Veterinarski glasnik 52, 129-132.

24. Stančić B, Radović I, Stančić I and Gagrčin M 2007. Sinhronizacija estrusa i fertilitet nazimica tretiranih različitim hormonskim preparatima. Savremena poljoprivreda 56, 8-13.

25. Stančić B and Vuković M 1995. Sinhronizacija estrusa i superovulacije u nazimica primenom prostaglandina i gonadotropina (abstrakt). VIII Savetovanje veterinara Srbije. Zlatibor, Srbija, 128 pp.

26. Stančić B 1979. Neka razmatranja o mogućnosti primene prostaglandina $F_{2\alpha}$ i njegovih sintetičkih analoga u regulaciji reproduktivnog procesa svinje i goveda. Veterinaria 28, 127-131.

27. Stančić I. Bošnjak D, Radović I, Gvozdić D, Savić B and Stančić B 2013. Fertility of gilts with prolonged preinsemination anestrus after progestagen-eCG treatment. Slov Vet Res 50, 111-116.

28. Stančić I 2010. Indukcija estrusa i fertilitet nazimica sa prolongiranom preinseminacionom anestrijom, tretiranih preparatima gonadotropina i progestina. PhD, Univerzitet u Novom Sadu, Poljoprivredni fakultet.

29. Stančić LB 2002. Biotechnology in swine reproduction: A review of our investigations. Buletinul Univ Sci Agric Med Vet Cluj (Romania) 57, 232-234.

30. Stančić BI, Bošnjak VD, Radović BI, Stančić LB, Harvey BR and Anderson CR 2012. Ovarian reaction and estrus manifestation in delayed puberty gilts after treatment with equine chorionic gonadotropin. Reproductive Biology and Endocrinology 10, 61.

31. http://www.ncbi.nlm.nih.gov/pubmed?term=Stancic%20IB"

32. Tummaruk P, Atthaporn Roongsitthichai A and De Rensis F 2011. Ovulation Induction in Swine. Thai J Vet Med Suppl 41, 19-23.

Original paper

TESTES WEIGHT IS NOT A RELIABLE TOOL FOR DISCRIMINATING IMMUNOCASTRATES FROM ENTIRE MALES

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Abstract

In view of the criticism regarding the piglet castration as currently practiced, one of the alternatives is the active immunisation against the hypothalamic GnRH hormone referred to as immunocastration. This method is effective in prevention of boar taint in pork and has the advantage of avoiding the pain associated with castration (performed without anaesthesia and analgesia). However, in some rare cases the immunocastration may not be effective and such pigs (so called non-responders) present a risk for boar taint. It is therefore important to have a reliable indicator of the effective immunocastration for the use on the slaughter line. Determination of boar taint substances (androstenone and skatole) is time consuming and expensive, whereas the size of reproductive organs could serve as an indicator of successful immunocastration. Present study provides results for 76 immunocastrates (IC) and 55 entire males (EM) varying in body (or carcass) weight and delay between immunocastration and slaughter, in which testes and accessory sex glands (vesicular gland, bulbourethral gland) were dissected and weighed. Gathered data were used to distinguish IC and EM by discriminant analysis. The results show better discrimination of IC than EM. Testes weight is less reliable indicator of successful immunisation than the weight of accessory sex glands and that the best discrimination was achieved when using all three measurements.

Key words: *discriminant analysis, immunocastration, pig, reproductive organs*

Introduction

Surgical castration of male piglets is a traditional practice in pig production used to avoid boar taint, an unpleasant odour and flavour of meat from entire male pigs. It has been ascribed to the presence of androstenone (Patterson, 1968) and skatole (Walstra and Maarse, 1970). Surgical castration as practiced nowadays (according to EU legislation it can be performed without anaesthesia and analgesia within first 7 days after birth) is being criticised and a ban of such practice is presently under consideration by the European Union. The alternatives include surgical castration with anaesthesia and/or analgesia, raising entire males, sperm sexing and immunocastration. Up to now the best alternative to prevent boar taint appears to be the immunocastration i.e. a vaccination against the gonadotropin-releasing hormone (GnRH) which induces the formation of specific antibodies that bind and neutralise GnRH. As a consequence, the hypothalamic-pituitary-

gonadal axis is disrupted resulting in a decreased pituitary release of LH and FSH, inhibition of testicular steroid synthesis, regression of reproductive organs and clearance of boar taint compounds. The first commercial product for immunocastration of male pigs (Improvac®) has been released in Australia and New Zealand in 1998 and registered for use in 53 countries since then, including the European Union in 2009. Before the immunisation becomes effective (two vaccinations are needed), the pigs exhibit performance of entire males i.e. improved feed efficiency and lean meat deposition (Batorek et al., 2012a). To benefit as much as possible from the advantages of entire males the late castration strategy is practiced. The first vaccination is usually performed when the animals enter the growing-finishing unit resulting in low antibody titres with animals physiologically remaining similar to an entire male. The second dose, resulting in effective castration, is injected 4-6 weeks before slaughter. This delay is recommended by vaccine producer to assure the clearance of boar taint compounds and is referred to as late immunocastration. In the case of an earlier immunocastration the advantage is that its efficiency can be easily demonstrated (e.g. by checking testes development), however the advantage in fattening performance comparatively to surgical castration is lost. In some cases the immunisation may be ineffective and such pigs present a risk for boar taint. It is therefore important to have a simple but reliable indicator of the efficacy of immunocastration for the use at slaughter line, because determination of boar taint substances (androstenone and skatole) is not practical (time consuming and expensive). Assessment of testes size has been suggested as an indicator of successful immunocastration, however, Bonneau (2010) questioned its reliability and suggested vesicular gland as better indicator of effective immunocastration. The mentioned study was based on published literature, whereas the aim of the present study was to test this suggestion on measurements collected from 131 pigs (76 immunocastrates and 55 entire males of varying carcass weight and delay between immunocastration and slaughter) from four experiments in which testes and accessory sex glands were dissected and weighed.

Materials and methods

Data on weight of testes, vesicular and bulbourethral gland was collected from previously published experiments on EM and IC (Škrlep et al., 2010; Škrlep et al., 2012; Batorek et al., 2012b; Kubale et al., 2012). The pigs used for the study were commercial crosses of different breeds (Large White × Landrace, Large White × Landrace × Duroc, Large White × Landrace × Pietrain) varying in body (103.6±13.5) and carcass weight (76.8±11.1 kg). In IC pigs the delay between second vaccination and slaughter varied from 2 to 9 weeks. Reproductive organs were collected at the slaughter line and taken to the laboratory for dissection and weighing. Samples of subcutaneous fat were also taken at the level of last rib for androstenone and skatole determination (HPLC methods) as described in previously mentioned articles. Data analysis was performed with statistical package IBM SPSS 21.0 for Windows using discriminant analysis to categorize pigs into EM or IC based on either one of the classifying factors (i.e. weights of testes, vesicular gland or bulbourethral gland) or using all three weights in a single step. Cross–validation of discrimination was also performed using leave-one-out method of SPSS.

Results and discussion

Figure 1 presents basic statistics and distribution for the reproductive organs weights used in the discriminant analysis. To show that IC and EM were similar in regard to body weight the distribution and basic statistics of the later is also shown. Although the two sex

groups differ considerably in regard to the size of reproductive organs (being strongly regressed in IC) some overlapping in distribution could be observed. There are several possible factors to explain this situation. Firstly there is one IC that could be considered a non-responder (pig no. 20 with androstenone level 0.34 μg/g and weight of reproductive organs similar to EM i.e. testes weight 413 g, vesicular gland weight 107 g and bulbourethral gland weight 96 g). Secondly, for some pigs the delay between the effective vaccination and slaughter was only 2 weeks, which may not be enough to reduce the size of the organs. Namely, it is known that approximately one week is needed after the second vaccination for the metabolic response to take place (Claus et al., 2007). Thirdly the pigs included in the study were of different crossbreeds and also varied considerably in body weight (in entire males a correlation between the size of organs and carcass weight was in the range 0.45-0.53; data not shown) i.e. those entire males with lower body weight were retarded in growth and likely delayed in reaching sexual maturity, exhibiting low reproductive organ size, closer to those of IC. Such circumstances, i.e. variability of crossbreeds, body weight, some pigs being retarded in growth, are usual in everyday pig production practice.

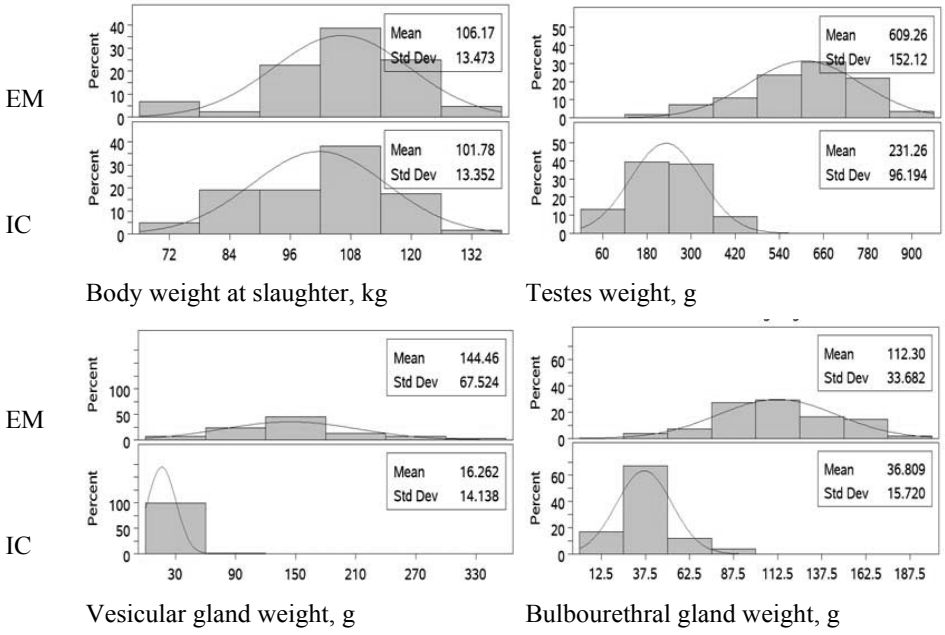

Figure 1. *Distribution of data for slaughter weight and weight of reproductive organs*

When using testes weight as a criterion to distinguish between EM and IC (Table 1) the results of discriminate analysis showed a 91.6 % success rate, with misclassification being higher in EM (12.7%) than IC (5.3%). Cross-validation results were the same as in the calibration step. Looking individually on misclassified pigs in EM group it could be observed that these were all Pietrain crossbred pigs (weighing between 71-108 kg) with two of them retarded in growth. It could be mentioned that Pietrain crosses had similar slaughter weight as pigs of other two crossbreeds whereas testes weight (520 g) in EM of Pietrain crossing was lower ($P<0.001$) than in other two crossbreeds (682 g and 701 g for

Landrace×Large White and Landrace×Large White×Duroc crosses, respectively; data not shown). Concerning IC, it could be argued that misclassified pigs were non-responders, however, their androstenone levels does not support that since it was below detection limit of the method (<0.24 μg/g fat) in all cases. Misclassified IC pigs were all crosses of Landrace×Large White (had the smallest regression of testes weight among the three crossbreeds), weighing between 100-130 kg and with 5 weeks delay between second vaccination and slaughter (recommended standard). As it was observed already in EM, lighter testes weight was also evidenced for immunocastrated Pietrain crosses.

Table 1. *Classification results[a,c] of discriminant analysis for testes weight as criterion*

			Predicted		Total	Missclassified pigs
			IC	EM		
Original	Count	IC	72	4	76	2, 3, 66, 68
		EM	7	48	55	105, 108, 109, 110, 111, 117, 120
	%	IC	94.7	5.3	100.0	
		EM	12.7	87.3	100.0	
Cross-validation	Count	IC	72	4	76	2, 3, 66, 68
		EM	7	48	55	105, 108, 109, 110, 111, 117, 120
	%	IC	94.7	5.3	100.0	
		EM	12.7	87.3	100.0	

[a]91.6% of original grouped cases correctly classified.

[c]91.6% of cross-validated grouped cases correctly classified.

When using bulbourethral gland weight (Table 2) as a criterion to distinguish between EM and IC the results of discriminate analysis were slightly better as in the case of testes weight and showed a 93.1% success rate, with misclassification being higher in EM (10.9%) than IC (3.9%). Cross-validation results were the same as in the calibration step.

Table 2. *Classification results[a,c] of discriminant analysis for bulbourethral gland weight as criterion*

			Predicted		Total	Missclassified pigs
			IC	EM		
Original	Count	IC	73	3	76	20, 37, 55
		EM	6	49	55	82, 104, 111, 117, 119, 120
	%	IC	96.1	3.9	100.0	
		EM	10.9	89.1	100.0	
Cross-validation	Count	IC	73	3	76	20, 37, 55
		EM	6	49	55	82, 104, 111, 117, 119, 120
	%	IC	96.1	3.9	100.0	
		EM	10.9	89.1	100.0	

[a]93.1% of original grouped cases correctly classified.

[c]93.1% of cross-validated grouped cases correctly classified.

We did not observe any effect of crossbreed on the weight of bulbourethral gland (data not shown). It is noteworthy that all the EM misclassified as IC showed also low androstenone levels (three of them below detection limit, others from 0.247 to 0.629 μg/g fat) which indicates that the reason for misclassification to IC group is likely due to the sexual immaturity of these EM pigs.

Table 3. *Classification results[a,c] of discriminant analysis for vesicular gland weight as criterion*

			Predicted		Total	Missclassified pigs
			IC	EM		
Original	Count	IC	75	1	76	20
		EM	11	44	55	85, 88, 90, 91, 95, 104, 105, 117, 118, 119, 120
	%	IC	98.7	1.3	100.0	
		EM	20.0	80.0	100.0	
Cross-validation	Count	IC	75	1	76	20
		EM	11	44	55	85, 88, 90, 91, 95, 104, 105, 117, 118, 119, 120
	%	IC	98.7	1.3	100.0	
		EM	20.0	80.0	100.0	

[a]90.8% of original grouped cases correctly classified.

[c]90.8% of cross-validated grouped cases correctly classified.

When using vesicular gland weight (Table 3) as a criterion to distinguish between EM and IC the result of discriminate analysis was overall the lowest with 90.8% success rate, which was due to the high misclassification of EM (20%). On the other hand the recognition rate was excellent (98.7%) in the case of IC where only one pig was misclassified as EM; in that particular case (pig no. 20); this pig was likely a non-responder which in the end denotes a correct classification into EM.

Table 4. *Classification results[a,c] of discriminant analysis using all three criteria (testes, bulbourethral gland, vesicular gland)*

			Predicted		Total	Missclassified pigs
			IC	EM		
Original	Count	IC	75	1	76	20
		EM	4	51	55	105, 117, 119, 120
	%	IC	98.7	1.3	100.0	
		EM	7.3	92.7	100.0	
Cross-validation	Count	IC	75	1	76	20
		EM	5	50	55	105, 111, 117, 119, 120
	%	IC	98.7	1.3	100.0	
		EM	9.1	90.9	100.0	

[a]96.2% of original grouped cases correctly classified.

[c]95.4% of cross-validated grouped cases correctly classified.

When using all three organs (Table 4) as a criterion to distinguish between EM and IC the results of discriminate analysis were overall the best with 96.2% success rate (95.4 % in cross-validation). Only 7.3% EM and 1.3% IC (i.e. one pig) have been incorrectly classified. As already mentioned IC pig (no. 20) was likely a non-responder. Out of five misclassified EM, only one had androstenone level typical for EM (1.0 μg/g fat) and above sensory threshold level (0.5 μg/g fat) whereas the other four pigs had low androstenone levels (<0.27 μg/g).

A higher degree of misclassification observed for EM than IC can be related to higher variability due to differences in (cross)breeds and growth rate affecting the onset of sexual maturity (e.g. delayed sexual maturity in slow growing pigs, resulting in underdeveloped testes and accessory reproductive glands). In agreement with Bonneau (2010), vesicular gland (or alternatively all reproductive organs) turned out as the most reliable indicator for IC discrimination. This result has a high practical relevance because it denotes that reliable on-line detection of non responders is possible. According to Bonneau (2010) vesicular gland undergoes a fast weight regression, whereas in testes or bulbourethral gland the response will take much longer. This can be explained by the tissue structure. In the vesicular gland grape-like acini, filled with serous secrete, are more prone to leakage than in the case of more condensed structure of bulbourethral gland, with smaller acini, filled with highly viscous content. A vesicular gland, a very reliable indicator for IC pigs, seems problematic for correct classification of EM. In the majority of vesicular gland based EM false classifications, the bulbourethral gland and/or testes were not regressed.

Conclusion

In conclusion, the correctness of the classification was high and much better for IC than EM. The lowest success rate in IC was obtained when using testes weight (87%) and the highest when using vesicular gland weight or alternatively all reproductive organs (one pig was false negative which was likely a non-responder) denoting faultless discrimination. For entire males using vesicular gland was the least reliable (80%) whereas using all reproductive organs provided the best classification correctness (93%).

Acknowledgement

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References

1. Batorek N, Čandek-Potokar M, Bonneau M, and Van Milgen J 2012a. Meta-analysis of the effect of immunocastration on production performance, reproductive organs and boar taint compounds in pigs. Animal 6, 1330-1338.
2. Batorek N, Škrlep M, Prunier A, Louveau I, Noblet J, Bonneau M and Čandek-Potokar M 2012b. Effect of feed restriction on hormones, performance, carcass traits, and meat quality in immunocastrated pigs. Journal of Animal Science 90, 4593-4603.
3. Bonneau M 2010. Accesory sex glands as a tool to measure the efficacy of immunocastration in male pigs. Animal 4, 930-932.
4. Claus R, Lacorn M, Danowski K, Pearce MC, and Bauer A 2007. Short-term endocrine and metabolic reactions before and after second immunisation against GnRH in boars. Vaccine 25, 4689-4696.

5. Kubale V, Batorek N, Škrlep M, Prunier A, Bonneau M, Fazarinc G, and Čandek-Potokar M 2013. Steroid hormones, boar taint compounds, and reproductive organs in pigs according to the delay between immunocastration and slaughter. Theriogenology 79, 69-80.
6. Patterson RL 1968. 5a-Androst-16-ene-3-one: Compound responsible for taint in boar fat. Journal of Science and Food Agriculture 19, 31–37.
7. Škrlep M, Šegula B, Zajec M, Kastelic M, Košorok S, Fazarinc G and Čandek-Potokar M 2010. Effect of immunocastration (Improvac) in fattening pigs I: Growth performance, reproductive organs and malodorous compounds. Slovenian Veterinary Research 47, 57-64.
8. Škrlep M, Batorek N, Bonneau M, Prevolnik M, Kubale V, Čandek-Potokar M 2012. Effect of immunocastration in group-housed commercial fattening pigs on reproductive organs, malodorous compounds, carcass and meat quality. Czech Journal of Animal Science 57, 290-299.
9. Walstra P and Maarse H 1970. Onderzoek geslachtgeur van mannelijke mestvarkens. Researchgroep Vlees en Vleesvare. T.N.O., Rap. C-147 and 2, 1–30. The Netherlands Zeist.

Review paper

NEW EVIDENCES ON DOMESTICATION OF THE HORSE (*Equus ferus caballus)* AND ORIGINE OF DOMESTIC MOUNTAIN PONY

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Abstract

The results of draft genome research of permafrost horse bone dated approximately 560-780 (kyr BP) have been published recently. According to the data representing the oldest full genome sequence compared to the modern genomes of domesticated horse, Przewalskii and donkey it was suggested that the Equus lineage gave rise to contemporary horses, zebras and donkeys some 4.0 to 4.5 million years ago, twice increasing the assumed time to the common ancestor. Upon the phylogenetic and divergence analyses it was estimated that the Przewalskii and domestic horse populations diverged some 38-72 kyr BP and that there are no evidences that admixtures between Przewalskii and domestic horse populations occurred post-divergently thus Przewalskii's involvement in the process of horse domestication has been excluded.

The new data on ancient equide genome question the conventional theory on the origin of Domestic mountain pony. Upon the protein variability of the Domestic mountain pony the influence of diluvial genome on the population was confirmed and this finding differed from the conventional theory stating *E. f. Przewalskii* and *E. ferus caballus* as ancestors of the autochthonous population. The role of different ancestral horses in evolution of our autochthonous population is therefore unclear.

The questions emerging from the most recent genetic and proteomic research in concern with the autochthonous populations are presented in the paper.

Key words: *fossil DNA, horse, wild ancestors*

Introduction

Although the fossil findings of equine ancestors made these mammals the model for evolutionary processes, the domestication process remained unsatisfactory cleared until recently. The evidences on horse domestication, found in grassland at the space between nowadays Russia and Ukraine, were calibrated to be 5-6 million years old, while the artifacts speaking of the utilization of these animals belong to the period 6,000-3,000 years B.C. Other centers of horse domestication were determined on different geographic regions so it was considered that domesticated horse arose through independent domestication events that have involved different ancestral subspecies of wild horse. The multicentric and polyphyletic origin can be the major factor contributing extensive diversity in domesticated

horse. It was also recognized that mutations occurred rarely during domestication so separate equine gen pools were geographically differentiated (Ellegren, 2002).

Anyway the importance of the horse in transportation an human migrations through history led to crossing between the separate domesticated populations and thus influencing the gene pool of the original domesticated breeds and types (Kaminski and de Andres Cara, 1986).

The origin of horse populations in Europe, North Africa and in West Asia is traceable back to the prehistoric forms. Therefore the contemporary horse breeds are classified as occidental, oriental and horses of East and South-East Asia. The pre-domestication horses belonged to the four (4) original types, i.e.: horse of North-West Europe, which assembled to contemporary Exmore pony (type1); a larger pony of North Europe and Asia similar to Highland pony (type 2); horses inhabiting Central Asia (type 3) probably ascending Akhal-teka and similar breeds; and horses of West Asia (type 4) as probable progenitor of Kaspian pony and Arab. Oriental horses descend from two ancestral forms: Tarpan *(E. ferus ferus)* and Przewalskii *(E. Przewalski),* while occidental horses have developed from diluvial forest pony. The dualistic evolutive approach has been undoubtedly incorporated into the existing theory on autochthonous horses of the Balkan (Savić et al., 2007).

Studies on polymorphism in horses lead to the development of evolutive-genetics as new discipline allowing testing and clarification of the evolutive process including effects of domestication and selective breeding. The data obtained by testing of genetic variability could be applied for characterization of the breeds and interpretation of evolutive-genetic relationship between the different population and breeds, even in absence of pedigree data (Langlois, 2005). The fact that wild ancestors of domesticated horses have been extinct their traits could be traced only by comparisons of the different domesticated breeds as carriers of the original wild genetic variance (Sponenberg, 2000). Only recently, the variability of the DNA molecule allowed investigation of the variability in the fossils, so allowing the comparison of the ancient and contemporary animals on the level other than comparison of the skeletons (Ludovic et al., 2013; Trailović et al., 2013a and 2013b).

Genetic variability applied in the studies of the equine evolution

Genetically controlled variability is profoundly studied in domestic animals and applied in characterization of different breeds and types, aiming to evaluate origin and course of domestication, and to establish population data necessary for preservation and/or conservation of rare and unique traits that can influence adaptation and unpredictable selective challenges in the future. The data concerning genetic characterization are obtained by evaluation of biochemical, physiological and DNA polymorphism (Trailović et al., 1994 and 1998; Trailović, 2009). Genetic polymorphism has been applied in the research of genetic variability in wild equines, while DNA variability and sequencing allowed insight in genetic constitution of the equines before domestication (Ellengren, 2002; Ludovic et al., 2013). Polymorphism of the different proteins, blood groups, MHC markers, DNA markers together with genome mapping and research of the genome sequence allowed studies of the interbreed divergence. The genome sequencing and equine gene mapping gave rise to the profound investigation of the genetic traits of the ancient equines by DNA analyses of the fossil remnants (Ludovic et al., 2013).

The evaluations of genetic variability on different levels show that some breed specific alleles reflect the heritage and therefore indicate possible ancestry of the modern breeds of horses (Trailović, 2009, Trailović et al., 2013a and 2013b). Although numerous allelic

forms in modern equine species are common, some alleles are species specific and therefore reflect divergence of the species (Put and Whitehouse, 1983; Nozawa et al., 1998; Cho and Cho, 2004). Research of genetic polymorphism and collection of the data gave rise to the debate on the role of Przewalskii horse during domestication.

Evaluations of the biochemical polymorphism revealed high degree of homology between Przewalskii and domestic horse breeds, although frequent alleles in Przewalski are rare in any of the domestic horse population studied and some Przewalski characteristic alleles have been revealed both by blood typing and by genotyping of the remaining wild horse (Putt and Whitehouse, 1983; Bowling and Ryder, 1987). It was suggested that Przewalski and domesticated horses had separated before domestication events and that the separate evolution gave rise to speciation of the two horse species.

During the last decade equine gene mapping allowed presentation of the medium dense gene map and revealed genome sequence in horses. The methodology of DNA studies has developed enough to allow amplification and sequencing of ancient DNA.

By comparison of genomic data obtained on Late Pleistocene horse remnants and modern genomes of five domestic horse breeds (*E. ferus caballus)*, Przewalskii *(E. Przewalski)* and donkey *(E. asinus)* the evolution of different equines was recalibrated and the role of the different ancient horses in domestication was clarified (Ludovic et al., 2013). The discovery and sequencing of the genomic DNA in Late Pleistocene horse fossil confirmed that Przewalskii is the last surviving wild horse population. The comparative analyses of the genome sequence of the Late Pleistocen horse and modern genomes of five domestic breeds, Przewalskii horse and donkey suggested that *Equus* lineage that gave rise to all contemporary horses, zebras and donkeys originated 4.0-4.5 million years before present (myr BP), twice the time that were accepted as the distance to the most recent common ancestor of the genus *Equus.* It was estimated that Przewalskii's and domesticated horse populations diverged 38-72 kyr BP and no admixture between the two diverging populations was evidenced. The results obtained by Ludovic et al. (2013) exclude Przewalskii horse from the domestication process.

The novel finding on *Equus* evolution posed several questions on ancestry of our autochthonous Yugoslav mountain pony, as well as the other horses on the Balkan.

The ancestry of autochthonous Yugoslav Mountain pony

Yugoslav Mountain pony is autochthonous horse breed that inhabits mountains South of Sava and Danube on West Balkan. It is believed that all mountain ponies of former Yugoslavia descended from Tarpan (*Equus ferus*) and Przewalskii (*Equus Przewalskii*).

Although the dispersed natural habitat of this breed is biogeographically divided by natural barriers, all ponies on the mountains in Serbia and Montenegro were considered to belong to autochthonous Yugoslav Mountain pony breed and the data concerning biogeographic differentiation of local ecotypes are missing. There is evident lack of interest reflected in few scientific data about this very adaptive breed which indicates the degree of endangerment of these unique animals (Mitrovic et al., 2011). On the other hand mountain ponies in B&H have been described in details by the beginning of the 20th century and besides numerous ecotypes, Bosnian pony was developed as highly selected breed.

Recently, some physiological, morphological, and reproductive characteristics of Autochthonous Yugoslav mountain pony have been described (Trailović et al., 1998;

Trailović, 2009; Đermanović et al., 2010; Djermanovic et al., 2012; Trailović et al., 2013a and 2013b).

The functional polymorphism within the breed was evaluated in aim to confirm the dualistic origin of autochthonous breed upon biochemical variability, to investigate genetic divergence between original population of Yugoslav Mountain pony and descending selected breed Bosnian pony, and also in aim to establish the data for further evaluation of physiology of the breed and to obtain information on functional variability in aim to promote conservation (Trailović, 2009).

During the investigation polymorphism in eight protein systems: albumin, transferring, protease inhibitors, Xk, vitamin D binding protein, esterase, phospho-glucose dehydrogenase and glucose-phosphate isomerase was established. A total of 33 functional alleles was segregating in Domestic mountain pony compared to 26 in Bosnian pony. The observed loss of functional polymorphism in descending population reveals restriction of the gene pool that could be the consequence of the limited number of founding animals and selective breeding. However none of the Przewalskii horse specific biochemical markers was determined in autochthonous population, thus the dualistic origin could not be confirmed upon the investigated biochemical polymorphism. Unexpectedly the rare Al^I isoprotein, which is considered to be the heritage of diluvial ancestors (Sandberg, 1972) was determined in Domestic mountain pony. The ancestral influence of the diluvial genome on gene pool of autochthonous horse in Former Yugoslavia was not described until our investigation. The introduction of diluvial horses to the Balkans could occur during prehistoric nomad migrations and certainly in Roman Empire.

Conclusion

Very recent evidences obtained by genome sequence analyses of early Middle Pleistocene horse allowed recalibrating of evolution of the genus *Equus* and profound comparison of the prehistoric and contemporary horses thus clarifying the lineage of all contemporary horses. These results also indicated that no recent admixture between the domestic horse breeds and Przewalskii occurred after the divergence of the species some 38-72 kyr BP (Ludovic et al., 2013), so that it was cleared that Przewalskii horse could not be involved into the horse domestication.

The novel finding therefore poses questions on the existing theory concerning the evolution of autochthonous Yugoslav mountain pony especially when Przewalskii is involved in ancestry. However the ancestral role of Tarpan is not questionable, but the original finding of the distribution of the diluvial genome markers in the autochthonous population of Yugoslav mountain pony however demands further research on routes and period of introduction of diluvial horse genome on the Balkans.

References

1. Bowling AT, Ryder OA 1987. Genetic studies of blood markers in Przewalskii horses. Journal of Heredity 78, 75-80.
2. Cho BW, Cho GJ 2004. Analysis of red cell and blood protein typing in the Mongolian horse. Journal of Animal Science and Technology 46, 887-896.
3. Djermanovic V, Mitrovic S, Djordjevic N 2012. Exterior characteristics of the horse breed population of „Posavac“ bred in the region of Eastern Serbia. Proceedings of the 47th Croatian and 7th International Symposium on Agriculture, Opatija, Croatia, 699-673.

4. Đermanović V, Mitrović S, Novaković M, Đorđević N, Ivanov S, Topolac M 2010. Qualitative properties of equidae grown in Central Serbia. Proceedings of Research Papers 16 (3-4), 179-188.
5. Ellegren H 2002. It took many mares to form the domestic horse. Trends in Genetics 18, 501-502.
6. Kaminski M, de Andres Cara DF 1986. Electrophoretic markers of Andalusian horses: comparison of Spanish and Lusitanian lineages. Comparative Biochemistry and Physiology 83B, 575-588.
7. Langlois B 2005. A revuew on the methods of parentage and inbreeding analysis with molecular markers. In Bodo I., Anderson L., Langlois B. (Eds) Conservation Genetics of Endangered Horse Breeds. EAAP Scientific Series, 116, 35-54.
8. Ludovic O, Ginolhac A, Zhang G, Frorese D, Albrechtsen A, Stiller M, Schubert M, Cappellini E, Petersen B, Moltke I, Johnson PLF, Fumagalli M, Vilstrup TJ, Raghavan M, Korneliussen T, Malaspinas AS, Vogt J, Syklarczyk D, Kelstrup DC, Vinther J, Dolocan A 2013. Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocen horse. Nature 499, 74-78.
9. Mitrovic S, Djermanovic V, Trailovic R, Trailovic D 2011. Equine selective breeding in Serbia: Status and Perspectives. Proceedings of the 2nd Regional Symposium: "Horsville - Science and Profession", Novi Sad, 47-53.
10. Nozawa K, Shotake T, Ito S, Kawamoto Y 1998. Phylogenetic relationships among Japanese Native and alien horses estimated by protein polymorphisms. Journal of Equine Science 9, 53-69.
11. Putt W, Whitehouse DB 1983. Genetics of plasma protein loci in *Equus przewalskii*. Animal Blood Groups and Biochemical Genetics 14, 7-16.
12. Sandberg KA 1972. Third allele in the horse albumin system. Animal Blood Groups and Biochemical Genetics 9, 169-174.
13. Savić M, Jovanović S, Vegara M 2007. Stočarstvo: farmske i socijalne životinje. Čigoja, Beograd, 304 pp.
14. Sponenberg DP 2000. Genetic resources and their conservation. In: The Genetics of the Horse. (eds A T Bowling, A Ruvinski), pp 387-410. CABI Publishing.
15. Trailović R 2009. Phylogenetic evaluation of Yugoslav Mountain pony upon genetic markers. PhD thesis. Faculty of Veterinary Medicine, University of Belgrade.
16. Trailović R, Đermanović V, Savić M, Dimitrijević V, Mitrović S, Ivanov S, Beckei Ž 2013a. Autochthonous breeds of horses and donkeys in Serbia: Status and Perspectives. Proceedings of Fourth Regional Symposium „Breeding, reproduction and health care of horses", Novi Sad, 47-53.
17. Trailović R, Jovanović S, Savić M 1994. Efficiency of some serum protein systems in parentage control in Yugoslav Trotter horse. Acta Veterinaria 4, 233-238.
18. Trailović R, Jovanović S, Savić M 1998. Some characteristics of the isolated populations of Yugoslav Mountain Pony. Proceedings of the 1998 International Conference of Endangered Animal Breeds of Danubian Countries, Budapest, 57-59.
19. Trailović R, Savić M, Dimitrijević V, Beckei Ž 2013b. Protein polymorphism as indicator of influence of diluvial ancestry on domesticated mountain pony population in Serbia. Proceedings of the 13th International Congress of the World Equine Veterinary Association WEVA, Budapest, Hungary, http://www.ivis.org/proceedings/weva/2013/posters/122.pdf?LA=1.

Original paper

RELATIONSHIPS BETWEEN THE UTERUS HORNS LENGTH AND PUBERTY ATTAINTMENT IN GILTS

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Abstract

Intensive pig production represents one of the basic requirements in order to fulfill conditions necessary to achieve the effective reproduction or to timely provide sufficient number of good pregnant gilts in order to replace and repair the sow herd base. Reproductive efficiency of gilts and the gilt management is one of the primary factors of a successful production of piglets. This efficiency, among other things, expresses the number of pregnant gilts, desirable genetic traits, body weight, age at first estrus, the status of sexual maturity, longevity and a good general health.

The aim of this study was to determine the correlation between length of uterus horns and the time of reaching puberty in gilts. The data used during the comparative analyses of morphological parameters were the length of uterus horns without weight, ligaments, cervices and ovaries. For the testing data 592 animals were used. The studies were conducted in warm and cool periods of the year. The animals were sacrificed at 210 days of age. On the basis of morphological structure of the ovary and the stage of sexual maturity the following has been determined: the animals before reaching puberty, animals with first and second estrus cycle.

It was determined that the gilts that have made a second estrus cycle have greater significant difference ($p<0.01$) in length of uterus horns (172.90cm) compared to others. The results show that the female fattening gilts in relation to breeding gilts had significantly greater ($p<0.01$) length of uterus horns (131.31cm). According to the studies based on the season a statistically significant ($p<0.01$) difference in the length of uterus horns was determined. In first and second estrus cycle the higher percent of animals that have reached puberty was achieved in breeding gilts (38.38 and 39.47%).

Key words: *gilts, puberty, uterus horns*

Introduction

Reproductive efficiency of gilts and the gilt management is one of the primary factors of a successful production of piglets. This efficiency, among other things, expresses the number of pregnant gilts, desirable genetic traits, body weight, age at first estrus, the status of sexual maturity, mating or conception in gilts which is associated with their subsequent reproductive performance, longevity and good general health.

In practice, the above mentioned condition is difficult to achieve. The reason for this can be seen as a result of the strong influence of paragenetic factors such as diet, season, contact with sexually mature boars, environmental conditions, stress factors, treatment with exogenous hormones and health (Stančić et al., 2003; Peltoniemi et al., 2005).

The main reason for the occurrence of prolonged anoestrus preinsemination was that that estrus was not detected even after 8 months of age (Tummaruk et al., 2007; Patterson et al., 2010; Stančić et al., 2010).

According to Tummaruk, Tantasuparuk and Kunavongkrit (2008), the age of puberty in gilts is usually defined as "the time of the first estrus and ovulation with a continuation of regular estrus cycles".

One of the most common problems present in modern pig production is a high percentage of culled sows, which is around 30% on our farms and up to 50% worldwide (Gagrčin et al., 2009). Most of the gilts and young sows were removed from herds due to reproductive failure (Engblom et al., 2007).

It has been demonstrated that gilts with a high growth rate attain puberty earlier than those with low growth rate (Young et al., 2008; Tummaruk et al., 2009).

Gilts with a delayed age at first mating (>260 days) have a shorter lifetime performance than gilts mated at a younger age. Gilts attaining puberty at a younger age also produce more piglets during the first 3 parities than those attaining puberty at an older age (Young et al., 2008).

Infertility in the summer and autumn months varies from farm to farm, field to region and year to year. Finally, what we are most interested in is to achieve maximum fertility and reproductive performance regardless of season or location (Holyoake et al., 2005). Infertility during the summer period covers a wide range of problems including anoestrus in gilts and sows, the problem with detection of estrus, conception and decreased level of embryo survival (Donald, 2007).

The aim of the present study was to determine that the length of uterus horns and the season affect the attaining puberty in gilts.

Material and methods

The aim of this study was to determine the correlation between the length of uterus horns and the time of reaching puberty in gilts. The data used during the comparative analyses of morphological parameters were the length of uterus horns without weight, ligaments, cervices and ovaries. Examination was made on a large farm in Vojvodina. For testing we used data of 592 gilts that were randomly assigned to four groups (two groups of female fattening gilts and two groups of breeding gilts). The testing was performed in both the warm (May-October) and cool (November-April) season.

The animals were sacrificed at an average of 210 days of age. After sacrifice the observation of the morphometric parameters of the gilts was made in the Laboratory in the Department of Animal Science at the University of Novi Sad. Breeding and female fattening gilts were kept under different housing and feeding regime. Based on the number of ovarian follicles and size all the animals were divided into three stages, namely: before reaching puberty (Pp), at first

estrus cycle (1E), or second estrus cycle (2E). The measurement was performed with the meter. The average values of the length of the uterus horns were expressed in centimeters. Descriptive statistics was performed using the software package Statistics 12th.

Results and discussion

It is very important to know the factors that affect the intensity of reproduction as well as the timely detection of estrus, insemination and the time of attaining puberty in gilts. To achieve these requirements it is of primary importance that breeding gilts achieve optimum age and weight, as well as the optimal ratio of muscle mass and fat body reserves, as at the time of attaining puberty and in the fertile time of insemination (Radović et al., 2007).

Table 1. *Distribution of stages of sexual maturity depending on season and category*

Stage of sexual maturity	(Cool season)				Warm season			
	Category							
	Breeding gilts		Female fattening gilts		Breeding gilts		Female fattening gilts	
	N	%	N	%	N	%	N	%
PP	22	28.95	181	75.74	46	46.46	129	72.47
1E	24	31.58	54	22.59	38	38.38	28	15.74
2E	30	39.47	4	1.67	15	15.16	21	11.79
Total	76	100	239	100	99	100	178	100
Total 592								

In Table 1 we can see that the highest percentage (75.74%) was in the female fattening gilts before reaching puberty in the cool season and the lowest (1.67%) in the second estrus cycle also in cool season. The highest percentage (38.38%) of gilts with one estrus cycle was in breeding gilts in the warm season in relation to the breeding gilts (39.47%) in the second estrus cycle in the cool season.

Similar results regarding the influence of seasonal photoperiod were given by Stančić et al., 1990. According to the study cited by Cotton, 2001, from 23 to 60% gilts attain puberty lower between June and September than in the period from October to May. In pigs, the seasonal influence on both fertility and production traits is well documented (Tummaruk et al., 2004; Tummaruk et al., 2010).

Furthermore, based on the fecal progesterone results, the proportion of gilts exhibiting first standing estrus without ovulation is more common during hot season compared to any other period (Tummaruk et al., 2007) which is in harmony with our study that shows that the percentage of gilts that reach first estrus was highest in warm period (38.38%) in breeding gilts.

In Table 2 we can see that the gilts that have made a second estrus cycle have greater significant difference ($p<0.01$) in length of uterus horns (172.90 cm) compared to the others. The results show that the female fattening gilts in relation to breeding gilts had significantly greater ($p<0.01$) length of uterus horns (131.31cm). According to the study based on the

season in cool and warm period of year a statistically significant ($p<0.01$) difference in the length of uterus horns was determined.

Table 2. *Average value and variability of the length of uterus horns*

	N	$\overline{X}$	SE	SD	CV	P
Category						
Breeding gilts	417	52.45	1.38	28.08	53.54	0.000**
Female Fattening gilts	175	130.31	4.55	59.97	46.02	
Seasons						
Cool	315	77.01	3.32	58.90	76.48	0.000**
Warm	277	73.52	2.82	46.90	63.79	
Stage of sexual maturity						
PP	377	47.18	1.00	21.36	45.25	0.000**
1E	145	101.78	2.80	33.59	33.01	
2E	70	172.90	7.82	65.47	37.86	
All population	**592**	**112.75**	**3.75**	**91.21**	**80.90**	

A morphometrical evaluation of the reproductive system of gilts before reaching sexual maturity could be a useful indicator for the estimation of early potential and actual fertility of sows (Kapelański et al., 2012).

Several experiments have suggested that uterine length influences uterine capacity (Wu et al., 1988; Chen and Dziuk., 1993; Wu and Dziuk., 1995). It has also been shown that gilts vary profoundly in the length of the uterine horns (Chen and Dziuk, 1993).

According to the research of the foreign authors the length of the uterus horns was 37 cm in gilts before reaching puberty and 244.1 cm in gilts that have reached first estrous cycle. This is in harmony with our research where the gilts before reaching puberty had 47.18 cm of the uterus horns to 101.78 cm at the time of reaching first estrus cycle. Between various races and crossbreds there are differences in the length of the uterus horns. For example, the length of uterus horns in China race Mainshan had a significantly lower length than the European white race Yorkshire. In addition, there are differences in the length of uterus horns between Swedish Landrace race (66cm) in comparison to race Mangulica (61cm). This is also shown in our research where we can see that the length of uterus horns is higher in the fattening gilts (130.31cm) compared to breeding gilts (52.45cm).

There is little research evidence that could clearly demonstrate the correlation between the length of the uterus horns before and after the attainment of sexual maturity and during pregnancy (Christenson et al., 1987; Wu and Dziuk, 1988). Furthermore, there is no research evidence that seasonal period affects the length of uterine horns which is in harmony with our research, but the length of uterus is the important feature that influence the uterus capacity.

Conclusion

Based on the results obtained in this work it can be concluded that the categories of female fattening gilts in relation to breeding gilts showed statistically significant difference in the length of uterus horns (131.31 cm compared to 52.45cm). Furthermore it can be seen from research that there was significant difference between the length of uterus horns observed by influence of season and also category that affected the length of uterus horns.

The highest percentage of animals which achieved puberty was in the first and second estrus cycle. Although our research shows that female fattening gilts have greater length of uterus in relation to breeding gilts the higher percentage of breeding gilts that reached puberty was in the first and second estrus cycle. From the aim of this work we can see that the length of uterus horns does not influence the puberty attainment in gilts.

References

1. Chen ZY and Dziuk PJ 1993. Influence of initial length of uterus per embryo and gestation stage on prenatal survival, development and sex ratio in the pig. J. Anim.Sci 71:7, 1895-1901.
2. Christenson RK, Leymaster HA and Young LD 1987. Justification of unilateral hysterectomy-ovariectomy as a model to evaluate uterine capacity in swine. J. Anim. Sci 65,738–744.
3. Cotton B 2001. Reproductive Development in Gilts. Manitoba Agriculture and Food (Livestock), May, 2001, pp.1-2.
4. Donald G Levis 2007. Designs to Reduce Seasonal Effects On Reproduction, National Hog Farmer. Retrived Jun 2 2014, from http://nationalhogfarmer.com/mag/farming_gestation_sow_housing.
5. Engblom L, Lundeheim N, Dalin A-M and Andersson K 2007. Sow removal in Swedish commercial herds. Livest. Sci. 106, 76–86.
6. Gagrčin M, Stančić I, Božić A, Stančić B, Anderson R and Hervey R 2009. Reproduktivna performansa nazimica na velikim vojvođanskim farmama, Veterinarski glasnik 63, 1-2, 77-86.
7. Holyoake T 2005. What do we know of pregnancy loss during seasons infertility? In 'Proceedings of the Australian Association of Pig Veterinarians'. Gold Coast, Australia, pp. 13–17.
8. Kapelanski W, Jankowiak H, Bocian M, Grajewska S, Dybata J and Zmudzinska A 2012. Morphometric characteristics of the reproductive system in polish large White and polish landrace gilts at 100 kg body Weight. J. Anim. Sci. 13, 45-53.
9. Patterson JL, Beltranena E and Foxcroft GR 2010. The effect of gilt age at first estrus and breeding on third estrus on sow body weight changes and long-term reproductive performance. J. Anim. Sci. 88, 2500–2513.
10. Peltoniemi OAT, Tast A, Virolainen JV, Karkamo V, Heinonen M and Andersson MA 2005. Night-time Melatonin Secretion and Seasonally Delayed Puberty in Gilts. Reprod. Dom. Anim. 40, 224-227.
11. Stančić B, Šolić R and Šahinović R 1990. Ciklična ovarijalna aktivnost peripubertetskih nazimica u zimskom i proletnjem periodu. Stočarstvo 44, 1-2, 41-50.
12. Stančić B, Kovčin S and Gagrčin M 2003. Nazimica za priplod – Fiziologija i tehnologija reprodukcije (monografija). Univerzitet u Novom Sadu, Poljoprivredni fakultet,39-48pp.

13. Stančić I, Gagrčin M, Jovičin M and Jovanović S 2010. Occurrence and practical solvent possibility of gilts preinsemination anestrus. Biotech. Anim. Husbandry 26, 1-2, 37-46.

14. Tummaruk P, Tantasuparuk W, Techakumphu M and Kunavongkrit A 2004. Effect of season and outdoor climate on litter size at birth in purebred Landrace and Yorkshire sows in Thailand. J. Vet. Med. Sci. 66, 477–482.

15. Tummaruk P, Tantasuparuk W, Techakumphu M and Kunavongkrit A 2007. Age, body weight and backfat thickness at first observed oestrus in crossbred Landrace×Yorkshire gilts, seasonal variations and their influence on their subsequent reproductive performance. Anim. Reprod. Sci. 99, 167–181.

16. Tummaruk P, Tantasuparuk W and Kunavongkrit A 2008. Age at puberty in landrace, yorkshire, duroc and crossbred Landrace x Yorkshire gilts kept in evaporative cooling system in a commercial herd in Thailand. In Proceedings of the 15th Congress of FAVA - OIE Joint Symposium on Emerging Diseases, Bangkok, Thailand, 4pp.

17. Tummaruk P, Tantasuparuk W, Techakumphu M and Kunavongkrit A 2009b. The association between growth rate, body weight, backfat thickness and age at first observed oestrus in crossbred Landrace×Yorkshire gilts. Anim. Reprod. Sci. 110, 108–122.

18. Tummaruk P, Tantasuparuk W, Techakumphu M and Kunavongkrit A 2010. Seasonal influence on the litter size at birth of pig are more pronounced in the gilt than sow litter. J. Agri. Sci. 148, 421–432.

19. Wu MC and D z i u k PJ 1988. Ovarian influence on uterine growth in prepubertal gilts. J. Anim. Sci. 66, 2893–2898.

20. Wu CM and Dziuk JP 1995. Relationship of lenght of uterus in prepubertal pigs and number of corpora lutea and fetuses at 30 days of gestation. Anim. Reprod. Sci. 38,327-336.

21. Young MG, Tokach MD, Aherne FX, Dritz SS, Goodband RD, Nelssen JL and Loughin TM 2008. Effect of space allowance during rearing and selection criteria on performance of gilts over three paities in a commercial swine production system. J. Anim. Sci. 86, 3181-3193.

Original paper

COMPARATIVE STUDIES ON THE REPRODUCTIVE AND PRODUCTIVE TRAITS OF NEW HAMPSHIRE AND SOMBOR CRESTED CHICKEN BREEDS REARED IN SEMI-EXTENSIVE PRODUCTION SYSTEM

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Abstract

Research was conducted on New Hampshire (in further text NH) and Sombor Crested (in further text SC) breeds which were kept in semi extensive system. 56 birds of each breed were used (50 females and 6 males) in the experiment. 100 eggs from every chicken breed were used for natural hatching, and remained eggs were sold. From the total number of the naturally incubated eggs, 83 (NH) and 85 (SC) chickens were hatched, and they were used for the study of performance and related parameters.

Eggs and chickens originating from NH breed were statistically significantly heavier ($P<0.001$; $P<0.01$) compared with SC breed. Egg shape index and chicken percentage in egg weight were significantly higher at SC ($P<0.001$; $P<0.05$) compared to NH breed. Very strong positive correlation was determined between egg weight and chicken weight in both breeds. Very weak negative correlation was determined between egg weight and relative chicken intake in the egg weight. Similarly, between egg shape index and chicken weight, negative correlation coefficient was determined in both breeds. However, between egg shape index and chicken percentage, statistically significant ($P<0.001$) negative correlation coefficient was determined in NH breed. Significant ($P<0.05$) positive correlation for the same performances was determined in SC breed.

Key words: *New Hampshire (NH) breed, reproductive parameters, semi-extensive system, Sombor Crested (SC) breed*

Introduction

Based on available literature it can be noted that productive and reproductive performances of pure breeds and their mestizos have nowadays become subjects of research for economic reasons. This is particularly important regarding the adoption of the Law on organic animal husbandry (EU, EEC-Regulation 1804/1999 – supplementing regulation no. 2092/91), respectively animal production, which attaches more importance to pure races because they are more convenient for the organic poultry meat and eggs than some chicken hybrids.

In semi extensive or extensive breeding system, following breeds are being reared in the Republic of Serbia: New Hampshire, Naked Neck, White Leghorn, Rode Island Red, and two domestic breeds Black Svrljig and Sombor Crested. Research was conducted with the

goal to determine productivity of these breeds in semi extensive breeding system (Mašić et al., 1970; Žigić et al., 1970; Marinković et al., 1972; Apostolov, 1976; Apostolov N. and Apostolov D, 1976). Thorough researches, in order to determine fattening and slaughter parameters of above mentioned breeds reared in different breeding systems, were conducted by many authors (Milošević et al., 2005; Pavlovski et al., 2009; Mitrović et al., 2011a). Besides, incubation values of eggs originating from two chicken breeds (strains) (White Naked Neck - WNN and Black Svrljig strain - BSv) which were reared in rural parts of Serbia in extensive breeding systems were conducted in a lesser extent (Mitrović et al., 2011b).

Next to incubation parameter results, researching of physical egg parameters and their influence on incubation values highly contributes to poultry production and reproduction of different breeds and poultry genotypes (Farooq et al., 2001; Islam et al. 2002a and 2002b; Witt and Schwalbach, 2004).

It is well known that egg weight influences the chicken weight and chicken percentage in total egg weight. Similar statement was confirmed for different genotypes of parental flocks by majority of researchers (Luquetti et al., 2004; Vieira et al., 2005; Almeida et al., 2006; Miclea and Zahan, 2006; Enting et al., 2007; Wolanski et al., 2007; Đermanović, 2010; Đermanović et al., 2010). The highest relative share of chicken in egg weight was determined in 27 weeks old parents, and the lowest in 60 weeks old parents. Phenotype correlation among monitored parameters of hybrids was similar for pure breeds (Farooq et al., 2001; Islam et al. 2002a and 2002b). However, between age of parents and egg weight, egg weight and chick weight, positive phenotype correlation was determined, but between egg weight and percentage of the chicken in egg weight negative correlation was determined and it had different levels of significance.

Bearing in mind that New Hampshire and Sombor Crested breeds are being reared in significant extent in Serbia, the aim of this research was a comparative analysis of productive and reproductive traits of the above mentioned breeds. Basically, laying intensity in specific time frame, egg weight, phenotype quality characteristics of eggs (length, width and shape index), hatched chickens weight and relative chicken weight share in total egg weight were studied. Phenotype correlation coefficients and their correlation strength were determined among the most of the following parameters.

Material and methods

With the goal to determine productive and reproductive parameters for NH and SC breeds the analysis of their reproductive traits while being reared in semi extensive system was conducted. Two parental flocks were used as experimental material. Within each breed there were 50 female and 6 male birds (1♂ : 8♀), which were individually marked by leg rings. Both parental flocks were reared separately near the farmer house and were fed, besides natural pasture, with corn grain, corn grout, and wheat and concentrate mixtures. The experiment lasted 60 days. Productivity of both breeds was monitored, respectively; number of laid eggs was counted each day for each breed and each hen during that period. Based on that laying the intensity, (absolute and relative), was calculated. Moreover, weight of each egg was taken just after being laid, length and width were also measured and the shape index was calculated for each laid egg.

For reproduction of both chicken breeds, 100 eggs were chosen by random sample method and they were planted under the hens in specific time intervals. All eggs were marked with specific number which was written on the egg shell. Identity of each chick was easily

determined that way. Eggs which were not used for reproduction, as surplus were used as consumable eggs at the farm or at the market as eggs for incubation. Chicks were laid naturally using the brooding hens and for that purpose from each breed seven hens were used.

During the experimental period following parameters were determined: number of laid eggs, laying intensity [(number of eggs/60 days) x 100], laid eggs weight, length and width of laid eggs, egg shape index [Shape index = (width/length) x 100], egg weight before planting under the broody hen, weight of hatched chickens and relative share of the chicken in the egg weight [(chick weight/egg weight) x 100]. Besides, not fertilized eggs were measured as well as their length and width, and shape index was calculated.

Usual statistic parameters were calculated for all productive and reproductive parameters: arithmetic mean, arithmetic mean error, standard deviation, and variation coefficient. Determined differences between followed parameters were calculated by t-test. Phenotype correlation coefficients and correlation between egg weight (fertilized and not fertilized) and phenotype egg parameters (length, width, and egg shape index), were calculated with statistic program (SAS, 2000) as well as correlation between weight of incubation eggs and one day old chickens and chicken share in total egg weight.

Results and discussion

It has already been mentioned that for natural hatching (by using the hen) 100 eggs were used for each breed and 83 day old chicks of NH breed and 85 chicks from SC breed were hatched. Hatchability for NH was 83.00% and 85.00% for SC. However, some authors (Mitrović et al., 2011b) determined significantly lower hatchability for BSv (78.02%) and WNN (80.00%), while eggs of RIR, BPR, WLH and WR during artificial incubation (Witt and Schwalbach, 2004) determined lower hatchability of 88.16% and 95.08%.

Both studied breeds produced totally 3,699 eggs out of which 200 (100 – NH and 100 – SC) were used for natural hatching (research), while 3,499 were used for consummation. For given period NH parental flock produced totally 1,799 eggs (laying intensity 59.97%), while SC flock produced 1,850 eggs (laying intensity 61.67%). Obtained results are shown in Table 1.

Table 1. *Average value, variability and significant difference between observed parameters in analyzed race of chicken*

Parameters	Breed	n	Mean	SEM	SD	C.V.	Significance
Hatching eggs							
Eggs weight, g	NH	100	58.99	0.44	4.48	7.85	1.95***
	SC	100	57.04	0.34	3.45	6.05	
Eggs length, cm	NH	100	5.91	0.02	0.23	3.89	0.34***
	SC	100	5.57	0.02	0.16	2.87	
Eggs width, cm	NH	100	4.36	0.01	0.13	2.98	0.09***
	SC	100	4.27	0.01	0.09	2.11	
Egg shape index, %	NH	100	73.77	0.30	3.02	4.09	-2.84***
	SC	100	76.61	0.11	1.06	1.38	
Fertilized eggs							
Eggs weight, g	NH	83	59.48	0.49	4.48	7.53	2.63***
	SC	85	56.85	0.37	3.43	6.03	

Eggs length, cm	NH	83	5.93	0.02	0.22	3.71	0.36***
	SC	85	5.57	0.02	0.16	2.87	
Eggs width, cm	NH	83	4.37	0.01	0.13	2.97	0.10***
	SC	85	4.27	0.01	0.09	2.11	
Egg shape index, %	NH	83	73.80	0.32	2.89	3.92	-2.80***
	SC	85	76.60	0.12	1.11	1.45	
1-day-old chickens							
Chick weight, g	NH	83	42.23	0.36	3.30	7.81	1.31**
	SC	85	40.92	0.23	2.15	5.25	
Chick weight, %	NH	83	71.01	0.22	2.04	2.87	-1.03*
	SC	85	72.04	0.20	1.83	2.54	
Not fertilized eggs							
Eggs weight, g	NH	17	56.59	1.15	4.75	8.40	-1.54^{ns}
	SC	15	58.13	0.86	3.32	5.71	
Eggs length, cm	NH	17	5.85	0.07	0.28	4.79	0.25**
	SC	15	5.60	0.04	0.15	2.68	
Eggs width, cm	NH	17	4.30	0.03	0.11	2.56	0.01^{ns}
	SC	15	4.29	0.02	0.09	2.11	
Egg shape index, %	NH	17	73.61	0.89	3.68	5.00	-3.07**
	SC	15	76.68	0.45	1.74	2.27	

*P < 0.05; **P < 0.01; ***P < 0.001; ^{ns}-no significance.

NH breed average weight of laid eggs was 58.99 g, hatched eggs (fertilized) 59.48 g and not fertilized 56.59 g, while for SC breed average weight of laid eggs was 57.04 g, hatched (fertilized) eggs 56.85 g and 58.13 g for not fertilized eggs. Weight of eggs (hatching and fertilized) coming from NH breed was significantly higher (P<0.001) than eggs from SC breed, while weight of not fertilized eggs was higher for SC breed though it was not statistically significant (P>0.05). It can be seen from Table 1 that all eggs from SC breed had statistically significantly (P<0.001; P<0.01) higher (76.61%; 76.60%; 76.68%) shape index (width length ratio, %) compared to eggs coming from NH breed (73.77%; 73.80%; 73.61%). Average chicken weight for NH breed was 42.23 g, while for SC breed 40.92 g, therefore chickens coming from NH breed had statistically significantly (P<0.01) higher body weight by 1.31 g than chickens coming from SC breed (Table 1). Chicken share in weight of an egg was higher in SC breed (72.04%) than in NH breeds (71.01%), and difference was statistically significant (P<0.05).

Regarding the eggs weight for NH breed (58.29 g – 58.36 g) and shape index (72.86%), some authors (Mašić et al., 1970; Žigić et al., 1970) obtained similar results. However, in eggs of the White Rock breed (Mašić et al., 1970) higher egg weight was determined being between 61.03 g and 61.32 g, while for the same chick breed (Marinković et al., 1972; Apostolov, 1976) in similar breeding conditions lower egg weight was determined (57.18 g) and slightly higher shape index value (74.70%). Authors (Farooq et al., 2001; Islam et al., 2002a and 2002b; Mitrović et al., 2011b) determined the highest egg weight 61.27 g (Mitrović et al., 2011b), slightly lower in White Leghorn 59.48 g (Islam et al., 2002a and 2002b), and lowest egg weight in Rhode Island Red 53.94 g (Farooq et al., 2001), while the shape index was 1.33 [(width/length) x 100 = 75.19%].

During the reproductive parameters research attention is given to the quality of hatched chickens, to the relative share of the chicken in egg weight. During determination of the mentioned parameter different results were obtained, more than 70% (Witt and Schwalbach, 2004) and between 65.96% and 68.43% (Apostolov N. and Apostolov D., 1976; Islam et al., 2002a; Mitrović et al., 2011b).

Based on obtained results it can be noticed that egg weight had some influence on all monitored productive and reproductive parameters for both studied breeds (NH and SC). This statement is best ratified by calculated phenotype correlation coefficients between monitored parameters (Table 2).

Table 2. *Phenotype correlation coefficients for the studied parameters of the analyzed chicken breeds (New Hampshire, n = 83; Sombor Crested, n = 85)*

Param.	Breed	E.We.	E.L.	E.Wi.	E.S.I.	C.We. (g)	C.We. (%)
E.We.	NH	1.000	0.593^{***}	0.734^{***}	-0.011^{ns}	0.863^{***}	-0.084^{ns}
	SC	1.000	0.722^{***}	0.602^{***}	-0.241^{*}	0.787^{***}	-0.124^{ns}
E.L.	NH	-	1.000	0.557^{***}	-0.643^{***}	0.606^{***}	0.112^{ns}
	SC	-	1.000	0.801^{***}	-0.623^{***}	0.565^{***}	-0.281^{*}
E.Wi.	NH	-	-	1.000	0.281^{*}	0.611^{***}	-0.271^{*}
	SC	-	-	1.000	-0.033^{ns}	0.401^{***}	-0.186^{ns}
E.S.I.	NH	-	-	-	1.000	-0.130^{ns}	-0.380^{***}
	SC	-	-	-	1.000	-0.300^{**}	0.221^{*}
C.We. (g)	NH	-	-	-	-	1.000	0.300^{**}
	SC	-	-	-	-	1.000	0.251^{*}
C.We. (%)	NH	-	-	-	-	-	1.000
	SC	-	-	-	-	-	1.000

E.We. - Eggs weight (g); E.L. - Eggs length (cm); E.Wi. - Eggs width (cm); E.S.I. - Egg shape index (%); C.We. - Chick weight (g); C.We. (%) - Chick weight (%); *P < 0.05; **P < 0.01; ***P < 0.001; ^{ns}-no significance

Data from Table 2 show that by incubation eggs weight increasing the length, width and chicken weight were increased for both breeds statistically significantly (P<0.001), and very strong correlation was determined. Between eggs weight, shape index and chicken share in weight of an egg negative coefficients were determined for both breeds though they were not statistically significant (P>0.05), except for the egg weight and shape index correlation where phenotype correlation (r_p=-0.241) was calculated and it was statistically significant (P<0.05).

By determining correlation of studied parameters between different genotypes most of the authors (Farooq et al., 2001; Islam et al., 2002a and 2002b; Mitrović et al., 2011b) came to similar conclusion. Similar correlation values between egg weight, length and width (P<0.05) were determined for Rhode Island Red (Farooq et al., 2001; Islam et al., 2002a and 2002b; Mitrović et al., 2011b), as well as between egg weight and one day old chick weight (r_p=0.496). However, WNN and BSv strain (Mitrović et al., 2011b) had statistically positive phenotype correlation between egg weight and chick weight (P<0.001). Between egg weight and relative chick share in egg weight (Mitrović et al., 2011b) negative correlation was determined (r_p=-0.204) for WNN, while for BSv it was positive (r_p=0.058), where correlation coefficients were not statistically significant (P>0.05).

Many authors (Luquetti et al., 2004; Vieira et al., 2005; Almeida et al., 2006; Miclea and Zahan, 2006; Enting et al., 2007; Đermanović, 2010; Đermanović et al., 2010) concluded that age of parents influences the egg weight, that egg weight influences the hatched chick weight and percentage of the chicken in the egg weight for hybrids Ross SL 2000, Ross 308 and Cobb 500. Quoted authors state that with the age of broiler parents egg weight increases, and that with increase of the egg weight chicken weight increases as well as dynamics of egg weight loss during the incubation period (positive phenotype correlation

was determined), but percent of the chick in the egg weight decreases (negative correlation).

Finally it can be said that research results of quoted authors for pure breeds and hybrids during artificial incubation mainly match up with productive and reproductive traits of NH and SC breeds during natural egg incubation.

Conclusion

Conducted research and obtained results show that NH and SC while being kept in semi extensive breeding system show satisfying productive and reproductive traits, and that both breeds can be used in organic poultry production. Moreover, research results point that during the natural hatching compared to artificial incubation, better results were obtained, especially regarding hatchability percentage and relative share of the chicken in total egg weight.

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References

1. Almeida JG, Dahlke F, Maiorka A, Aria Filho DE and Oelke CA 2006. Effect of broiler breeder age on hatching time, chick permanence time in hatcher and chick weight. Archives of Veterinary Science 11,1, 45-49.
2. Apostolov N 1976. Comparative analysis of physical characteristics of eggs with whole and damaged shells in light and heavy type layers. Proceedings of "Poultry days 1976", Otesevo, Macedonia, 352-361.
3. Apostolov N and Apostolov D 1976. Relation between egg shell deformation and hatchability of White Rock eggs. Proceedings of "Poultry days 1976", Otesevo, Macedonia, 392-397.
4. Đermanović V 2010. Phenotype variability and correlation of productive and reproductive characteristics of heavy hybrid hen lines Cobb 500 and Ross 308. PhD, Belgrade Univ.
5. Đermanović V, Mitrović S and Petrović M 2010. Broiler breeder age effects carrying eggs intensity, brood eggs incubation values and chicken quality. Journal of Food, Agriculture & Environment 8, 3&4, 666-670.
6. Enting H, Boersma WJA, Cornelissen JBWJ, Van Winden SCL, Verstegen MWA and Van der Aar PJ 2007. The effect of low-density broiler breeder diets on performance and immune status of their offspring. Poultry Science 86, 282-290.
7. EU (European Union) 1999. Council Regulation (EC) № 1804/1999 of 19 July 1999 supplementing regulation (EEC) № 2092/91 to include livestock production. Off. J. Eur. Commun. 1.122, 1-28.
8. Farooq M, Durrani FR, Aleem M, Chand N and Muqarrab AK 2001. Egg Traits and Hatching Performance of Desi, Fayumi and Rhode Island Red Chickens. Pakistan Journal of Biological Science 4, 7, 909-911.

9. Islam MS, Howlider MAR, Kabil F and Alam J 2002a. Comparative Assessment of Fertility and Hatchability of Barred Plymouth Rock, White Leghorn, Rhode Island Red and White Rock Hen. International Journal of Poultry Science 1, 4, 85-90.
10. Islam MS, Howlider MAR, Uddin MS, Kabir F and Alam J 2002b. Study on Reproductive Parameters of Barred Plymouth Rock, White Leghorn, Rhode Island Red and White Rock Breed of Cock. Journal of Biological Science 2, 9, 605-607.
11. Luquetti BC, Gonzales E, Bruno LDG, Furlan RL and Macari M 2004. Egg traits and physiological neonatal chick parameters from broiler breeder at different ages. Brazilian Journal of Poultry Science 6,1, 13-17.
12. Marinković V, Šrajber L, Žigić Lj and Mašić B 1972. Examination of the effect of restricted feeding and simultaneous application of vitamin-antibiotic additive on the reproductive performance of heavy type breeders. Proceedings of "Poultry days 1972", Belgrade, Serbia, 347-353.
13. Mašić B, Žigić Lj, Šrajber L and, Marinković V 1970. Investigation of periodically forced molting of White Rock and New Hampshire hens in a two-year laying. Veterinaria 58-65.
14. Miclea V and Zahan M 2006. Eggs weight influence on the incubation of light hen breeds eggs. Technical bulletin № 63, USAMV-CN, 107-110.
15. Milošević N, Perić L, Strugar V, Žigić D and Pavlovski Z 2005. Rearing of fattening chickens on free range and extensively in chicken coop. Biotechnology in Animal Husbandry 21, 5-6, 217-221.
16. Mitrović S, Bogosavljević-Bošković S, Stanišić G, Đermanović V, Dosković V and Rakonjac S 2011a. Carcass characteristics of two strains of native broilers (White Naked Neck and Black Svrljig) fattened under a semi-intensive system. African Journal of Biotechnology 10, 70, 15813-1518.
17. Mitrović S, Đermanović V, Pupovac S, Ostojić Đ and Rajović M 2011b. Incubation value of eggs domestic strains grown in chicken rural areas our country. Proceedings of Research Papers, Belgrade, Serbia, 17 (3-4), 149-156.
18. Pavlovski Z, Škrbić Z, Lukić M, Vitorović D, Petričević V and Milošević N 2009. Naked Neck chicken of Serbian and foreign origin: carcass characteristic. Biotechnology in Animal Husbandry 25, 5-6, 1023-1032.
19. SAS Institute 2000. SAS (Statistical Analysis System). User's Guide: Statistics. SAS Institute Inc., Cary, NC.
20. Vieira SL, Almeida JG, Lima AR, Conde ORA and Olmos AR 2005. Hatching distribution of eggs varying in weight and breeder age. Brazilian Journal of Poultry Science 7, 2, 73-78.
21. Žigić Lj, Šrajber L, Mašić B and Marinković V 1970. The effect of forced molting on physical properties and inner quality of eggs layed by New Hampsire hens. Veterinaria 68-71.
22. Witt F and Schwalbach LMJ 2004. The effect of egg weight on the hatchability and growth performance of New Hampshire and Rhode Island Red chicks. South African Journal of Animal Science 34, 62-64.
23. Wolanski NJ, Renema RA, Robinson FE, Carney VL and Fancher BI 2007. Relationships among egg characteristics, chick measurements, and early growth traits in ten broiler breeder strains. Poultry Science 86, 1784-1792.

Original paper

INVESTIGATION OF BLOOD PARAMETERS AND OVARIAN MORPHOLOGY OF LAYING HENS FEEDING *LAVANDULA STOECHAS* ESSENCE

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Abstract

The objective of this study was to evaluate the effects of different levels of *Lavander stoechas* essence on blood parameters and ovarian morphology of laying hens for 8 weeks. This experiment was conducted with 160 Hy-Line (W36) laying hens (30 wks of age) randomly divided into 4 experimental groups, 5 replicates and 8 birds per each (2 cages for each replicate and 4 birds in each cage). Treatments were control (without *Lavander* essence), and levels of 200, 400, and 600 *ppm Lavander* essence. All hens were provided the layer diets (2870 kcal/kg ME and 15.5 % CP) ad- libitum and received 16 h of light/ 8 h of dark. At the end of experiment, 2 birds of each replicate were randomly selected and blood samples were collected by brachial venipuncture. Serum was harvested by centrifugation and frozen for future analysis of plasma lipid. After that birds were killed by cervical dislocation for morphological assessments of ovaries. The weights of the oviduct, ovary, and stroma were recorded. The stroma weight comprised the ovarian tissue remaining after the large yellow follicles (LYF) were counted and removed. The number of small yellow follicles (SYF) and postovulatory follicles (POF) was recorded. Blood triglycerides, glucose and HDL were not affected by the treatments but by adding *Lavender* essence to the diet, cholesterol and LDL concentration decreased significantly in comparison with control diet ($p<0.05$). In addition, the levels of *Lavander* essence had no effect on the relative weight of the ovary, oviduct, stroma and number of SYF and POF. However, the levels of 200 and 400 ppm of *Lavender* caused significant increase of number and weight of LYF. The results showed that the addition of essence of *Lavender* up to 400 ppm increased weight and numbers of LYF.

Key words: *blood parameters, Lavender essence, ovary morphology*

Introduction

In the commercial egg type chicken industry profits depend on the cost and nutritive value of the feed. One of the promoting enhances productive performance of layers are antibiotics. Antibiotics have been used as a growth promoting substance. However, the using of antibiotics as feed additives is risky due to, not only cross-resistance, but also to multiple resistances in pathogens (Bach Knudsen, 2001; Schwarz et al., 2001). Consequently, the animal feed industry is under increasing consumer pressure to reduce the use of antibiotics as a feed additive and to find substitutes for antibiotics in the diet (Hertrampf, 2001; Humphrey et al., 2002). Many scientists have searched for alternatives to antibiotics (Langhout, 2000; Mellor, 2000; Wenk, 2000; Kamel, 2001). Recently, it has

been found that natural additives such as herbs and edible plants have some properties as growth enhancers to replace antibiotics. These additives are given to animals or birds to improve their physiological and productive performance. The antimicrobial effect of the medicinal plants is well documented (Valero and Salmeron, 2003). Lavander stoechas is a flowering plant in the family of *Lamiaceae*. The medicinal parts are the essential oil from the fresh flowers and/or the inflorescence, the flowers collected just before opening and dried, the fresh flowers and the dried flowers. Lavender oil has been reported to contain more than 100 components. The essential oil (1 to 3%) of *Lavandula* is rich in linalool and linalyl acetate. Further aroma components are β-ocimene, cineol, camphor and caryophyllene epoxide. Linalyl acetate is the major compound found in flowers. The plant also contains rosmarinic acid and coumarin. Since no information is available about the administration of *Lavandula* in laying hens diet, this experiment was conducted to investigate the effect of using Lavander stoechas as an additive on productive performance and egg quality of laying hens.

Material and methods

Birds and housing

One hundred and sixty Hy-Line (W36) laying hens (30 wks of age) were individually weighed and randomly housed in cages and allotted for four dietary treatment groups of five replicate and eight birds in each replicate for ten weeks (four birds in each cage and two cages for each replicate). Two weeks were for adaptation and eight weeks for sampling. The birds were maintained under commonly 16 h light:8 h dark cycle throughout the experimental period. Hen house temperature was 17-20°C during the experiment. Feed and water were offered ad-libitum. Treatments were basal diet (Corn-Soybean diet with 2870 kcal/kg ME and 15.5 % CP) and increasing levels of Lavander stoechas essence (200, 400 and 400 ppm) added to basal diet. The experimental diets were in mash form and formulated to meet or exceed NRC (1994) recommendations.

Data collected

At the end of the experiment, two birds of each replicate were selected and 10-mL blood sample was collected by brachial venipuncture. Serum was separated by centrifugation at 3000 g for 10 min, and stored at -20ºC until the time of analysis. Serum samples were analysed for glucose (glucoseoxidase method; Trinder, 1969), triglycerides (glycerol-3-phosphate-oxidase paraamino-phenazone GPO-PAP colourimetric enzymatic method; Trinder, 1969), total cholesterol (cholesterol-oxidase para-aminophenazone (CHOD-PAP) colouri-metric enzymatic method; Roeschlau et al., 1974), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) (Zlatkis et al., 1953). At the end of the experiment, two birds from each replicate were killed by cervical dislocation and body weight was recorded. Then the birds were dissected, and weights of the oviduct, ovary, and stroma (ovarian tissue remaining after the LYF were counted and removed) were recorded. The LYF were counted, sorted by size (> 10 mm diameter) and individually weighted. The number of small yellow follicles (SYF) (5 to 10 mm diameter), large white follicles (LWF) (< 5 mm diameter) and postovulatory follicles (POF) on the stroma were recorded (Renema et al., 1999).

All the results were statistically analyzed by General Linear Models (GLM), one way analysis of variance, using SAS software (SAS Institute, 1999). Differences among the means were separated using Duncan's multiple range test (Duncan, 1955).

Results and discussion

Table 1. *Effects of treatments on blood parameters of laying hens*

Level of essence (ppm)	Glucose, mg/dL	Cholesterol, mg/dL	Triglycerides, mg/dL	LDL,[2] mg/dL	HDL,[1] mg/dL
0	193.38	146.0[a]	201.80	62.25[a]	45.4
200	191.23	90.6[b]	195.20	45.00[b]	46.8
400	194.54	130.2[ab]	189.00	51.50[b]	45.2
600	204.30	106.2[ab]	158.25	51.20[b]	43.6
SEM	3.32	7.18	9.90	1.50	0.88

[a,b] Column means with different superscripts differ significantly (P < 0.05); [1]High-density lipoprotein; [2]Low-density lipoprotein

As shown in Table 1, serum parameters such as glucose, triglycerides and HDL were not affected by the treatments (P>0.05). However, it was noted that the triglyceride values of the control group were numerically higher than those of the other treatments. Layers fed diets supplemented with *Lavandula* essence significantly (P<0.05) decreased total plasma cholesterol and LDL compared with those fed control diet (Table 1). Our results are in agreement with that reported by Abdalla et al. (2011) that showed that the addition of mixture of medicinal plants to layer diets decreased significantly total cholesterol compared to control group. El-Husseiny et al. (2002) also noticed that addition of fenugreek to broiler diet decreased significantly total cholesterol compared to control group. Moreover, El-Kaiaty et al. (2002) found that a fenugreek seeds extract containing steroid saponins induced hypocholesterolaemia. Such reduction is often related to the mode of action of fenugreek in bird metabolism, which includes competition with cholesterol at binding sites or interfere with the cholesterol biosynthesis in the liver. Hypocholesterolaemic effects of fenugreek are owing to increased conversion of hepatic cholesterol to bile salts due to the loss in the feces of complexes of these substances with fenugreek fiber and saponins.

Table 2. *Effects of treatments on reproductive morphometrics of laying hens*

Parameters	Level of essence (ppm)				
	0	200	400	600	SEM
Oviduct weight (g)	4.90	4.04	3.87	3.87	0.13
Ovary weight (g)	3.72	3.44	3.21	2.77	0.13
Stroma weight (g)	8.07	6.99	7.93	7.55	0.25
Number of SYF[1]	17.8	14.4	17.6	16.0	0.81
Number of LYF[2]	5.2[ab]	5.8[a]	5.7[a]	5.0[b]	0.1
Number of LWF[3]	38.6	41.8	36.6	42.2	2.15
Number of POF[4]	3.4	3.8	3.6	3.7	0.12
Total LYF weight (g)	39.38[ab]	46.08[a]	44.51[a]	32.85[b]	1.14

[a,b] Row means with different superscripts differ significantly (P < 0.05); [1]Small yellow follicle (5 to 10 mm diameter); [2]Large yellow follicle (>10 mm diameter); [3] Large white follicle (<5 mm diameter); [4]Postovulatory follicle.

Oviduct, ovary and stroma weights were not significantly affected by the treatments (P>0.05) (Table 2). However, the number of LYF and total LYF weight were significantly

affected by the treatments ($P<0.05$). There was an increase in the number of LYF and total LYF weight at levels of 200 and 400 *ppm* essence compared to the control and 600 *ppm*.

We found no study that shows the effect of *Lavandula* essence on reproductive morphometrics of laying hens in literature to compare our result. But Khazaei et al. (2011) reported significant increase in a total number of follicles by using *Foeniculum vulgare* compared to the control in female mice.

Conclusion

Lavandula essence supplementation in laying hen diets shows a significant positive effect on lipid parameters. Moreover, *Lavandula* essence supplementation in diet increased number of LYF and total LYF weight. The present study has elucidated the fact that *Lavandula* essence has a folliculogenesis effect in laying hens. But further studies are suggested for understanding the exact mechanism(s) underlying these actions and probable changes in hormonal levels.

References

1. Abdalla A, Mona A, Ahmed MI, Abaza M, Aly OM and Hassan Effat Y 2011. Effect of using some medicinal plants and their mixtures on production on productive and reproductive performance of gimmizah strain. Egypt poultry science 31, 641-654.
2. Bach Knudsen KE 2001. Development of antibiotic resistance and options to replace antimicrobials in animal diets. Proceedings of the Nutrition Society 60, 291-299.
3. Duncan DB 1955. Multiple range and multiple F-Test. Biometrics 11,1-42.
4. EL- Husseiny O, Shalash SM and Azouz HM 2002. Response of broiler performance to diets containing hot pepper, and / or fenugreek at different metabolizable energy levels. Egypt Poultry Science 22, 387-406.
5. El-Kaiaty AM, Soliman AZ and Hassan MSH 2002. The physiological and immunological effects of some natural feed additives in layer hen diets. Egypt Poultry Science 22, 175-183.
6. Hertrampf JW 2001. Alternative antibacterial performance promoters. International Journal of Poultry Science 40, 50-52.
7. Humphrey BD, Huang N and Klasing KC 2002. Rice expressing lactoferrin and lysozyme has antibiotic-like properties when fed to chicks. Journal of Nutrition 132, 1214-1218.
8. Kamel C 2001. Tracing modes of action and the roles of plant extracts in non-ruminants. In: Recent advances in animal nutrition. Eds. Garnsworthy, PC, Wiseman J, Nottingham University Press, Nottingham pp. 135-150.
9. Khazaei M, Montaseri A, Khazaei R and Khanahamadi M 2011. Study of foeniculumvulgare effect of folliculogenesis in female mice. International Journal of fertility and sterility 5, 122-127.
10. Langhout P 2000. New additives for broiler chickens. World Poultry-Elsevier 16(3), 22-27.
11. Mellor S 2000. Nutraceuticals–alternatives to antibiotics. World Poultry-Elsevier 16(2), 30-33.
12. NRC 1994. Nutrient Requirements of Poultry. ^{9}th revised Edition. National Academy Press. Washington, DC.

13. Renema RA, Robinson FE, Proudman JA, Newcombe M and McKay RI 1999. Effects of body weight and feed allocation during sexual maturation in broiler breeder hens: 2. Ovarian morphology and plasma hormone profiles. Poultry Science 78, 629–639.

14. Roeschlau P, Bernt E and Gruber W 1974. Enzymatic determination of total cholesterol in serum. Z. Klin. Chem. Klin. Biochem 12, 226.

15. SAS Institute 1999. SAS User1's Guide. SAS Inst. Inc., Cary, NC.

16. Schwarz S, Kehrenberg C and Walsh TR 2001. Use of antimicrobial agents in veterinary medicine and food animal production. International Journal of Antimicrobial Agents, 17, 431-437.

17. Trinder P 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry 6, 24-27.

18. Valero M and Salmeron MC 2003. Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth. International Journal of Food Microbiology 85, 73-81.

19. Wenk C 2000. Recent advances in animal feed additives such as metabolic modifiers, antimicrobial agents, probiotics, enzymes and highly available minerals. Review. Asian - Australasian Journal of Animal Sciences 13, 86-95.

20. Zlatkis A, Zak B and Boyle AJ 1953. A new method for the direct determination of serum cholesterol. Journal of Laboratory and Clinical Medicine 41,486–492.

Original paper

EFFECT OF ROOSTER MACROPHAGES ON THE MOTILITY CHARACTERISTICS OF SPERMATOZOA

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Abstract

The aim of our study was to detect macrophages in rooster semen and subsequently to asses their effect on the spermatozoa motility parameters. Semen samples were collected from Ross PM3 breeder males (n = 24) by the dorso-abdominal massage into prepared sterile tube. Macrophages were identified using fluorescent dye Alexa Fluor-AcLDL and evaluated under a Leica fluorescent microscope (Leica Microsystem, Germany). Roosters (n=8) with the occurrence of macrophages above 20% per samples were classified to the group R1 and roosters (n=16) with the occurrence of macrophages 0-10% were classified to the group R2. The mean content of macrophages was 23.43±1.82 vs. 7.59±1.39 (group 1 vs. group 2, $P<0.001$) during experiment. The concentration (10^9 per ml), percentage of motile spermatozoa (motility > 5 μm/s) and percentage of progressive motile spermatozoa (motility > 20 μm/s) of two heterospermic samples were measured using computer-assisted sperm analysis (CASA system, Sperm Vision™). We observed significantly ($P<0.05$) lower progressive motile of rooster spermatozoa in the group R1 in comparison to group R2 (25.04±5.98 vs. 46.38±3.28). Based on the observed preliminary results, we hypothesized that the higher presence of macrophages in semen may have a negative effect on the quality of rooster spermatozoa in terms of their motility. Further experiments are required in order to prove our hypothesis.

Key words: *CASA, macrophages, motility, rooster, spermatozoa*

Introduction

Monocytes-macrophages, cells belonging to the mononuclear phagocytic system, are considered as the first line of immunological defense. Being mobile scavenger cells, macrophages participate in innate immunity by serving as phagocytic cells. These cells arise in the bone marrow and subsequently enter the blood circulation as blood monocytes. Upon migration to various tissues, monocytes mature and differentiate into tissue macrophages (Qureshia et al., 2000). Activated macrophages, engaging in sperm phagocytosis (spermiophages), might represent a marker of innate immune system activation (Pelliccione et al., 2008). Macrophages are known to perform essential roles in a number of physiological processes including lipid metabolism, wound healing, scavenging, and host defense against microorganisms and neoplasms but no clearly defined role has been assigned to macrophages normally present in the reproductive tract. Spermiophagy has been reported to occur widely in various groups of vertebrates, e.g., teleosts (Porawski

et al., 2004), amphibians (Sever, 1992), reptiles (Akbarsha et al., 2007), mammals (Murakami et al., 1985; Goyal, 1982; Abou-Elmagd and Wrobel, 1990). In mammals, spermiophagy takes place in various portions of the male reproductive tract, such as the seminiferous tubules (Holstein, 1978), *rete testis*, efferent duct (Holstein, 1978; Goyal, 1982), ampulla of the *vas deferens* (Murakami et al., 1985), seminal vesicle (Murakami et al., 1978), and ejaculatory duct (Abou-Elmagd and Wrobel, 1990). In avian species, spermiophages have been identified in the semen of guinea fowl (Hess et al., 1986) and in the *rete testis* of normal cockerels (Aire and Malmqvist, 1979). Aire (2000) reported active spermiophagy by the non-ciliated cells in the epithelial lining of the epididymal efferent ducts of the normal chicken.

The macrophages are capable of engulfing numerous spermatozoa (Hughes et al., 1981), and such phagocytosis could represent a process for removal of ageing spermatozoa (Tomlinson et al., 1992). However, current evidence indicates that it can also be pathological (Bronson, 1999; Turek and Lipshultz, 1994).

The aim of the study was to detect macrophages in rooster semen using fluorescent staining and to asses their effect on the spermatozoa motility and concentration using CASA method.

Materials and methods

Animals

Sexually mature (37 - 40 weeks old) Ross PM3 breeder males (n = 24) reared in a private breeding facility (Liaharenský podnik Nitra Ltd., Močenok Slovak Republic) were used in experiments. The roosters were housed in individual cages, under a constant photoperiod of 14 h of day light and were fed commercial standard diet with water given *ad libitum*.

Semen collection

The semen samples were collected from all roosters by dorso-abdominal massage into prepared sterile tubes. All the semen samples were at first analyzed for the presence of macrophages using fluorescent dye. The samples were divided into two groups on the basis of macrophage's content as follows: roosters (n=8) with the occurrence of macrophages above 20% per samples were classified to the group R1 and roosters (n=16) with the occurrence of macrophages 0-10% were classified to the group R2. Then heterospermic samples were routinely collected from these roosters twice a week into two prepared sterile tubes. The heterospermic pools were transported to the laboratory for fluorescent and CASA analysis.

Analysis of macrophages

Semen samples were washed in a saline solution (Sodium chloride 0.9 %, B. Braun Medical Ltd. Bratislava, Slovak Republic) and centrifuged at 300 x g for 3 min from the rest of seminal fluid. Pellets were resuspended in 2 $\mu g.ml^{-1}$ of Alexa-AcLDL (Acetylated Low Density Lipoprotein, Molecular Probes, USA) in saline solution and incubated in incubator for 2 hours. The samples were subsequently centrifuged at 300 x g for 3 min and resuspended in a cold saline solution. The suspension was afterwards placed on to a microscope slide, mixed with an equal volume of Vectashield antifade medium (Vector Laboratories, Burlingame, CA) containing DAPI fluorochrome. DAPI was used to identify the whole spermatozoa population. The drop was covered with a coverslip. Samples were evaluated under Leica fluorescent microscope (Leica Microsystem, Germany). The

percentage macrophages were determined for each heterospermic sample as the percentage of stained macrophages within the 400 spermatozoa counted.

CASA

The samples were diluted at the ratio of 1:100 in a saline solution. The concentration and motility characteristics of diluted rooster spermatozoa with different macrophage content were analyzed using CASA system (Sperm Vision™; MiniTüb, Tiefenbach, Germany). A subsample of this solution (4 μL) was placed on a Standard Count Analysis Chamber Leja 20 micron (MiniTüb, Tiefenbach, Germany) and evaluated using the CASA system under a Zeiss Axio Scope A1 microscope. In each sample the following parameters were evaluated: the concentration (10^9 per ml), percentage of motile spermatozoa (motility > 5 μm/s), percentage of progressive motile spermatozoa (motility > 20 μm/s), VCL (velocity curved line, μm/s), VSL (velocity straight line, μm/s), STR (straightness - VSL:VAP, velocity average path), LIN (linearity - VSL:VCL), BCF (beat cross frequency, Hz).

Statistical analysis

The experiment with heterospermic samples was replicated 4 times. Observed results were evaluated statistically using t-test by means of SigmaPlot software (Systat Software Inc., Germany) and expressed as the means ± SEM. P-values at $P<0.05$ were considered as statistically significant.

Results and discussion

The semen quality of the rooster is affected by different factors like age, breed, feed, environmental stressors, temperature and humidity (Zhang et al., 1999; Karaca et al., 2002; Shanmugam et al., 2012). The assessment of semen quality characteristics of poultry birds gives an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs (Peters et al., 2004). Among the parameters evaluated in the seminal analyses, the white blood cell count is considered, as are sperm concentration, motility and morphology. It has been determined that neutrophils and macrophages constitute 95% of seminal leukocytes (Cavagana et al., 2012).

The macrophages in rooster semen were detected by fluorescent dye Alexa-AcLDL in this work. If the LDL (Low-Density Lipoprotein Complexes) has been acetylated, the LDL complex no longer binds to the LDL receptor, but rather is taken up by macrophages that possess “scavenger” receptors specific for modified LDL. The superior fluorescence output by Alexa Fluor AcLDL provides easier identification of macrophages and in mixed cell population. The experimental roosters were divided into two groups on the basis of macrophages content, that was observed using fluorescent dyes Alexa-AcLDL (green stained) and DAPI (blue stained). Roosters (n=8) with the occurrence of macrophages above 20% per samples belong to the group R1 and roosters (n=16) with the occurrence of macrophages 0-10% belong to the group R2. The average content of macrophages was 23.43±1.82 vs. 7.59±1.39 (group 1 vs. group 2, $P<0.001$) during experiment.

Present work evaluates effect of rooster macrophages on selected sperm parameters. Tomlinson et al. (1992) suggested the possibility that macrophages may play a positive role in the control of semen. The authors postulated that phagocytes might shape the quality of the human ejaculate by phagocytising morphologically abnormal spermatozoa. In our study, there was no statistically significant difference in motility of spermatozoa between R1 and R2 group. On the other hand, the progressive motility was significantly

lower (P<0.05) in the group R1 (25.04±5.98) in comparison to the group R2 (46.38±3.28). There were no significant differences in other motility parameters (VCL, VSL, LIN, STR, BCF) between analyzed groups (Table 1).

Table 1. *CASA parameters of rooster heterospermic samples in the groups R1 and R2*

Semen sample	R1	R2
Concentration (10^9 per ml)	3.15±1.29	3.69±1.07
% of motile spermatozoa (motility μm/s)	43.99±3.88	55.51±8.43
% of progressive motile spermatozoa (motility 20 μm/s)	25.04±5.98*	46.38±3.28
VCL (μm/s)	87.06±6.73	86.09±6.11
VSL (μm/s)	31.15±1.23	30.29±2.27
LIN (VSL/VCL)	0.36±0.02	0.35±0.17
STR (VSL:VAP)	0.66±0.03	0.64±0.01
BCF (Hz)	26.84±1.53	28.63±1.61

Results are expressed as means ± SEM; * statistically significant at P<0.05

Pelliccione et al. (2008) reported a significant positive correlation between macrophage counts in men semen and sperm tail defects, acrosome damage and high sperm deformity index. The presence of spermiophages was associated with a lower total sperm count and sperm concentration, a lower forward motility and a high percentage of disrupted sperm compared to ejaculates without macrophages. Nevertheless, in our study no significantly negative effects of the macrophages on the concentration of rooster spermatozoa were observed (Table 1).

The polymorphonuclear macrophages are the main components of seminal leukocytes which can generate significantly higher (>100-fold) quantities of ROS, overwhelming the ROS-scavenging mechanisms in seminal plasma and resulting in oxidative stress and damage to spermatozoa (Sharma et al., 2001). Immune activation induced a steeper decline in sperm swimming velocity, thus highlighting the potential costs of an induced immune response on sperm competitive ability and fertilizing efficiency. Oxidative damage negatively correlated with sperm swimming velocity as reported for Great Tit (Losdat et al., 2011). Oxidative damage to spermatozoa can reduce fertility in domestic animals and humans (Tremellen, 2008; Aitken, 1999), and has been demonstrated recently in a wild bird species to affect sperm quality by a reduction of sperm motility and swimming velocity (Helfenstein et al., 2010). But on the contrary, the addition of macrophages to normal turkey semen had no effect on fertilizing capacity, hatchability, or embryonic mortality of unstored semen or semen stored for 6 h (Barnes et al., 1996). Seshadri et al. (2012) analyzed the effect of human leucocyte subpopulations on fertilization rates in an IVF cycle and they reported that the macrophages and the monocytes were significantly elevated in the good fertilizers group in comparison with the poor fertilisers.

Conclusion

These preliminary results may indicate the negative effect of higher macrophages presence in semen on the quality of rooster spermatozoa. Nevertheless, it is necessary to more precisely determine the effects of seminal macrophages on the quality parameters of rooster semen and fertilization.

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References

1. Abou-Elmagd A and Wrobel KH 1990. The epithelial lining of the bovine ejaculatory duct. Acta Anatomica (Basel) 139, 60-65.
2. Aire TA 2000. Active spermiophagy in the initial part of the proximal efferent duct of the epididymis of normal domestic fowl (*Gallus domesticus*). Research in Veterinary Science 68, 135-140.
3. Aire TA and Malmqvist M 1979. Macrophages in the excurrent ducts of the testes of normal domestic fowl *(Gallus domesticus)*. Anatomia, Histologia, Embryologia: Journal of Veterinary Medicine Series C 8, 172-176.
4. Aitken RJ 1999. The Amoroso Lecture The human spermatozoon - a cell in crisis? Journal of Reproduction and Fertility 115, 1-7.
5. Akbarsha MA, Kadalmani B and Tamilarasan V 2007. Efferent ductules of the fan-throated lizard *Sitana ponticeriana* Cuvier: light and transmission electron microscopy study. Acta Zoologica (Stockholm) 88, 265-274.
6. Barnes DA, Thurston RJ, Scott TR and Korn N 1996. Effect of Added Spermiophages in Pooled Turkey Semen on Fertility, Embryonic Mortality, and Hatchability. Poultry Science 75, 943-948.
7. Bronson RA 1999. Sperm antibodies: a critical evaluation and clinical guidelines. Journal of Reproductive Immunology 45, 159-183.
8. Cavagna M, Oliveira JB, Petersen CG, Mauri AL, Silva LLI, Massaro FC, Baruffi RLR and Franco JG 2012. The influence of leukocytospermia on the outcomes of assisted reproductive technology Reproductive Biology and Endocrinology 10, 1-10.
9. Goyal HO 1982. Light microscopic and ultrastructural evidence of epithelial phagocytosis of sperm in the rete testis and ductuli efferentes in the bull. American Journal of Veterinary Research 43, 785-790.
10. Helfenstein F, Losdat S, Møller AP, Blount JD and Richner H 2010. Sperm of colourful males are better protected against oxidative stress. Ecology Letters 13, 213-222.
11. Hess RA, Hughes BL and Thurston RJ 1986. Frequency and structure of macrophages and abnormal sperm cells in guinea fowl semen. Reproduction Nutrition Development 26, 39-51.
12. Holstein AF 1978. Spermiophagy in the seminiferous tubules and excurrent ducts of the testis in rhesus monkey and man. Andrologia 10, 331-352.
13. Hughes L, Ryder TA, McKensie ML, Pryse-Davies J, Stedronska J and Hendry WF 1981. The use of transmission electron microscopy to study non-spermatozoal cells in semen. In Oligozoospermia-recent progress in andrology (eds F Frajese, EFE Hafe, C Conti, A Fabbrini), pp. 65-75. Raven Press, New York, USA.
14. Murakami M, Sugita A, Shimada T and Yoshimura T 1978. Scanning electron microscope observation of the seminal vesicle in the Japanese monkey with special reference to intraluminal spermiophagy by macrophages. Archives of Histology Japan 41, 275-283.
15. Murakami M, Nishida T, Shiromoto M and Inokuchi T 1985. Phagocytosis of spermatozoa and latex beads by epithelial cells of the ampulla vasis deferentis of the rabbit: a combined SEM and TEM study. Archives of Histology Japan 48, 269-277.

16. Karaca AG, Parker HM and McDaniel CD 2002. Elevated body temperature directly contributes to heat stress infertility of broiler breedermales. Poultry Science 81, 1892-1897.

17. Losdat S, Richner H, Blount JD and Helfenstein F 2011. Immune Activation Reduces Sperm Quality in the Great Tit. Plos One 6, 1-10.

18. Qureshia MA, Heggena CL and Hussainb I 2000. Avian macrophage: effector functions in health and disease. Developmental and Comparative Immunology 24, 103-119

19. Pelliccione F, D'Angeli A, Cordeschi G, Mihalca R, Bocchio M, Ciociola F, Mancini M, Necozione S, Francavilla F and Francavilla S 2008. Seminal macrophages in ejaculates from men with couple Infertility. International Journal of Andrology 32, 623-628.

20. Peters SO, Omidiji EA, Ikeobi CON, Ozoje MO and Adebambo OA 2004. Effect of Naked Neck and Frizzled Genes on Egg Traits, Fertility and Hatchability in Local Chicken. Proceedings of the 9th Annual Conference of Animal Science Association of Nigeria. Nigeria, Abakaliki : Ebonyi State, p. 262-264.

21. Porawski M, Wassermann GF and Achaval M 2004. Localization of acid phosphatase activity in the testes of two teleostean species (*Oreochromis niloticua* and *Odonthestes perugiae*). Brazilian Journal of Biology 64, 853-858.

22. Seshadri S, Flanagan B, Vince G and Jones DIL 2012. Leucocyte subpopulations in the seminal plasma and their effects on fertilisation rates in an IVF cycle. Andrologia 44, 396-400.

23. Sever DM 1992. Spermiophagy by the spermathecal epithelium of the salamander *Eurycea cirrigera*. Journal of Morphology 212, 281–290.

24. Shanmugam M, Rajkumar U, Reddy MR and Rama Rao SV 2012. Effect of age on semen quality in naked neck and dwarf chicken under tropical climatic conditions. Animal Production Science 52, 964-968.

25. Sharma R, Pasqualotto F, Nelson D, Thomas A, Agarwal A 2001. Relationship between seminal white blood cells counts and oxidative stress in men treated at an infertility clinic. Journal of Andrology 22, 575-583.

26. Tremellen K 2008. Oxidative stress and male infertility - a clinical perspective. Human Reproduction Update 14, 243-258.

27. Tomlinson MJ, White A, Barratt CLR, Bolton AE and Cooke ID 1992. The removal of morphologically abnormal sperm forms by phagocytes: a positive role for seminal leukocytes. Human Reproduction 7, 517-522.

28. Turek PJ and Lipshultz LI 1994. Immunologic infertility. Urologic Clinics of North America 21, 447-470.

29. Zhang X, Berry WD, McDaniel GR, Roland DA, Liu P, Calvert C and Wilhite R 1999. Body weight and semen production of broiler breedermales as influenced by crude protein levels and feeding regimensduring rearing. Poultry Science 78, 190-196.

Original paper

REPRODUCTIVE PARAMETERS AND BIRTH TYPE INFLUENCE ON SANSKA GOAT KIDS BODY WEIGHT*

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Abstract

In this paper Sanska goat reproductive parameters after the first kidding are shown as well as influence of birth type on kid body weight at birth. At first insemination average age of young goats was 252 days. Average gestation period for goats that carried twins was 150.19 days and for goats that carried singles 151.10 days. Determined difference in gestation length of 0.91 days in favor of goats that carried singles was not statistically significant (P>0.05). Goat fertility after first kidding was 164.70%. Kid body weight at birth was analyzed based on birth type (singles-twins). Singles had higher body weight at birth than twins by 0.91kg (34.60%) which was statistically very significant (P<0.01). Average body weight of singles was 3.54kg, and twins 2.63kg, twins were approximately 74.29% of body weight of singles. Gender based differences at birth in body weight were present in favor of male kids, but they were not statistically significant (P>0.05). Based on obtained results in this research we can conclude that the results which were obtained for researched parameters are within average limits for Sanska goat breed.

Key words: *age, birth type, body weight at birth, fertility, Sanska breed*

Introduction

Republic of Serbia, even though it has favorable conditions for goat herding development (geographic position, relief, ground composition, flora, environment) is in deficit with goat meat, milk and milk products (Mekić et al., 2011).

Goats are being reared in Europe mainly because of milk production and two breeds of goat which have the highest milk yield originate from Europe (Park, 2001). In the European Union, France is in the first rank for its goat milk production, it averagely produces 534 millions of liters of goat milk (Jean-Claude Le Jaouen, 2005).

Goats as genetic resource have very important socio-economical roles in many rural areas of the world (Ogola and Kosgey, 2012). In the developing countries, goat productivity level is low (Abdel Aziz, M. 2010). Today, it is considered that goat herding is very significant part of sustainable production, rural development and poverty reduction (Haen Lein, 1998).

Fact is that exceptional characteristics of goat milk – good nutritive value, digestibility and lack in allergens – make it recommendable for kids and reconvalescents (Ribeiro and Ribeiro, 2011). In the past two decades these facts had positive influence on goat herding interest increase in Serbia, especially for high milk yield of pure breeds. However, because in Serbia there are no such breeds, in several occasions Alpine, Sanska and German fawn were imported because of their good genetic potential for milk production and high fertility (Ćinkulov et.al., 2007).

When it comes to high milk production in Europe Sanska and Alpine breed have one of the highest potentials. Productive traits of those two breeds are almost the same. Sanska breed has slightly higher milk yield and fertility rate (Rako, 1981). Both genotypes are excellent for rearing in our ecological conditions.

Milk yield is influenced by genetic and paragenetic factors. Yield and milk quality depend on the breed (Sung et al., 1999), lactation stage (Ciappesoni et al., 2004); kidding season (Crepaldi et al., 1999) and all these factors have a very significant influence on the final product quality (Fekadu et al., 2005).

Amount and milk composition is a breed genetic characteristic, however, it is also influenced by physiological and environmental factors (Crepaldi et al., 1999). The most significant physiological factors according to Dimassi et al. (2005) are lactation length, fertility, kidding season, while most significant environmental factors that have influence on the amount and milk composition are diet and milking.

It is well known that the amount of milk increases with the age of the goat till the fourth, sometimes, till the fifth lactation (Steine, 1975; Crepaldi et al., 1999; Spath and Thume, 2000).

The aim of this paper was to determine phenotype variability of reproductive traits of Sanska breed after first kidding and the influence of birth type (singles-twins) on kid body weight at birth.

Materials and methods

Reproductive parameters were monitored at the farm which keeps Sanska breed. Fertility parameters were monitored for young goats that were kidding for the first time. Parameters that were monitored were: young goat age at first insemination, gestation period length, goat fertility, body weight of kids at birth depending on birth type (singles-twins).

Data processing was done by usual statistical methods for this type of research. Influence of the birth type and gender of newborn kids on body weight at birth was processed using analysis of variance. Researched treatments comparison was conducted using the least significant difference test (LSD – test).

Results and discussion

Reproductive parameters and goat fertility were unevenly expressed with different goat breeds and considered as their important racial trait. Average age at first insemination was 252 days and at first kidding it was 402.6 days.

Gestation period is a biological trait and it is influenced by many factors such as breed, season, number and gender of kids and goat age. Gestation length results for the first pregnancy depending on type of birth (singles-twins) are shown in Table 1.

Table 1. *Average values and gestation length variability depending on birth type (days)*

Birth type	Parameters				
	n	$\bar{x}$	$S_{\bar{x}}$	SD	CV(%)
Singles	75	151.10	0.46	3.95	2.61
Twins	52	150.19	0.49	3.54	2.36
Average	127	150.64	0.30	3.43	2.28

From given data (Table 1) it is visible that for the first gestation period average gestation length was 150.64 days. Determined difference of 0.91 days in gestation length between the singles and twins in favor of singles was not statistically significant (P>0.05).

Urošević et al. (1999) determined that the age during first insemination in Sanska goat herd was 265.59 days and average gestation length was 149.84 days. Ćinkulov et al. (2009) quote that average age for German fawn during the first insemination was 242 days, while gestation length was 151.6 days, which is similar to our results.

Our research results are in compliance with the research of Duygu Ince (2010) where it was determined that gestation period length for Sanska goat was averagely 150.1 days and slightly higher value of 152.87 days of the same parameter and the same breed was determined by (Mekić et al., 2012).

Ćinkulov et al. (2009) in their research determined that gestation length for German fawn was averagely 152 days.

Determined gestation length of 150.64 days for Sanska breed in our research is in compliance with the research conducted by many authors and published in literature (Amoah, E.A. et al., 1996; Göncü et al., 2005; Moaeen UD-DIN-M. et al., 2008).

Fertility of Sanska breed after first kidding was averagely 164.70%.

Fertility of Sanska breed according to literary data is between 1.20 up to 2.14 kids, Taskin et al. (2003), Göncü et al. (2005), Moaeen UD-DIN-M. et al. (2008). Mekić et al. (2012), conducted a research and average fertility of Sanska breed in consequent kidding was 159.98%, and Ćinkulov et al. (2007) quote that fertility of German fawn was 1.96%, which is a higher value compared to our research.

Lower fertility for Sanska breed aged two years was determined by Duygu Ince (2010), where fertility value was 1.13%, and for goats older than five years it was 1.47%. Therefore, the average fertility of 164.70% obtained in our research for Sanska breed after the first kidding can be considered satisfying and in accordance with literature data, while Rako (1981) points out that fertility high milk yield goat breeds is 1.8 kids.

Kid body weight at birth

Determined body weights of kids at birth were analyzed from the birth type aspect (singles-twins). It was concluded (Table 2) that difference between the body weight of singles and twins at birth was 0.91kg in favor of singles while twins were approximately 74.29% of body weight of singles. Determined difference was statistically very significant (P<0.01).

Table 2. *Average values and variability of body weight of one day old kids (kg)*

Birth type	Gender	Parameters				
		n	$\bar{x}$	$S_{\bar{x}}$	SD	CV(%)
Singles	♂	35	3.57	0.16	0.98	27.45
	♀	40	3.51	0.30	0.83	23.65
	♂+♀	75	3.54	0.11	0.92	25.99
Twins	♀	58	2.66	0.12	0.89	33.46
	♀	46	2.60	0.12	0.81	31.15
	♂+♀	104	2.63	0.08	0.81	30.80

Body weight of kids at birth is very variable and it mainly depends on the breed. On average it represents 1/15 of grown goat weight, Morand-Fehr (1981). Majid et al. (1993) quote that body weights of kids at birth for five goat breeds in southern part of the USA (Alpine 3.8 kg; La Mancha 3.3 kg; Anglo Nubian 3.3 kg; Sanska 3.8 kg and Toggenburg 3.5 kg). Within the breed, kid body weight at birth depends on litter size, gender, kidding order, constitution and age of the mother, gestation length, diet, kidding season, health condition of the goat, etc. (Laes-Fettback and Peters, 1995). Jančić and Antunac (1986) have determined significantly higher body weight for Alpine breed singles compared to twins (3.95:2.92 kg).

In the research by Duygu Ince (2010), Sanska breed kids body weight was 3.06 kg; Göncü et al. (2005) determined for Turkish Sanska breed that kid body weight at birth ranges from 3.0 to 3.2 kg; Mioč (1998) has determined that value of Sanska goat kids body weight at birth was 3.26 kg while for Alpine it was 3.55 kg; Ćinkulov et.al. (2009) determined body weight of 3.38 kg for German fawn.

Amoah et al. (1996) quotes that average body weight of kids at birth for milking breeds of goat is within interval of 3.24±0.643 kg and that body weight drops down significantly if the number of kids increases in the litter.

When it comes to the gender of newborn kids within the birth type, there were differences and they were in favor of male kids but they were not statistically significant ($P>0.05$). Male kids have bigger body weight than female by 200 to 500 grams (Mavrogenis et al., 1984). Otuma and Osakwe (2008) point out that body weight of kids at birth is under very significant influence of the season, birth type and gender, therefore determined values for body weight in our research is in compliance and within expected values for Sanska goat.

Conclusion

Based on the obtained results about reproductive parameters for Sanska breed after the first kidding following conclusions can be made:

1. Average age at first insemination was 252 days.
2. Average gestation period length was 150.64 days and determined difference of 0.91 day between singles and twins was not statistically significant ($P>0.05$).
3. Goat fertility was averagely 164.70%.
4. Average body weight of singles was 3.54 kg, and twins 2.63 kg, twins were approximately 74.29% of body weight of singles. Difference in favor of singles was statistically very significant ($P<0.01$).

Based on presented reproductive parameters of Sanska breed after the first insemination it can be concluded that satisfying results were achieved for fertility and that body weight of

kids at birth can be even higher if the breeding conditions were to be improved, especially diet, during the entire productive cycle, as that is the only way to express genetic predispositions which Sanska goat has.

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References

1. Abdel Aziz M 2010. Present status of the world goat populations and their produtivity. Lohman information 45(2), 42-45.
2. Amoah EA, Gelaye S, Guthrie P, Rexroad CE IE 1996. Breeding season and aspects of reproduction of female goats. J. Animal Sci. 74, 723-728.
3. Ciappesoni G, Přibyl J, Milerski M, Mareš V 2004. Factors affecting goat milk yield and its composition. Czech Journal of Animal Science 49(11), 465-473.
4. Crepaldi P, Corti M, Cicogna M 1999. Factors affecting milk production and prolifacy of Alpine goats in Lombardy (Italy). Small Ruminant Research, 32(1), pp. 83-88.
5. Ćinkulov M, Nebesni A, Krajinović M, Pihler I, Žujović M 2009. Reproductive Traits of German Fawn Goats in Vojvodina. Biotechnology in Animal Husbandry 25 (1-2), 119-124.
6. Ćinkulov M, Trifunović S, Krajinović M, Popović-Vranješ A, Pihler I, Porcu K 2007. Osobine mlečnosti nemačke šarene koze u prve tri laktacije. Savremena poljoprivreda, 56, 1, 32-36.
7. Dimassi O, Neidhart S, Carle R, Mertz L, Migliore G, Mane – Bielfeldt A, Vale Zarate 2005. Cheese production potential of milk of Dahlem Cashmere goats from rhedogical point of view. Smal. Rum. Res. 57, 31-36.
8. Duygu Ince 2010. Reproduction Performance of Saanen Goats Raised Under Extensive Gonditions. African Journal of Biotechnology 9(48), 8253-8256.
9. Fekadu B, Soryal K, Zeng S, Van Hekken D, Bah B, Villaquiran M 2005. Changes in goat milk composition during, Lactation and their effect on yield and guality of har and semi-hard cheeses. Small Ruminant Research 59 (1), 55-63.
10. Göncü C, Yurtman IY, Savas T 2005. Besleme düzeyinin disi cebiclerde büyüme ve üreme özellikleri üzerine etkilerri. Süt Kecicliği Ulusal Kongresi, Sayfa, pp. 95-101, Izmir.
11. Haenlein GFW 1988. The value of goat and sheep to sustain mountain farmers. Int. J. Anim. Sci. 13, 187-194.
12. Jančić S, Antunac N 1986. Neka osnovna saznanja o importiranim alpina kozama. Poljoprivredna znansvena smotra 7, 371-381.
13. Jean-Claude Le Jaouen 2005. Proizvodnja kozjeg mleka i sira u Francuskoj. Simpozijum proizvodnja i prerada kozjeg mleka zbornik radova, str. 43-47. Poljoprivredni fakultet, Beograd-Zemun.
14. Leas-Fattback C, Peters KJ 1955. A comparative Study of performance of Egyptian goat breeds II. Growth performance and productivity. Arhiv für Tierzucht 38 (5), 363-575.
15. Majid AM, Cartwright TC, Yazman JA, Fitz-Hugh HA, J.R. 1993. Performance of five breeds of dairy and maturing pattern. World Reviem of Animal Production 28 (2), 15-23.

16. Mavrogenis AP, Constantinou A and Louca A. 1984. Environmental and genetic cause variation in preoduction traits of damastus goats 1. Pre-weaning and post-weaning gromth. Anim. Prod. 38, 91-97.

17. Mekić C, Petrović PM, Perišić P, Novaković Zorica 2012. Age Influence on Reproductive Indicators of Sanska Goat Breed. V International Symposium of Livestock Production. Hotel „Kontinental“, 5-7. IX 2012 Skopje, Macedonia. Book of abstracts, (43) ISBN 978-9989-9688-8-4, COBISS. MC-ID 92030730.

18. Mekić C, Trifunović G, Hristov S, Novaković Zorica 2011. Mlečne rase koza, nutritovna i tehnološka vrednost kozjeg mleka. Ekonomika poljoprivrede, God. Vol. LVIII, CB, Si 1 pp. 340-349.

19. Mioč B 1998. Povezanost pasmine i intenziteta rasta s kemijskim sastavom jarećeg mesa. Poljoprivredno znanstvena smotra, vol. 63, br. 4, pp. 179-186.

20. Moaeen-Du-Din M, Yanf LG, Chen SL, Hawg ZR, Xiao JZ, Ven QY, Dai M 2008. Reproductive performance of Matou goat Under sub-tropical monsoonal climate of Central China. Trop. Anim. Helth. Prod. 40, 17-23.

21. Morand-Fehr P 1981. Growth. In C. Coll (Editior), Goat Production. Academic Press London, pp. 253-283.

22. Ogola TDO, Kosgey IS 2012. Breeding and development of dairy goats: Eastern Africa Experience, Livestock Research for Rural Development 24(1).

23. Otuma OM, Osakowe II 2008. Estimation of genetic parameters of growth traits in Nigeria Sahelian goats, Res. J. Anim. Sci. 2 (3), 83-86.

24. Park YW 2001. Proteolysis and Lipolysis of Goat milk Cheese. J. Dairy Sci. 84 (E. Suppl), E. 84-E 92.

25. Rako A 1981. Razvoj intenzivne kozarske proizvodnje (farme mlečnih koza). Stočarstvo 35 (7-8), str. 209-218, Zagreb.

26. Ribeiro ELA, Ribeiro HJSS 2001. Uso nutricional e terapêutico do leite de cobra. Ci. Agrárias, Londrina, 22, 229-235.

27. Spath H, Thume O 2000. Ziegen halten, 5. Authage, Eugen Ulmer Verlag, Stuttgart.

28. Steine TA 1975. Faktorar med innverknad pa okonomisk viktige eig inskopar hos geit. Meldinger fra Norges Landbrukshegskole, vol. 54(2), pp. 1-29.

29. Sung YY, Wu TI, Wang PH 1999. Evaluation of milk guality of Alpinc, Nubian, Saanen and Toggenburg breeds in Taiwan. Small Ruminant Research 33(1), pp.17-23.

30. Taskin T, Demirören E, Kaymakci M 2003. Saanen ve Bornova Kecilerinde oğlak veriminin üretkenliği ve etkin liği Ege Üniversiteisi Ziraat Fak. Dergisi, 40(2), 33-40.

31. Urošević M, Skalicki Z, Šakić V 1999. Fenotipska varijabilnost reproduktivnih osobina koza rase francuska alpina. Veterinarija, God. Vol. 48, sv. 1-2, Str. 111-117, Sarajevo.

Original paper

EFFECT OF APPLIED PMSG DOSE ON REPRODUCTIVE PARAMETERS FOR IMPROVED SJENICA SHEEP IN ANESTROUS SEASON

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Abstract

This paper shows the results of the effects of higher dose 700 i.u. of natural gonad tropic hormone PMSG application in comparison with usual 500 i.u dose which is used at the farms during the induction and synchronization of estrus in anestrous season in Sjenica improved sheep. This experiment included 90 grown sheep divided into two groups (control group 30 sheep and experimental group 60 sheep). Both groups received polyurethane sponges impregnated with 20 mg of Fluor Geston Acetate (FGA). After 14 days both group sponges were removed. First group received 500 i.u of PMSG, usual dose, and second (experimental group) received 700 i.u of PMSG. Insemination was conducted 48-72 hours after gonad tropic hormone application. Number of sheep that lambed compared to the number of sheep that were treated was 93.33% (control) and 88.33% experimental group. Average fertility of control group sheep was 150.00% while experimental sheep had average fertility of 209.43%, which is more than 59.43% higher compared to control group. Higher dose of PMSG led to higher number of triplets by 7.34%, quadruplets by 14.41% and quintuplets by 4.50% in the experimental group. Higher dose by 200 i.u of PMSG had a positive effect and increased fertility by 59.43%, which negates zero hypothesis that there is no difference between researched treatments and it proved hypothesis that higher dose of PMSG has a positive effect on Sjenica improved sheep during anestrous season.

Key words: *Fluor Geston Acetate (FGA), PMSG dose, sheep, sheep fertility*

Introduction

Main goals of modern animal husbandry production are how to increase animal fertility, extend their usage period and get and grow highest possible number of high quality offspring. Sheep are seasonal poly estrous animals. A seasonal sexual activity phenomenon is influenced by different length of sunlight and dark cycle in specific seasons (Spring, Summer, Autumn, Winter). A mating season for sheep starts when days become short, which in our northern hemisphere happens in the period from second half of August till the first half of January (Stančić, 2008).

Complex neural-endocrine apparatus is under a direct influence of differences in photoperiods during the year, so it is being manifested as mating season (estrous season) and sexual inactivity season (anestrous) (Nenadić et al., 1993).

In anestrous season sheep are not sexually active, they do not have estrus cycles and ovulation, and they do not show estrus signs. Today intensive sheep production is based on genetic potential and life cycle of the animal, mainly on increasing the biologic base of reproductive cycle (Mekić et al., 1997, 2012, 2013). Methods that are used with goal to increase the reproductive efficiency of sheep are mainly oriented towards shortening the seasonal anestrous time, increasing the number of born lambs per sheep and introduction of young sheep in reproduction.

Evocation and synchronization of estrus in sheep by applying hormonal substances has important role among the methods for sheep reproduction increase, therefore it has important role for total sheep production increase. For example, if the sheep is being treated with intra vaginal sponges during 12-14 days of anestrous season, and they receive injection of PMSG after sponge removal, evocated ovulation is followed by visible estrus signs (Stančić, 2008).

Progesterone hormones are in wide usage for small ruminants with the goal to induce estrous synchronization (Zeleke et al., 2005; Dogan and Nur, 2006). In anestrous season estrous induction and synchronization effect in sheep by using Progesterone and PMSG were researched by (Mekić and Stojković, 2002; Dogan and Nur, 2006; Zonturlu et al., 2011; Moradi et al., 2012). During the productive cycle sheep breeders have to know if their sheep are in proper condition or if they are too skinny or too fat. Body weight in each phase of productive cycle represents an excellent parameter. Sheep body weight is not constant throughout the year. It changes with the change of production phase (Mekić et al., 2007).

Polyovulation is expressed more often in heavy and well fed sheep than in weaker and inadequately fed sheep that also have high prenatal mortality rate. In the research by (Allison, 1978) influence of body weight on induction, ovulation and number of follicles was researched. It has been determined that heavier sheep for entire estrous cycle time had higher number of follicles compared to lower weight sheep. Robinson, 1983 and Chursh, 1986, quote that there is a specific correlation between body weight (condition) and ovulation, success in sheep insemination. Body condition of the sheep is connected with specific body weight during the mating season which makes the base for number of egg cells that will be released for fertilization during the ovulation. For estrus evocation mostly used method is an application of intra vaginal sponges that are applied on 12^{th} or 14^{th} day into the sheep vagina (Amer and Hazzaa, 2009).

The aim of this paper was to give contribution to enlighten the questions regarding the influence of different dose of gonad tropic hormone application influence on fertility of Sjenica sheep in anestrous season.

Materials and methods

Research of different dose of natural gonad tropic hormone influence on sheep fertility in anestrous season was conducted at a private sheep farm in Valjevo rural area. Researched sheep was Sjenica improved breed. Induction and synchronization of estrus was conducted in anestrous season by using polyurethane sponges impregnated with 20mg of Fluor Geston Acetate (FGA), and they were kept inside sheep vagina for 14 days.

Experiment included total number of 90 sheep. Two groups of sheep were formed: control group (30 sheep) which after removal of polyurethane sponge received the injection of 500 i.u of PMSG (Foligon) which is a usual application dosage. Experimental group (60sheep) received dose of 700 i.u of PMSG.

After 48-72 hours from sponge removal and PMSG application all sheep were individually inseminated.

Main data processing was done using usual mathematical and statistical methods for this type of research.

Results and discussion

Reproductive parameters: Reproduction represents most important and limiting factor for increasing the production and profitability of sheep herding. In wide practice by controlling estrus with hormonal treatments fertility can be significantly increased and lambing can be precisely synchronized.

The results of the effects of different doses of PMSG hormone on sheep reproductive parameters are shown in Table 1. From the data shown in Table 1 it can be seen that the percent of sheep that were inseminated compared to the sheep that were treated was equal and it was 96.67%. The percent of sheep that lambed compared to the number of sheep that were inseminated was for control group 96.5% and for experimental 91.38%, meaning that sheep in experimental group lambed 5.17% more.

Table 1. *Sheep reproductive parameters depending on applied PMSG dose*

N°	Parameters			PMSG dose	
				Control 500 i.u.	Experimental 700 i.u.
1.	Number of treated sheep			30	60
2.	Number of inseminated sheep compared to number of treated			29	58
	%			96.67	96.67
3.	Number of sheep that lambed compared to number of inseminated			28	53
	%			96.55	91.38
4.	Percent of sheep that lambed compared to the number of treated sheep			93.33	88.33
5.	Total number of lambs			42	111
	Type of birth	Singles	♂	9	8
			♀	7	6
		Twins	♂	11	25
			♀	9	27
		Triplets	♂	2	17
			♀	4	7
		Quadruplets	♂	-	9
			♀	-	7
		Quintuplets	♂	-	3
			♀	-	2
6.	Sheep fertility, %			150.00	209.43

For experimental sheep group higher fertility by 59.43% was determined. Higher fertility for experimental group can be explained by the fact that experimental group had more triplets by 7.34%, quadruplets by 14.41% and quintuplets by 4.50% than control group.

Based on given results we can conclude that higher dose of PMSG by 200i.u per sheep had positive effect on increase of the number of ovulated egg cells on ovaries, which resulted with birth of higher number of lambs per sheep in the experimental group.

Research by Mutavelić et al. (1989) confirms that higher dose of PMSG has positive effect on increasing the sheep fertility. In his experiment he treated sheep with 750 i.u of PMSG and compared it with control group that received 500i.u of PMSG. Sheep that were treated with higher dose of PMSG had higher percent of lambing and higher fertility than control group. Mekić et al. (2012) determined higher fertility for Ile de France breed by 23.17% for sheep that received 750 i.u. of PMSG compared to group that received 500 i.u. In earlier research by Mekić et al (2009) Sjenica improved sheep were treated by 1000 i.u of PMSG and then fertility rate of 192.00% was achieved.

Based on above results we can conclude that increased dose of 700 i.u of PMSG compared to usual dose of 500 i.u of PMSG had positive effect on fertility of treated animals by 59.43% compared to experimental group. Higher fertility of experimental group was explained by higher number of lambs per sheep (triplets, quadruplets and quintuplets) compared to control group.

Besides the above mentioned parameters, fertility rate depends largely from the number of factors including heritage, breed, season, age, environment, diet, health condition, female hormonal status, sperm quality (Webb et al., 1994; Lewis et al., 1996; Beck et al., 1996; Husein et al., 1996, 1998; Yavuzer, 2005).

Conclusion

Based on conducted research of the effect of the application of induction and estrus synchronization for Sjenica improved sheep using sponges impregnated with 20mg of Fluor Geston Acetate (FGA) and different doses of gonad tropic hormone PMSG (500 i.u for control group and 700 i.u for experimental group) following conclusions can be made:

1. Number of sheep that lambed compared to the sheep that was treated for control group was 96.55% and for experimental group 91.38%.
2. Average fertility for sheep of the control group was 150.00% while for experimental group it was 209.43%, meaning that experimental group had fertility rate higher by 59.43%. Higher fertility was expressed through higher number of triplets, quadruplets and quintuplets than in control group.

This research clearly shows that application of higher dose of gonad tropic hormone by 200 i.u led to increased number of lambs at birth and higher fertility what has a direct influence on higher production of mutton per sheep thus increasing the economy and profitability of sheep production.

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References

1. Allison J 1978. Effect of nutritionally induced leiweight diferences on ovulation rates and the population of ovarian folicles in ewes. ABA, 46 (2).
2. Amer HA, Hazzaa AM 2009. The effect of different progesterone protocols on the reproductive efficiency of ewes during the non-breeding season. Veterinarski arhiv 79(1), 19-30.
3. Church DC 1986. Livestock feeds and feeding. Second ed. O.B. books inc. Usa.
4. Dogan I, Nur Z 2006. Different estrous induction methods during the non-breeding season in Kivircik ewes. Vet. Med-Czech 51, 133-138.
5. Ilker S, Gunes S, Murat Y, Funda K, Ceylan A 2010. The Effects of Body Weight, Body Condition Score, Age, Lactation, Serum Trygliceride, Cholesterol and Paraoxanase Levels on Pregnancu Rate of Saanen Goats in Breeding Season. Jurnal of Anilan and Veterinary Advances 9(13), 1680-5593.
6. Karaca F, Ataman MB, Coyan K 2009. Synchronization of estrus with chort-and long-term progestagen tretments and use of GnRH prior to short-term progestagen treatment in ewes. Smoll Rum. Res. 81, 185-188.
7. Mekić C, Grubić G, Krajinović M, Petrović PM, Stojković M 1997. Mogućnost primene novih biotehnoloških metoda u reprodukciji ovaca. Zbornik radova sa međunarodnog simpozijuma „Naučna dostignuća u stočarstvu '97", Subotica, 105-111.
8. Mekić C, Stojković M 2002. Povećanje ekonomičnosti ovčarske proizvodnje primenom novih biotehnoloških metoda u reprodukciji. Savremena poljoprivreda 51(3-4), 147-149.
9. Mekić C, Latinović D, Grubić G 2007. Odgajivanje, reprodukcija, selekcija i ishrana ovaca (monografija). Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd.
10. Mekić C, Vujić MR, Novaković Z, Vujić PR 2012. Sexual Activity and Sheep Reproductive Traits Stimulated With Differnt Dosage of Natural Gonadotropic Hormone in Anestric Period. Faculty of agriculture, University of Belgrade, Serbia. The First International Symposium On Animal Science, Book I, 111-115.
11. Moradi N, Sadeghi S, Ziaei N 2012. Comparison reproductive Performance in Kermani ewes Treated with two synchronization methods and Subsequent eCG treatment out of the breeding season. International Journal of Biological & Medical Research 3(2), 1485-1489.
12. Mutavelić A, Podžo M, Hajdarević F, Ferizbegović J, Šahmanović S, Divanović A 1989. Indukcija estrusa ovaca brdsko-planinskih područja izvan spolne sezone chrono-gest postupkom. XIV savetovanje Nove i savremene metode u razmnožavanju ovaca i koza. Ohrid, Makedonija, 13-15. septembar, 107-112.
13. Robinson JJ 1983. Nutrient reguirement of the breeding ewe. In Recent advances in animal nutrituon. Butterworths, London.
14. Stančić B 2008. Reprodukcija domaćih životinja. 3. izd. Novi Sad: Univerzitet u Novom Sadu, Poljoprivredni fakultet.
15. Zeleke M, Greyling JPC, Schwalbach LMH, Muller T, Erasmus JA 2005. Effect of progestagen and PMSG on oestrous synchronization and fertility in Dorper ewes during the transition period. Small Ruminant Res 56, 47-53.
16. Zonturlu AK, Özyurtlu N, Kaçar C 2011. Effect of Different doses oses PMSG on Estrus Synchronization and Fertility in Awassi Ewes Synchronized with Progesterone During the Transition Period. Kafkas Univ Vet Fak Derg 17 (1), 125-129.

Invited paper

FEATURES AND POSSIBILITIES FOR OPTIMIZING FARM MILK PRODUCTION IN SERBIA

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Abstract

Milk production in Serbia is organized on farms of different capacities that mutually vary in relation to agro-ecological, zootechnical and production conditions. The aim of this paper is to present a part of the most important results that have been obtained so far, in the implementation of the project "Optimization of technological procedures and zootehnical resources on farms with the goal to upgrade sustainability of milk production". The results that are presented relate to the most important features of farm cow milk production in Serbia, as well as the possibility for optimization of the most important technological procedures and zootechnical resources on which milk production is based. The structure of dairy farms in Serbia, in terms of size and number of animals, is not satisfactory, because the predominated types are small and medium scale farms. However, there are numerous possibilities for optimization of farm and zootechnical conditions and resources in order to improve the sustainability of milk production. These possibilities can be divided into optimizing selection and breeding methods, optimizing nutrition manners and physical form of forage and complete meals in the nutrition of dairy cows, optimizing housing conditions, welfare and health care in order to improve the sustainability of milk production, as well as optimizing milking and handling the milk after milking.

Key words: *animal housing, animal welfare, breeding method, dairy farm, nutrition*

Introduction

Milk production in Serbia has a long tradition and represents one of the most important branches of livestock production with a great impact on the economic development of a particular geographic area. Milk production in Serbia is organized on farms of different capacities that mutually vary in relation to agro-ecological, zootechnical and production conditions. According to the research performed by Bogdanović et al., 2012, and Bogdanović et al., 2014, the largest number of cattle farms in Serbia are small capacity farms and they keep up to 10 cows, while the number of medium, and especially large farms, is significantly lower. Despite the available natural resources, farm fragmentation leads to large heterogeneity in their exploitation, and therefore in achieved production results, which significantly affects the unevenness in the quantity and quality of produced and supplied raw milk.

Researches of very complex farming systems for milk production require significant engagement of different resources, so researches of this type, as a rule, are very complex in terms of research activities that must be implemented, they are long lasting and rather expensive. This imposes the requirement for them to be financed from public funds i.e. from the budget of the Republic of Serbia, through the relevant Ministry of Education, Science and Technological Development.

The aim of this paper is to present a part of the most important results that have been obtained so far, in the implementation of the project "Optimization of technological procedures and zootehnical resources on farms with the goal to upgrade sustainability of milk production" which is funded by Ministry of education, science and technological development of the Republic of Serbia. The results that are presented relate to the most important features of farm cow milk production in Serbia, as well as the possibility for optimization of the most important technological procedures and zootechnical resources on which milk production is based.

State of the art of dairy cattle production sector in Serbia

Cattle production has traditionally been one of the most important branches of livestock production. Since the first official Simmental (SM) cattle imports in the second half of the nineteenth century until today it has passed through various stages of development. However, despite several decades of development, the current state of livestock production is not completely satisfactory. Generally speaking, the production of milk and meat is carried on with a significantly reduced number of cattle and is, generally, intended to meet the needs of the domestic market, which has relatively low purchasing power. A negative trend has been visible for more than two decades in the numbers of cattle, and all forecasts indicate that this tendency will continue in the future. The situation is further aggravated by the existence of a large number of non-commercial operators, mostly elderly households, with poor equipment and motivation. In the presence of these, it is not possible to organize a modern and efficient production sector, or to make long-term production plans (Perišić et al., 2006).

Based on the surveys that were performed by Bogdanović et al. 2012 and 2014, it was found that about 59% of farms that are primarily registered for livestock production and which are commercially engaged in milk production have a size of up to 20 ha (of which about 11% of the farms have a size up to 5 ha, about 25% of the farms have a size from 5 to 10 ha, about 14% of the farms have a size from 10 to 15 ha, and about 9% of the farms have a size of 15 to 20 ha), about 20% of the farms have a size of 20-50 ha, about 6.5% of the farms have a size between 50 and 100 ha, about 2.5% of the farms have a size from 100 to 200 ha, while about 6% of the farms that engage in commercial milk production have a size of more than 200 ha. Some 6% of surveyed farmers did not respond to this question.

The average size of farms in the first group with up to 20 ha is about 10.15 ha, the average size of the farms in the group between 20 to 50 ha is about 29.8 ha, farms in the group from 50 to 100 ha have an average size of about 66.4 ha, while in the group of farms with a size from 100 to 200 ha the average size is about 131 ha.

The most common cattle breeds are SM and HF. The SM breed exists mainly in central Serbia, while HF is more prevalent in Vojvodina, i.e. in north region of Serbia. However, due to its greater robustness the SM breed is gradually increasing in Vojvodina.

The usual way of managing cows in milk production is in free stalls, rarely in tied-up systems. Stables with the free system are open or semi-open with appropriate outlets, while keeping the cows in pasture is seldom practiced.

The average number of cows varies considerably from farm to farm. About 55% of farms have up to 15 cows and heifers (of which about 26% of farms have up to 5 cows, about 19% of farms have 5 to 10 cows and 10% of farms have 10 to 15 cows), about 15% of farms have 15 to 30 cows, about 5% of farms have 30 to 100 cows, about 5% of farms have 100 to 200 cows, while about 3% of the farms have more than 200 cows in the production. A total of 17% of surveyed farmers did not answer this question.

Even though the results of this study cannot be generalized to all the farms that continuously or occasionally supply milk to the dairies, of which there are, according to various estimates, around 149,000, they indicate that there are still significant potentials for optimization and improvement of milk production both in terms of farmers, and in terms of optimum utilization of available farm resources. This certainly includes optimization of breeding and selection programs, optimization of cow nutrition in lactation period, as well as optimization of the conditions of keeping and care of animals.

Possibilities for optimization of most important technological procedures and zootechnical resources in milk production

Cow milk production in Serbia is mainly organized in semi-intensive and intensive production systems, while extensive systems are less common and mainly associated with undeveloped or mountainous areas. Common to all farmers who are engaged in specialized livestock production, compared to the average farmers engaged in mixed agricultural production, is a higher level of technological knowledge, greater openness to technological production improvements, larger farm areas and larger number of animals that are kept on the farm.

Numerous analyzes of the situation and the level of milk production on farms with dairy cows confirm that there is still a need for continuous organization and implementation of planned activities to improve milk production, primarily by improving the breeding composition of cattle. All estimates indicate that the number of cattle, especially cows, will decrease also in the period to come and the only answer to that is continuous improvement of the genetic potential of cattle intended for milk production. The main reason for this assumption is the constant closing down of a large number of small, mainly elderly households, as well as the reorientation of small farms and households to some other types of agricultural production. Intensive and sustainable milk production require, primarily, improving the genetic potential of milk production cattle. In accordance with the characteristics of the area (climate conditions, forage quality, breeders habits, altitude) the method that imposes for improving both domestic Simmental, and Holstein, is growing in pure breed with performing strict selection and application of artificial insemination with bull semen that is highly rated for their milk properties (Perišić et al, 2011a and 2011b). The goal of the breeding should be improving milk production traits, without compromising fattening traits, while retaining the resistance, regular fertility, good health and longevity. This type of breeding should contribute to the improvement of milk performance traits, from the average level which is currently, with the majority of milk producers, in the interval from 3,000 to 4,000 kg, to the average production level of the entire population of 5,000 kg and more. At the same time, feeding conditions, housing and care have to be improved in order for the improved cattle to fully express their genetic potential. It should be mentioned that the Simmental breed kept in the mountainous area of

some European countries (e.g., Austria, Germany) has an average milk production of 6,500-7,000 kg with over 4% fat, and quite often there are herds with an average of more than 8,000 kg of milk per cow. It is from these countries that bull semen should be imported in order to improve the genetic potentials for milk yield of our Simmental herds (Perišić et al, 2011a and 2011b).

Apart from this, one should have in mind that the size of the farm, together with applied farm management, can have a significant impact on the expression of, primarily, production traits of cows. In general, the tendency is that farms that raise more livestock achieve not only higher average milk yield, but have less manifested problems in the reproduction of animals. The results of the research by Kučević et al. 2011, indicate, however, that this trend is maintained only up to a certain farm size, i.e. number of livestock in the herd, after which there is a stagnation, and even decrease in production. In this regard, it is extremely important that every farmer independently estimates the optimal size of his herd in relation to the availability of necessary resources for milk production.

During the implementation of any program for the improvement of milk yield one should pay attention that the quality of milk and dairy products directly depends on its protein composition. The variability of kappa-casein (κ-CN) , as one of the four casein proteins in milk, is of particular importance in the selection of cattle. The research of Đedović et al., 2012 (unpublished results), conducted on the genotypes of Simmental breed cows, the crossbreed obtained by crossbreeding the Simmental with Red Holsteins and cows of the indigenous breed of Buša, showed that there are differences in the frequency of κ-CN. Genotype frequencies κ-CN for Simmental breed were: 42.8%; 47.6% and 9.6% for AA, AB and BB genotype, for crossbreeds: 75.0%; 25.0% and 0.0% and for Buša individuals: 41.7%; 50.0% and 8.3%, respectively. The frequencies of alleles A and B, for observed breeds, estimated on the basis of genotypic frequencies, were 0.667 and 0.333 for Simmental breed, 0.875 and 0.125 for crossbreeds and 0.667 and 0.333 for the indigenous cattle breed Buša, respectively. The research showed that the genotype κ-CN, statistically, significantly affects milk yield and highly significantly milk fat yield, but it does not affect the milk fat content of tested animals.

The research established the dominance of allelic A variant with all covered breeds, which particularly applies to crossbreeds obtained by crossbreeding Simmental and Red Holstein. The frequency of desirable B allelic variant κ-CN was lower and the same with cows of Simmental breed and Buša cows. Through identification of populations and cattle breeds with an increased proportion of B allelic variants κ-CN and their inclusion in selection programs, the opportunity to increase the frequency of favourable alleles would be gained, and thus the technological quality of cattle milk would be improved, which would increase the economic value of livestock production. Conducted research provides a starting point for further determination of breeders for keeping breeds and producing milk of a certain quality and quantity according to the needs of the dairy industry.

It is well known that the costs of cattle feeding in milk production can amount to 65-75% of total production costs. Therefore, all farmers involved in this survey pay special attention to proper feeding of all categories of cattle. All the farmers involved in this analysis adapt feeding to the physiological status of the animal, paying particular attention to milk yield and to reproductive status. About 92% of farmers prepare their own livestock feed, while 55% of farmers buy ready-made concentrates from animal feed manufacturers. Farmers that prepare their own feed, mainly produce the entire amount of forage and one part of the concentrated feed. In terms of forage, farmers prepare hay from clover or alfalfa, corn silage and, increasingly, haylage. When it comes to concentrated feed, farmers usually produce feeds such as corn, soybean or sunflower, while they only purchase

additives or necessary premixes. About 78% of surveyed farmers buy the vitamin and mineral supplements required in cattle diets.

What is, in the researches by various authors, more frequently expressed as a possibility for additional optimization of nutrition programs on dairy farms is to solve the problem of forage crumbling and primarily whole-plant corn silage and alfalfa haylage, as well as total mixed ration (TMR), for nutrition of cows in lactation. Based on the research by Stojanović et al. 2011a, 2011b and 2011c, and with the use of "Penn State Particle Separator" system of four sieves, various deviations were defined from the optimum predicted values of distribution of certain fractions of particles for both forage and TMR. The more uneven the crumbling of forage was, the more the fragmentation of TMR varied. The authors of these researches point out that the results indicate the importance of optimization of the physical form of forage during its preparation, in order to achieve the desired physical effectiveness of the entire meal. The optimum physical efficiency of forage can be achieved using various methods and procedures both during the grinding of the mass and during preparation of whole-plant corn silage, and alfalfa haylage.

High temperatures (as a seasonal factor) in the form of heat stress can significantly reduce the daily milk production, and exceptionally stop lactation, which is especially the case with poorer dairy cows. The selection of high yielding cattle breeds with the aim of improving the genetic predisposition for higher milk production and the quantity of consumed food, has led to a decrease in productive and reproductive potentials in periods of increased outside temperature, because not enough attention has been given to the thermoregulatory abilities of animals. In the conditions of heat stress, there is first a reduction in milk production, which has particularly become important in recent years. High yielding dairy cows are most sensitive to heat in early lactation and milk production reduces significantly when body temperature is higher than 39 degrees Celsius. Dairy cows in early lactation are unlikely to cope with heat stress, which is why it has the greatest impact on milk production in the first 60 days of lactation (Joksimović-Todorović et al., 2011).

One of the major problems of modern intensive farm milk production is a growing presence of mycotoxins, as a result of significant climate changes, which has been validated in Serbia in the past few years. Due to the harmful effects on human and animal health, mycotoxins are today called the "silent killers", "invisible thieves" or "natural toxicants". The main mechanisms of toxicity are the inhibition of protein synthesis, DNA, RNA, lipid peroxidation, changes in the structure and function of the membrane and the initiation of programmed cell death. In addition to inadequate preparation and storage of feed, poor nutritional status of animals and housing conditions may, also, affect the increase in the sensitivity of animals to mycotoxins. In that sense, the use of different antioxidants can potentially be very important in the protection against mycotoxins. Selenium and vitamin E are the key ingredients that act as antioxidant protection and farmers should pay attention to this when preparing the meals for feeding the cattle (Joksimović-Todorović and Davidović, 2011).

Finally, it should be noted that for the success of any optimization program or improvement of production parameters on dairy farms it is essential that farmers are interested in this and ready to implement certain new solutions on their farms. In this regard, the oldest surveyed farmer was born in 1928, and the youngest in 1990. About 25% of surveyed farmers were born in the period from 1928 to 1952, while 50% of surveyed farmers were born in the period from 1952 to 1973. If we consider all the birth years, 50% of surveyed farmers were born until 1964 and 50% were born in the period from 1964 to 1990. It is the age of surveyed farmers, who are also the owners of their farms, that

provides some "space" for the acceptance of new technological solutions or optimization of existing technological processes. This is supported by the fact that 71% of all surveyed farmers have regular, and 23% occasional contact with agricultural advisory service. Also, over 86% of surveyed farmers intend to expand the existing farm production, mainly by increasing the number of animals, stricter selection of animals, as well as by improving the feeding conditions and manners, housing, care and milking. About 6.5% of farmers do not currently intend to expand their production. No less important is the fact that about 75% of surveyed farmers expect certain benefits from future EU membership, even though they are aware that they, also, can expect a lot more adjustments. On the other hand, about 19% of farmers do not expect any benefits from the potential Serbian EU membership.

Conclusion

Based on everything that has been mentioned, it can be concluded that the structure of dairy farms in Serbia, in terms of size and number of animals, is not satisfactory, because the predominated type are small and medium scale farms. As Serbia moves closer to EU, these farms will soon face even greater market pressure. On the other hand, family farms of larger capacity, but also many farms of medium capacity, can become competitive on the market only if they continuously improve and innovate their production. Commercial milk production of today is extremely demanding and based on continuous improvement of all available farm conditions, technological processes and zootechnical resources that are important for this type of production. The possibilities for optimization of farm and zootechnical conditions and resources in order to improve the sustainability of milk production can be divided into optimizing selection and breeding methods, optimizing nutrition manners and physical form of forage and complete meals in the nutrition of dairy cows, optimizing housing conditions, welfare and health care in order to improve the sustainability of milk production, as well as optimizing milking and handling the milk after milking. How many farm and zootechnical conditions and resources will be optimized or improved in order to increase the sustainability of milk production does not only depend on the obtained research results, but also on the system and the manner of their dissemination, and the willingness of farmers to innovate their production.

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References

1. Bogdanović V, Đedović R, Perišić P, Stanojević D, Zarić V and Petrović MD 2014. An assessment of efficiency and prospects for the cattle sectors in Serbia. In Cattle Husbandry in Eastern Europe and China (eds. A. Kuipres, A. Rozstalnyy and G. Keane), EAAP Publication no. 135, 201-211, Wageningen Academic Publisher, The Netherland.
2. Bogdanović V, Đedović R, Perišić P, Stanojević D, Petrović MD, Trivunović S, Kučević D and Petrović MM 2012. An assessment of dairy farm structure and characteristics of dairy production systems in Serbia. Biotechnology in Animal Husbandry 28, 4, 689-696.
3. Joksimović-Todorović M, Davidović V 2011. The importance of antioxidants in the protection against mycotoxicoses in farm animals. 4th International Scientific Meeting

"Mycology, Mycotoxicology and Mycose", Novi Sad, Republic of Serbia, 20-22th April 2011. Zbornik Matice srpske za prirodne nauke 120, 261-267.

4. Joksimović-Todorović M, Davidović V, Hristov S and Stanković B 2011. Effect of heat stress on milk production in dairy cows. Biotechnology in Animal Husbandry 27, 3, 1017-1023.
5. Kučević D, Trivunović S, Radinović M, Plavšić M, Skalicki Z and Perišić P 2011. The effect of the farm size on milk traits of cows. Biotechnology in Animal Husbandry 27, 3, 951-959.
6. Perišić P, Skalicki Z, Bogdanović V and Petrović MM 2006. Simentalska rasa i pravci njenog razvoja. Biotechnology in Animal Husbandry 22, Special Issue, 231-244.
7. Perišić, P, Skalicki Z and Bogdanović V 2011a. The state in the sector of milk production in European Union and in our country. 3rd International Congress "New Perspectives and Challenges of Sustainable LivestockProduction", 5-7th October 2011, Belgrade, Republic of Serbia, Biotechnology in Animal Husbandry, 27, 3, Book 1, 315-327.
8. Perišić P, Topisirović G, Pešić-Mikulec D and Puđa P 2011b. Analiza faktora proizvodnje mleka na odabranim gazdinstvima Zlatiborskog okruga. Poljoprivredna tehnika, God. XXXVI, 4, 11–20.
9. Stojanović B, Grubić G, Đorđević N and Božičković A 2011a. Physically effectiveness of forages in rations for dairy cows and methods for determination. Proceedings of International Scientific Symposium of Agriculture "Agrosym Jahorina 2011". Faculty of Agriculture East Sarajevo (B&H), Faculty of Agriculture Univeristy of Belgrade (Serbia), Balkan Environmental Association B.EN.A. (Greece), Academy of Engineering Science of Serbia (Serbia), Institute of Agricultural Economics (Serbia), 469-476.
10. Stojanović B, Grubić G, Đorđević N, Božičković A and Ivetić A 2011b. Effects of forages and total mixed rations particle size on physical effectiveness and chewing activity of lactating cows. Biotechnology in Animal Husbandry 27, 3, 935-942.
11. Stojanović B, Grubić G, Đorđević N, Božičković A and Ivetić A 2011c. Uticaj sadržaja fizički efektivnih vlakana u obroku za mlečne krave na aktivnost žvakanja. Zbornik naučnih radova Instituta PKB Agroekonomik, 17, 3-4, 37-48.

Original paper

SOURCES OF VARIATION FOR MILK TRAITS IN REGIONS OF VOJVODINA

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Abstract

The study aimed to investigate different sources of variation for milk traits in dairy cows, in first lactation from three breeding regions of Vojvodina (Srem, Banat and Backa). For research purposes a total of 2767 complete and 305 days records of milk yield (MY), fat yield (FY) and milk fat content (MFC) of Holstein-Friesian (HF, black and red) dairy cows was used. All cows were involved in the official milk recording program in 2013 in Vojvodina. Milk traits were analyzed using the mixed linear model in order to explain total variation with bull-sire as a random effect, region, year of birth and calving season as fixed effects and length of lactation as covariates. The average values of MY, MF and MFC in the first lactation of 305 days were 6053.4 kg of milk, 225.24 kg of milk fat and 3.74% milk fat content. The effects of the bull-sire, calving season, year of birth and breeding region on all investigated milk traits were highly significant ($p>0.01$) during 305 days, but year of birth for complete records had no significant effect on these parameters ($P>0.05$).

Key words: *dairy cows, milk traits, regions*

Introduction

It is important to evaluate various sources of variation in regard to phenotypic and genetic variability of milk in order to ascertain which effects are major for a dairy production and future decision for selection and breeding program. Production traits were greatly affected by para-genetic factors such as season of calving, parity, age of cow, age at first insemination, etc. (Petrović et al., 2006), as well as significant influence of genetic components, Pantelić et al. (2011), Trivunović (2006).

In the last years, according to the official statistics, the total number of dairy animals in Serbia (including the province of Vojvodina) was reduced but it was not significantly reflected in total milk production at the country level. Indeed, in the last 10 years, milk production was stable and amounted to about 1.5 to 1.6 million tons of raw milk per year. Significant increase of yield per cow was realized in herds involved in the official milk recording program (Kučević et al., 2011). Increase in row milk production was achieved due to genetic improvement and selection program by the Center for the breeding of domestic animals in Vojvodina. In relation to the other cattle population, HF breed is the most present in Vojvodina and makes around 78%. This breed is specialized for milk production and breeding goals are adjusted to achieve sufficient quantities of milk according to the quality standards requested by the European Union (Bogdanović et al., 2007). According to the Center's Annual Report in 2013, the average milk yield of

recorded and controlled dairy cows of the total population of HF breed in Vojvodina was around 6307 kg with 237 kg of milk fat and average milk fat content of 3.76%.

Objective of this study was to analyse different sources of variation for milk traits in HF dairy cows in first lactation from three regions of Vojvodina (Srem, Banat and Bačka).

Materials and methods

This research included 2767 HF first calvers involved in the official milk recording program in Vojvodina. We examined the variability of complete and 305 days records of MY, FY and MFC. Data were collected from three breeding regions in Vojvodina: Srem (1), Banat (2) and Bačka (3). In order to explain different sources of variation for milk traits a mixed model with a sire as a random effect, the region, year of birth and season as fixed effects and length of lactation (in days) as covariates were used. The effects of the various factors influencing milk traits were evaluated by fitting a mixed model of least squares with fixed and random effects (LS-Least Squares), procedure of *STATISTICA* ver.10 (StatSoft Company, 2013). Factors included in the model were based on their significance and impact on the studied traits, which represent systematic fixed part of the model. Systemic effects were combined in various ways. Determination of model for investigated traits ranged from 19% to 33%, which is in line with their biological characteristics. The following model was used for complete records:

$Y_{ijklm} = \mu + R_i + Y_j + S_k + B_l + b_l (x_1 - x_1) + eijklm$

where:

Y_{ijklm} - phenotypic value of observed traits

μ – general mean

R_i – fixed effect of breeding region (1-3)

Y_j – fixed effect of year of birth (1-5)

S_k – fixed effect of season of calving (1-3)

B_l– random bull-sire effect (1-72)

$b_l(X_1-X_1)$ – linear regression effect of length of lactation

e_{ijklm} – random error

For the records of 305 day the same model but without length of lactation was used.

Results and discussion

In the Table 1 results of the descriptive statistical analysis of the studied traits are presented.

Table 1. *The average values and variability of investigated traits*

Traits	$\bar{x}$	S $\bar{x}$	SD	min	max	CV %
MY, kg	7131	38.27	2013.4	3121	13491	28.2
MF, kg	276.72	1.53	80.85	115	682	29.2
MFC, %	3.88	0.006	0.34	2.10	7.67	8.9
MY 305, kg	6053.4	26.94	1417.6	2048	12557	23.4
MF 305, kg	225.24	0.99	52.43	69.00	709.0	23.2
MFC 305, %	3.74	0.008	0.43	2.06	7.74	11.5

Milk yield =MY; Milk fat =MF; Milk fat content =MFC; MY for 305 days =MY 305; MF for 305 days =MF 305; MFC for 305 days =MFC 305

Based on studied sample, average values of investigated parameters for complete lactation (on average 359 days) amounted to 7131 kg of MY, 276.72 of MF and 3.88 of MFC whereas during standard lactation of 305 days it was produced 6053.4 of MY, 225.24 of MF and 3.74 of MFC.

The lowest recorded milk yield during 305 days included in the study was 2048 kg and the highest was 12557 kg, resulting in high value of the variation width of even 10509 kg of milk. Values of the variation coefficient (CV) and standard deviation (SD) for milk yield were also very high and amounted to CV=23.4% and SD=1417.6. Contrary to the established values of CV and SD for MY, the value obtained for MFC was significantly lower CV=11.5% and SD=0.4. These lower values indicate that the variability of this trait is more under the influence of biological and breed characteristics of dairy cows than on rearing conditions present on the farm. Similar data on variation of milk traits were obtained by Kučević et al. (2011) and Radinović et al. (2011).

Using the mathematical-statistical model we identified genetic and environmental factors which influenced expressions of investigated traits. The obtained factors in the study are shown in tables 2 and 3.

Table 2. *The effect of genetic and environment factors on investigated milk traits*

Factors	DF	MY	MF	MFC
		F-value	F-value	F-value
Region	2	27.36**	14.97**	5.98**
Year of calving	4	1.35 ns	1.83 ns	0.90 ns
Season	2	8.04**	3.10*	3.73*
Sire	71	11.56**	9.42**	4.37**

DF-degree of freedom; F-values; **-P<0.01-statistically highly significant effect; ns-P>0.05-no significant effect

Table 3. *The effect of genetic and environment factors on investigated milk traits in 305 days*

Factors	DF	MY 305	MF 305	MFC 305
		F-value	F-value	F-value
Region	2	27.15**	14.04**	4.65**
Year of calving	4	2.13**	4.80**	3.76**
Season	2	8.01**	3.62*	6.54**
Sire	71	14.12**	7.45**	6.69**

DF-degree of freedom; F-values; **-P<0.01-statistically highly significant effect; ns-P>0.05-no significant effect

Data presented in tables 2 and 3 show highly significant effect (p>0.01) of the sire, calving season and breeding region on all investigated milk traits. Only age for complete records had no significant effect on these parameters (p>0.05). The research is in accordance with the results obtained by Perišić (1998), Petrović et al. (2006) and Sekerden (1997), which stated that calving season highly significantly affected milk yield, milk fat, and protein in standard lactation. Petrović et al. (2009) confirmed that quantitative milk traits of first calving cows under the influence of region had highly significant deviation from general average. If we observe production of milk in the region of Vojvodina it may be pointed out that dairy production is organized in very different rearing and farm management conditions, therefore statistically highly significant sources of variation in regard to breeding region was expected. Considering that the heritability values for milk

performance traits range from very low to medium, applied technology and management, which includes nutrition, housing, care, milking, etc., express strong effect on phenotypic variability of traits.

Conclusion

In the studied population of Holstein-Friesian dairy cows, involved in the official milk recording program in the three regions of Vojvodina, the variability of complete and 305 days records of milk traits as well as different sources of genetic and para-genetic variation was examined. The average values of MY, MF and MFC in the first lactation of 305 days were 6053.4 kg of milk, 225.24 kg of milk fat and average milk fat content amounted to 3.74%. Based on obtained results it was established that effects of the bull-sire, calving season, year of birth and breeding region were highly significant ($p>0.01$) on all investigated milk traits. Year of birth had no significant effect for complete records on these parameters ($p>0.05$). It can be concluded that investigated factors significantly affect milk traits therefore it is necessary to work on further optimization of the environmental factors, as well as on genetic improvement, primarily of bull-sires in the population of HF dairy cows.

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References

1. Bogdanović V, Đedović R, Perisić P, Petrović MM 2007. Dairy cattle breeding and production in small farms in Serbia. Book of Abstracts No 13, 58th Annual Meeting of the European Association for Animal Production, Dublin, Ireland, 26–29 July.
2. Kučević D, Trivunović S, Radinović M, Plavšić M, Skalicki Z and Perišić P 2011. The effect of the farm size on milk traits of cows, Biotechnology in Animal Husbandry, v.27, 3, 951-958.
3. Pantelić V, Plavšić M, Trivunović S, Aleksić S, Sretenović LJ, Ostojić-Andrić D and Nikšić D 2011. The evaluation of breeding value of simmental bulls for milk performance in Serbia, Biotechnology in Animal Husbandry 27 (2), 127-135.
4. Perišić P 1998. Reproduktivne i proizvodne osobine različitih genotipova krava simentalske rase. Magistarska teza, Poljoprivredni fakultet, Beograd–Zemun.
5. Petrović DM, Đoković R, Bogosavljević-Bošković S and Kurćubić V 2006. Uticaj paragenetskih faktora na proizvodne osobine standardnih laktacija kod krava simentalske rase. Savremena poljoprivreda vol. 55(1–2), 138–143.
6. Petrović MM, Sretenović LJ, Aleksić S, Pantelić V, Novaković Ž, Perišić P and Petrović MD 2009. Investigation of the heritability of phenotypes of fertility and milk performance of simmental cattle breed in Serbia. Biotechnology in Animal Husbandry 25 (5-6), 285-292.
7. Radinović M, Trivunović S, Kučević D, Đedović R and Bogdanović V (2011). Assesment of breeding values of bulls for somatic cell count in milk. Biotechnology in Animal Husbandry, 27 (3), 1059-1066.

8. Sekerden Ö 1997. Effects of Calving Season, Lactation Order and Stage on Milk Yield, Milk's Components and Yields Cattle. Book of Abstracts of the 48th Annual Meeting of the European Association for Animal Production, Vienna, Austrija, 25–28 August.
9. Trivunović S 2006. Genetski trend prinosa mleka i mlečne masti u progenom testu bikova za veštačko osemenjavanje. Doktorska disertacija. Poljoprivredni fakultet, Novi Sad.

Review paper

THE STATE AND DEVELOPMENT PROSPECTS OF GOAT PRODUCTION IN THE WORLD

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Abstract

Goats are the most popular animals in the world and goat meat and milk consumption are most widely distributed in the world. Goats are popular with small holders because of their efficient conversion of feed into edible and high-quality meat, milk and hide. Goats are also used as holistic tools for land vegetation management and fire fuel load control. With proper grazing management, goats can eliminate noxious weeds, restore native grasses and prevent fires through fuel load reduction. In the world the number of goats is increasing. However, when it comes to growing goats, the conditions prevailing today are fairly complex. Problems and prospects vary by region, which consequently have different cultural and economic implications. Both global and regional approaches to livestock farming are important from the economic aspect of developed and developing nations. Rapidly increasing goat populations in developing countries point to the goat assisting in solving some of the needs created by the rising human population. Goat farming in Europe also has a more positive outlook after nearly a century of a negative reputation. Dairy-goat farming is significant to the economies of the Mediterranean countries. Dairy goats produce about 15.2 million metric tons (MT) of milk, accounting for about 2% of the world total amount of milk produced by livestock species. The developing countries produce approximately 83% of the total amount. In Europe, goat breeding is strongly oriented towards milk production, with only 3% of the world goat population producing about 15% of the world's goat milk, which is mostly used for cheese production. Goat meat is widely consumed in the developing countries. According to FAOSTAT (2008), total meat inventory is about 280 million MT. Goat meat represents only 2% of this total. The total amount of goat meat produced in 2008 was 4.9 million MT. The developing countries produced approximately 97% of this amount, reflecting the great importance of goat meat to feed millions of people in these countries. The top ten countries producing goat meat are all from Asia and Africa. China is a world leader in producing goat meat, accounting for 38% of the world total goat meat produced. Goat meat production has been increasing from 2.65 million MT in 1990 to 4.93 million MT in 2008.

Key words: *goats, milk, meat, number of goats, production*

Introduction

Globally, the number of goats is constantly increasing. However, despite the fact that this branch of livestock production had the highest growth rate in last 20 years, global situation in regard to goat production in the world shows contradictory situation. On one hand, there are well organized private sectors in several countries which have clearly defined breeding and selection goals in goat production, and have constant progress in production of milk, meat and thread. On the other hand, most of the goat population is mainly used for production of milk, meat, leather or threads in developing countries but without any greater tendencies towards quantitative and qualitative improvement of production. So, milk, and primarily meat, meets the nutritional requirements of rural population in developing countries, especially in those regions with rapid growth of human population (Boiazoglu et al., 2005; Dubeuf et al., 2004; Devendra, 2007). This means that increase of number of goats is no indicator of positive development of productivity, but simply reflects the fact that many people living in rural regions of developing countries are trying to survive by keeping small ruminants, such as goats (Dubeuf and Boiazoglu, 2009; Žujović et al., 2011). Dairy-goat farming is significant to the economies of the Mediterranean countries. Previously, in developed countries, a limited number of high quality goats was raised as a complementary activity. Recently, the market for high-quality cheeses made from goat's milk has been developing and there has been growing interest in intensive cultivation of goats with high milk yield. An example of this is France, with about 1.2 million dairy goats and production of about 40.000 tones of cheese per year, while the production of meat and skin of goats and kids is of secondary importance in the total income of goat production.

Goats live either in small or large herds or in different areas and environments ranging from plains, desert areas, mountain and alpine areas. Table 1 shows the numerical strength of the goats in different parts of the world, as well as their representation in the percentage of the total number (FAOSTAT, 2010). The number of goats has increased by 18.91% in the analyzed period, reaching 867.9 million heads in 2009. Most of the livestock is in Asia (59.5%), followed by Africa (34%), the Americas (4.3%), Europe (1.8%) and Oceania (0.4%), which proves that the highest share is in developing countries where goat milk is a basic food especially for rural population (Table 1).

Table 1. *Number of goats in the world (FAOSTAT, 2010)*

Year	MU	Asia	Africa	America	Europe	Oceania	Total world
1999	Million heads	447.3	227.8	33.7	19.0	2.1	729.9
	%	61.3	31.2	4.6	2.6	0.3	100.0
2009	Million heads	516.7	294.8	37.1	15.9	3.4	867.9
	%	59.5	34.0	4.3	1.8	0.4	100.0

Source:FAOSTAT, 2010

Based on the data presented in Table 1, it is evident that the number of goats in the world is 867.9 million (FAOSTAT, 2010). However, there are huge variations in the distribution and number of goats among different parts of the world. The largest number of goats is grown in

Asia (China, India, Pakistan and Bangladesh have 59.5% of the total goat world population), followed by Africa, 34% of which represents 93.5% of the total number of goats in the world (Table 2).

Table 2. *Top 10 countries based on goat livestock (FAOSTAT, 2010.)*

Country	Number of goats (million heads)	% of the total number of goats in the world
India	154.0	16.7
China	150.7	16.4
Bangladesh	65.0	7.1
Pakistan	59.9	6.5
Nigeria	56.5	6.1
Sudan	43.4	4.7
Iran	25.7	2.8
Ethiopia	22.0	2.4
Indonesia	16.8	1.8
Mali	16.5	1.8
Total world	920.6	100.0

The number of goats in the world has been growing steadily since 1990 from about 1% to 4% annually. In the same period, the number of cattle has increased by 5%, while the number of sheep decreased by 10%. Goat is now considered "poor man's cow". Goats consume a smaller amount of food, take up little space, and according to their size, i.e. body mass, produce enough milk for the needs of an average family, and in relation to the keeping of cows, gain on an increasing popularity.

Milk production

Goat breeds according to milk production can be divided into three groups:

1. Breed of goats belonging to a highly productive improved breeds;
2. The second group of the breeds with an average production, with the potential for high production but whose actual production is decreased due to the conditions of nutrition, care and housing; and finally

3. The third breeds belong to the group with low production that are not regularly milked. Highly productive goat breeds are originating from the mountain range of the Alps and the Mediterranean area. Typical improved breed of goats are Saanen, Alpine and Toggenburg. Spanish Granada and Malaga which have sufficient milk production are also classified as a dairy breed, along with Nubian (Table 3). The examples of breeds with an average production is the Damascus from the Middle East, as well as the Indian breeds: Jamnapari, Beetal and Barbari. Most of the African, Asian and Latin American goat breeds fall into the third group of goats, where our local goat breeds and their crosses can also be introduced (Memiši, 2000; Memiši and Žujović, 2012).

Table 3. *Milk production of certain breeds of goats in the world*

Goat breeds	Milk production, kg	% milk fat	Authors
Saanen	720 – 1000	3.4	Fehse and Kunzi, (1998)
Toggenburg	700 – 900	3.3	Fehse and Kunzi, (1998)
Alpine	650 – 900	3.4	Gall (1982)
Appenzell	670	3.1	Fehse and Kunzi, (1998)
Chamois Coloured	758		Fehse and Kunzi, (1998)
German White Improved	1126	3.6	Gall (1982)
Poitou	700	3.0	Gall (1982)
Murcia Granada	500	4.0 – 4.5	Falagán and Mateos (1996)
Maltese	500 – 600		Gall (1982)
Israeli Saanen	650		Gall (1982)
Damascus	400 – 600	3.5	Constantinou (1985)
Jamnapari	540	3.5 – 4.5	Gall (1982)
La Mancha	800	4.0	Gall (1982)
Domestic breeds and genotypes of goats			
Domestic Balkan	180	3.7	Memiši (1998 and 2000)
Domestic Serbian White	400	4.0	Žujović (1988)
Domestic crossbreds	250 - 350	3.5 – 4.0	Memiši and Bauman (2002, 2003)

The use of goat milk as an excellent food source is undeniable. It has beneficial effects for health maintenance, physiological functions, nutrition of children and elderly people, and according to some authors, can be consumed without negative effects by people suffering cow milk allergy. Goat (*Capra hircus*) milk production is of significant importance to the economy and survival of large populations of many countries in the world: in developing countries (i.e. Asia, Africa, the Middle East and Mediterranean countries and South America) as well as in developed countries (i.e. Europe, North America and Oceania) (Billon, 2003; Albenzo et al., 2006).

Dairy goats produce about 15.2 million metric tons (MT) of milk, accounting for about 2% of the world total amount of milk produced by livestock species (FAOSTAT, 2008). The developing countries produce approximately 83% of the total amount. In Europe, goat breeding is strongly oriented towards milk production, with only 3% of the world goat population producing about 15% of the world's goat milk, which is mostly used for cheese production. Table 4 shows the amount of goat milk produced by the top ten countries in the world, along with the total number of dairy does and the average of milk produced per doe (FAOSTAT, 2008).

The largest amount of goat milk is produced in India, followed by Bangladesh and Sudan. China, India, Pakistan, Indonesia and Bangladesh together contribute 78% of the total small ruminant population in Asia (Talpur et al., 2009). In Pakistan, the Damani and Kamori breeds are popular, while in other countries popular breeds are Barbari, Beetal, Jamnapari, Malabar, Damascus, Barky and Black Bengal (Talpur et al., 2009).

Table 4. *The amount of goat milk produced in the top ten countries (FAOSTAT, 2008)*

Country	Goat milk production (million MT)	Number of dairy goats (million)	Milk produced per dairy doe (kg)
India	4.0	30.2	132.5
Bangladesh	2.2	27.1	80.0
Sudan	1.5	-	-
Pakistan	0.7	4.9	141.9
Spain	0.6	1.4	422.3
France	0.6	0.8	703.8
Greece	0.5	4.1	123.9
Iran	0.4	13.7	29.9
Somalia	0.4	6.6	59.7
China	0.3	1.4	194.8

Goat farming is of vital importance for the national economy in many countries in the Mediterranean and Middle East region, and is particularly well organized in France, Italy, Spain and Greece (Park et al., 2007). Among the European countries, Greece is the first in goat population (6,000,000 animals) and produces about 450,000 t of goat milk per year (Kondyli et al., 2012). France has nearly 1 million goats and occupies the first place in the European Community in terms of milk production. Turkey has the largest goat population in Europe (5,600,000 t in 2009) and is the fourth in total goat milk production (192,000 t in the year 2009) in the European and Mediterranean region, after France, Spain and Greece (FAOSTAT, 2009). In France, interest in dairy goats has led to the establishment of organized programs for selection, processing and commercialization of goat milk, which is produced mainly from Saanen and Alpine breeds. France leads the list in terms of the annual milk production per dairy doe, while Iran reports the lowest milk production per dairy doe. China has the largest total number of goats in the world with 195.6 million, but they are mainly kept for meat production (Olivijer et al., 2005), followed by India with 120.0 million, and Pakistan 56.7 million.

Table 5. *Increase in the production of goat milk and cheese in the period from 1985 to 2005, Source: FAOSTAT (2006)*

	2005		1985	
	Cheese	Milk	Cheese	Milk
Europe	180	2.5	132	1.7
Asia	99	6.7	113.7	4.1
America	17.8	0.37	33.96	0.48
North and Central A.	13.8	0.19	30.06	0.32
South A.	4.0	0.18	3.9	0.16
Africa	122	2.8	35	1.8
Total in world	437.8	12.4	343.32	8.4

By the amount of milk produced per goat (194.8 kg), China is on the third place just behind France and Spain. China officially reports 1.4 million dairy goats producing 0.3 million MT of milk (FAOSTAT, 2008). Since 1990, interest in dairy goats has been steadily increasing, as

manifested by the increase in milk production from about 10 million MT in 1990 to about 15.2 million MT in 2008. The dairy goat industry has great potential for further growth.

Meat production

In many countries, especially in Asia and Africa, goat and caprine meat have great importance in the diet of the population. Because of its high biological value, caprine meat is increasingly required in highly developed countries (Todaro et al., 2004). Production of goat meat in the world, although it is four times smaller than the meat of sheep, is of great importance for many countries, especially in Asia, Africa and South America (Dubeuf et al., 2004). In EU countries the production of goat meat is of much less importance and scope, especially in the countries where there are grown dairy goat breeds in which the meat is a by-product. It is estimated that the goat meat in Europe makes about one tenth of the total quantity of sheep meat. Greece, Spain, Italy and France are the largest producers of these types of meat products because they produce two thirds of the total quantity of goat meat in Europe (Webb et al., 2005).

Table 6. *The amount of goat meat produced in the top ten countries, number of animals slaughtered and the average amount of meat produced per animal (FAOSTAT, 2008)*

Country	Total meat (million MT)	Number of animals slaughtered (million)	Average meat produced per animal (kg)
China	1.8	133.3	13.7
India	0.5	47.8	10.0
Nigeria	0.3	21.3	12.7
Pakistan	0.3	15.4	17.0
Bangladesh	0.2	30.0	7.0
Sudan	0.2	14.5	13.0
Iran	0.1	7.6	14.0
Indonesia	0.1	6.6	10.0
Ethiopia	0.1	7.6	8.5
Niger	0.1	4.4	12.0

Due to the long-standing prohibition of goats keeping which has been in force since 1954 (unique law in the world that has been adopted in the former Yugoslavia), on the territory of the Republic of Serbia there has not been paid special attention to the production of goat meat, nor any statistical data on the number of production of goats have been produced. Although on the territory of Serbia, the basic product is a goat milk, meat production should not be ignored either. It should properly utilize high biological potential of goats for good fertility (Memiši et al., 2001). Goat is known as the most prolific ruminant, which many breeders use to increase a caprine meat production by forcing goat fertility and creating a race that can on average produce 2-3 young goats annually. This ability in goats may very well take advantage of the sequential kidding, and this is where the caprine meat production is more profitable than milk production, and where, there may be the reason for a lack of interest for the production of milk, or the conditions for organized sale of large quantities of milk or for a milk processing (Memiši and Bauman, 2002a and 2007; Memiši et al., 2004).

Goat meat has about the same nutritional value and digestibility as sheep milk (to put it more exact: more protein and less fat compared to sheep meat). It is still less appreciated because of specific smell and flavor, especially if the animal is older. On the basis of chemical composition, caprine and goat meat, in terms of nutritional and biological value is not inferior to other types of meat in other types of livestock for slaughter.

Table 7. *Top exporters of goat meat*

Country	Goat meat production (million tonnes)	%
Australia	16.431	50
China	3.999	12.1
France	2.628	8
New Zealand	1.198	3.6
Industrialized countries	21.194	64
World	33.087	

Source: FAOSTAT (2005)

Goat meat is widely consumed in the developing countries. According to FAOSTAT (2008), total meat inventory is about 280 million MT. Goat meat represents only 2% of this total. The total amount of goat meat produced in 2008 was 4.9 million MT. The developing countries produced approximately 97% of this amount, reflecting the great importance of goat meat to feed millions of people in these countries. The top ten countries producing goat meat are all from Asia and Africa (Table 6). China leads the world in producing goat meat accounting for 38% of the world total goat meat produced. Goat meat production has increased from 2.65 million MT in 1990 to 4.93 million MT in 2008.

Table 8. *Top importers of goat meat*

Country	(MT)	%
U.S.	9.551	18.2
China	5.709	10.9
Italy	1.451	2.8
Canada	1.374	2.6
France	1.151	2.2
Industrialized countries	16.097	30.7
World	52,477	

Source: FAOSTAT (2005)

Australia leads goat meat export in the world with 16.431 MT and 50% of the total world market. China, France and New Zealand claim 12.1, 8.0 and 3.6% of the world market respectively (Table 7).

Total goat meat imported in the world is 52,477 MT. Top importers of goat meat are the United States with 18% of the market, followed by China with 10.9% and Italy, Canada and France, each about 2% of the market (Table 8).

Conclusion

Geographical conditions that have been deteriorating due to global warming will probably further increase the importance of goat industry in the future. Although the goats have been the most defamed domesticated animals in many countries they have played an important role in human nutrition, wellbeing and survival. In Europe, the decline in goat number is compensated by an increased milk and meat performance with a positive impact on the continent contribution to the world goat milk.

While in the developing countries goat milk and meat will continue to be a basic food for rural population, in the developed ones the goat milk processing will continue to be a priority because of the increased demand for dairy products.

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References

1. Albenzo M, Caroprese M, Marino R, Muscio A, Santillo A and Sevi A 2006. Characteristics of Garganica goat milk and Cacioricotta cheese. Small Rumin. Res. 64, 35-44.
2. Billon P 2003. Milking management. In: Roginski H, Fuquay JW, Fox PF (Eds.), Encyclopedia of Dairy Sciences, pp. 1243-1253. . Academic Press, Cornwall.
3. Boyazoglu J, Hatziminaoglou Y and Morand-Fehr P 2005. The role of the goat in the Society : past present and perspective for the future. Small Rumin. Res. 60 (1–2), 13–23.
4. Constantinou A, Beuing R and Mavrogenis AP 1985. Genetic and phenotypic parameters for some reproduction and milk production characters of the Damascus goat. Journal of Animal Breeding and Genetics 102, 1-5, 301–307.
5. Devendra C 2007. Perspectives on animal production systems in Asia. Livest. Sci. 106, 1–18.
6. Dubeuf JP and Boyazoglu J 2009. An international panorama of goat selection and breeds. Livestock Science 120, 225–231.
7. Dubeuf JP, Morand-Fehr P and Rubino R 2004. Situation, changes and future of goat industry around the world. Small Rumin. Res. 51, 165–173.
8. Food and Agriculture Organization of the United Nations 2005. FAOSTAT database, available at www.fao.org/waicent/portal/statistics
9. Food and Agriculture Organization of the United Nations 2006. FAOSTAT database, available at from http://faostat.fao.org/site/409/default.aspx.
10. Food and Agriculture Organization of the United Nations 2008. FAOSTAT database, available at http://faostat.fao.org/site/362/DesktopDefault.aspx.
11. Food and Agriculture Organization of the United Nations 2009. FAOSTAT database, available at Agricultural Statistics (online: http://www.fao.org)

12. Food and Agriculture Organization of the United Nations 2010. FAOSTAT database, available at www.fao.org
13. Fehse R and Kunzi R 1998. Die Ziegenzucht in de Schweiz.
14. Falagán A and Mateos E 1996. La producción de leche en la cabra. Zootecnia Bases de la Producción Animal 9, 131-143.
15. Gall C 1982. Ziegezucht. Verlag Eugen Ulmer, Stuttgart.
16. Kondyli E, Svarnas C, Samelis J and Katsiari MC 2012. Chemical composition and microbiological quality of ewe and goat milk of native Greek breeds. Small Rumin. Res. 103, 2, 194-199.
17. Memiši N, Božović V, Bauman F and Latinović D 1998. Varijabilnost eksterijernih i proizvodnih osobina domaće balkanske koze sa područja šarplaninskih župa. Contemporary Agriculture 46, 3-4, 75-80.
18. Memiši N. 2000. Quantitative analysis of body development and production traits of domestic Balkan goat. PhD, University of Belgrade.
19. Memiši N, Bauman F, Činkulov M and Žujović M 2001. The influence of years and lactation in order on fertility of domestic Balkan goat. Contemporary Agriculture 51, 3-4, 63-67.
20. Memiši N and Bauman F 2002. Goat. Agricultural library. Belgrade.
21. Memiši N and Bauman F 2002a. Tovna i klanična svojstva jaradi domaće balkanske koze. Poljoprivredne aktuelnosti, 5-6, .
22. Memiši N and Bauman F 2003. Domaće rase koza. Poljoprivredne aktuelnosti 5-6, 41-56.
23. Memiši N, Bauman F, Stojanović S. Pavlov B and Jovanović S 2004. Production characteristics of domestic Balkan goats. Animal Genetic Resources Information 35, 87-94.
24. Memiši N and Bauman F 2007. Nutrition of goats. Admiralbook. Belgrade.
25. Memiši N and Žujović M 2012. Goat breeds. Institute for animal husbandry, Belgrade-Zemun.
26. Olivier JJ, Cloete SWP, Schoeman SJ and Muller CJC 2005. Performance testing and recording in meat and dairy goats, Small Rumin. Res. 60, 1-2, 83-93.
27. Park YW, Juárez M, Ramos M and Haenlein GFW 2007. Physico-chemical characteristics of goat and sheep milk. Small Rumin. Res. 68, 88-113.
28. Talpur FN, Bhanger MI and Memon NN 2009. Milk fatty acid composition of indigenous goat and ewe breeds from Sindh, Pakistan. Journal of Food Composition and Analysis 22, 59-64.
29. Todaro M, Corrao A, Alicata ML, Shinelli, R, Giaccone P and Priolo A 2004. Effects of litter size and sex on meat quality traits of kid meat. Small Rumin. Res. 54, 191-196.
30. Webb EC, Casey NH, and Simela L 2005. Goat meat quality. Small Rumin. Res. 60, 153-166.
31. Žujović M. 1988. Oplemenjivanje populacije koza gajenih na farmi »Bačevsko polje« u Dimitrovgradu. Master' s Theses, University of Belgrade.
32. Žujović M, Memiši N and Ivanović S 2011. Present status, possibilities and perspective of development of goat production in the Republic of Serbia. Biotechnology in Animal Husbandry 27 (3), 431-443.

Original paper

BODY MEASURES IN ENDANGERED DOMESTIC BALKAN DONKEY BREED

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Abstract

Information regarding morphology of endangered autochthonous donkey breed in Serbia is insufficient. The aim of the study was to establish 18 morphometric values for the Balkan donkey breed and to explore possible age dependence of the parameters tested. In addition, inter-breed similarity of morphometric parameters was assessed by comparing the data obtained for the Balkan donkey with available morphometric measurements of several previously characterized donkey breeds. The study population included 74 donkeys, divided in two age groups (group A $\leq$ 3 years; group B > 3 years). Significant morphometric differences ($p<0.05$) in body length, head length, chest circumference and body weight were found between two age groups. Significant differences in morphological parameters were revealed among the Balkan donkey and other donkey breeds (Catalonian, Croatian and Albanian). However, the smallest distance was between the Balkan donkey and Albanian donkeys as demonstrated by the results of cluster analysis. The results of morphometric analyses showed consistency of the obtained values within the breed, and diversity as compared to other donkey breeds, and, thus, could be taken as referent for the Domestic Balkan donkey. The information gained through this comprehensive morphometric characterization of the Balkan donkey breed provides a basis for conservation and development of the breed standard.

Key words: *breed characterization, Domestic Balkan donkey, morphological measures*

Introduction

Local donkey breeds are important genetic resources. However, many of them are at risk of extinction as a result of intensive agrarian production and the socio-economic changes in the rural areas. Protection of genetic resources is one of the crucial issues aimed at biodiversity conservation as autochthonous breeds are vanishing at disturbing rate (Allendorf and Luikart, 2007). Genetic or morphological data for the most of donkey breeds are lacking. Information regarding main morphological and genetic characteristics should be acquired in initial investigations in order to preserve breeds.

Domestic Balkan donkey (Balkan donkey) is an endangered autochthonous breed widespread across the Serbian territory (Official Gazette of RS, no. 38/10, Kugler et al., 2008). Over the last decades, the population of Balkan donkey has been constantly declining due to the loss of their economic importance. It is estimated that current number of sexually mature Balkan donkeys in Serbia is as low as 250-300 and they are under some form of surveillance and protection (Trailovic et al., 2011).

The initial research in the process of donkey breed preservation relies on the phenotype study including the geographical distribution and morphometric characterization, along with the age differences (Folch and Jordana, 1997; Ivankovic et al., 2000; Rischkowsky and Pilling, 2007; Papa and Kume, 2012). Scientifically based data on donkey population in Serbia are deficient. The only available data are basic body measures (Trailovic et al., 2011) and major hematological parameters (Vucicevic et al., 2011). Both these surveys, however, included small number of Balkan donkeys located in Stara Planina Mt. region. The aim of our study was to establish morphometric values for the endangered Balkan donkey breed by using representative sample acquired from two age categories. In order to assess inter-breed similarity within a context of morphometric parameters, the data obtained for the Balkan donkey were compared with morphometric parameters of several previously characterized domestic donkey breeds.

Material and Methods

The Balkan donkey population

The majority of donkey population in Serbia is situated in following locations: the Special Nature Reserve "Zasavica" (SNR "Zasavica", $44^0$57′ 32,2″ N $19^0$31′32,7″ E), the Stara Planina Mt. region (43°06′37″N 22°57′14″E) and in Kovilj village ($45^0$25′16,7″ N $19^0$83′69,4″ E) near Novi Sad. Besides that, small number of adult donkeys and foals is distributed over the country in individual households. In total, 74 heads have been included in this survey: 39 donkeys from SNR "Zasavica", 16 donkeys from Stara Planina Mt. and 19 donkeys located in Kovilj village. The study population included 6 males and 68 females. In accordance with the previous investigations (Folch et al., 1997; Jordana et al., 1998), the study population consisted of two age groups. The group A included 22 young donkeys (≤ 3 years of age) while group B included 52 adult donkeys older than 3 years. The oldest animals in group B were 10 years old.

Morphometric measurements

In total, 18 body measures were selected for morphometric characterization of Balkan donkey. For six body variables (chest circumference, carpal circumference, tarsal circumference, tibia circumference, ear length and head length) a measuring tape was used. The additional 11 parameters (wither height, back height, hip height, chest depth, carpal height, tarsal height, body length, head width, chest width, croup length and croup width) were measured by the Lydtin stick. The body weight (BW) for each animal was calculated according to the formula:

$$BW\ (kg) = (CC^2 \times BL) / 11877$$

where CC and BL represent chest circumference and body length, respectively (Pejić, 1996). Microchip number, followed by age, coat color, specific distinctive signs and possible external defects were recorded for each animal.

The five body measures (wither height, chest depth, body length, chest width and chest circumference) were selected for inter-breed analysis among Balkan donkey and Catalonian donkey (Folch and Jordana, 1997), Croatian donkeys (Ivankovic et al., 2000) and Albanian donkeys (Papa and Kume, 2012).

Statistical analysis

The data recorded in this study were presented through mean, median, quartile range and coefficient of variation (CV). Shapiro-Wilk's W test was used in order to assess the normal distribution of the data followed by Levene's test for determination of the homogeneity of variances. Variables with normally distributed data and homogenous variances were compared between groups through the t-test, otherwise, the Mann-Whitney U-test was used. Similarity of Balkan donkey with other donkey breeds was examined through the cluster analysis, based on Euclidean distances and Ward's method of linkage. The statistical differences for the five selected morphological parameters among donkey breeds were determined by using t-test. Differences were considered as significant for p values <0.05. Statistical analysis of the results obtained in the experiment was carried out using software STATISTICA v. 6 (StatSoft, Inc., Tulsa, OK, USA).

Results

The values of body measures displayed homogeneity (CV<30%) in both age categories (Table 1). Most of the body measures for adult donkeys followed normal distribution, while majority of the variables for young donkeys indicated not-normal data distribution (Shapiro-Wilk's W test, p<0.05). Between groups A and B, there was a significant difference (p<0.05) in four out of eighteen parameters: body length, head length, chest circumference and body weight.

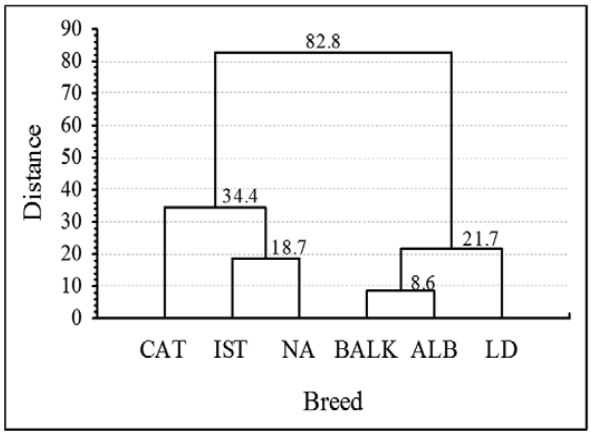

Figure 1. *Dendrogram of the cluster analysis showing relationships among the Domestic Balkan donkey and previously described European donkey breeds, based on the morphometric data.*

The results of t-test (Table 2) for five selected body measures showed the significant differences (p<0.01) when Balkan donkey (BALK) was compared to Catalonian donkey

(CAT) and Croatian donkeys (LD - Littoral-Dinaric donkey; NA - North-Adriatic donkey; IST - Istrian donkey). Comparison of Balkan donkey with Albanian donkey population (ALB) revealed no significant differences in body length values ($p>0.05$); however, values of chest depth ($p<0.05$), wither height ($p<0.01$), chest width ($p<0.01$) and chest circumference ($p<0.01$) were significantly different (Table 2). Results of hierarchical clustering demonstrated the smallest distance between Balkan donkey and Albanian donkeys (Figure 1).

Table 1. *Morphometric measurements for the Domestic Balkan donkey breed*

Parameters	Age group A				Age group B				
Body variables (cm)	Mean	Median	Quartile range	CV (%)	Mean	Median	Quartile range	CV (%)	p
Wither height	103.3	103.5	101.0-107.0	5.7	104.9	104.0	99.0-108.5	8.5	0.906
Back height	101.9	101.7	99.0-106.0	5.9	102.8	102.5	97.5-106.7	8.4	0.873
Chest depth	47.1	46.5	45.0-51.0	10.4	47.3	46.5	44.0-50.7	9.9	0.859
Hip height	106.8	107.0	105.0-111.0	5.0	107.2	106.7	102.0-112.0	7.9	0.731
Tarsal height	40.7	40.5	39.0-42.0	5.8	41.3	41.0	38.0-44.0	9.2	0.541
Carpal height	32.0	31.5	30.0-34.0	6.6	32.4	33.0	30.0-34.5	11.7	0.757
Body length	110.3	111.5	107.0-116.0	8.7	117.2	117.0	112.0-121.5	8.2	**0.002**
Head length (t)	47.6	48.0	45.0-51.0	7.7	49.6	49.7	47.0-52.0	7.3	**0.038**
Head width	21.0	21.0	20.0-22.0	6.8	22.1	21.7	20.7-23.0	12.4	0.145
Ear length (t)	26.1	26.0	25.0-27.0	8.2	25.6	25.0	24.0-27.0	8.4	0.318
Croup length	29.4	31.5	25.0-33.0	17.1	29.9	32.0	24.0-35.0	21.3	0.674
Croup width	33.7	34.0	33.0-36.0	12.3	35.0	36.0	32.5-38.0	12.5	0.198
Chest width	23.9	24.0	23.0-25.0	11.0	24.3	24.2	22.5-27.0	11.9	0.609
Chest circumference	114.2	114.7	112.5-118.0	8.0	119.6	118.5	114.5-124.5	8.8	**0.030**
Carpal circumference	21.0	21.0	20.5-22.0	6.8	21.4	21.0	19.7-22.5	11.5	0.896
Tarsal circumference	27.3	27.0	26.0-29.0	7.7	28.6	28.0	27.0-29.0	10.3	0.142
Tibia circumference	23.9	24.0	23.0-25.0	10.7	25.0	25.0	23.7-26.2	12.1	0.275
Body Weight (kg)	124.9	122.0	111.0-132.0	16.9	143.4	138.5	123.5-155.0	24.8	**0.005**

(t) – t-test; for the rest of the variables U test was used

Table 1. *Morphometric measurements for the Domestic Balkan donkey breed*

Parameters	Age group A				Age group B				
Body variables (cm)	Mean	Median	Quartile range	CV (%)	Mean	Median	Quartile range	CV (%)	p
Wither height	103.3	103.5	101.0-107.0	5.7	104.9	104.0	99.0-108.5	8.5	0.906
Back height	101.9	101.7	99.0-106.0	5.9	102.8	102.5	97.5-106.7	8.4	0.873
Chest depth	47.1	46.5	45.0-51.0	10.4	47.3	46.5	44.0-50.7	9.9	0.859
Hip height	106.8	107.0	105.0-111.0	5.0	107.2	106.7	102.0-112.0	7.9	0.731
Tarsal height	40.7	40.5	39.0-42.0	5.8	41.3	41.0	38.0-44.0	9.2	0.541
Carpal height	32.0	31.5	30.0-34.0	6.6	32.4	33.0	30.0-34.5	11.7	0.757
Body length	110.3	111.5	107.0-116.0	8.7	117.2	117.0	112.0-121.5	8.2	**0.002**
Head length (t)	47.6	48.0	45.0-51.0	7.7	49.6	49.7	47.0-52.0	7.3	**0.038**
Head width	21.0	21.0	20.0-22.0	6.8	22.1	21.7	20.7-23.0	12.4	0.145
Ear length (t)	26.1	26.0	25.0-27.0	8.2	25.6	25.0	24.0-27.0	8.4	0.318
Croup length	29.4	31.5	25.0-33.0	17.1	29.9	32.0	24.0-35.0	21.3	0.674
Croup width	33.7	34.0	33.0-36.0	12.3	35.0	36.0	32.5-38.0	12.5	0.198
Chest width	23.9	24.0	23.0-25.0	11.0	24.3	24.2	22.5-27.0	11.9	0.609
Chest circumference	114.2	114.7	112.5-118.0	8.0	119.6	118.5	114.5-124.5	8.8	**0.030**
Carpal circumference	21.0	21.0	20.5-22.0	6.8	21.4	21.0	19.7-22.5	11.5	0.896
Tarsal circumference	27.3	27.0	26.0-29.0	7.7	28.6	28.0	27.0-29.0	10.3	0.142
Tibia circumference	23.9	24.0	23.0-25.0	10.7	25.0	25.0	23.7-26.2	12.1	0.275
Body Weight (kg)	124.9	122.0	111.0-132.0	16.9	143.4	138.5	123.5-155.0	24.8	**0.005**

(t) – t-test; for the rest of the variables U test was used

Table 2. *Comparison of morphological parameters of the Domestic Balkan donkeys and other donkey breeds and populations based on t-test*

Breed	Wither height			Chest depth			Body length			Chest width			Chest circumference		
	Mean	t	p	Mean	t	p	Mean	t	p	Mean	t	p	Mean	t	p
LD	96.9	7.9	<0.01	42.0	9.5	<0.01	102.6	10.6	<0.01	23.1	3.4	<0.01	112.7	4.3	<0.01
NA	115.2	-11.4	<0.01	50.9	-22.9	<0.01	121.1	-5.0	<0.01	27.3	-9.4	<0.01	131.8	-11.2	<0.01
IST	124.0	-20.7	<0.01	54.7	-13.6	<0.01	131.3	-13.6	<0.01	29.8	-17.1	<0.01	144.0	-21.3	<0.01
ALB	107.8	-3.5	<0.01	46.1	2.1	<0.05	113.4	1.5	**0.13**	26.7	-7.4	<0.01	125.2	-5.8	<0.01
CAT	136.2	-33.6	<0.01	59.3	-21.9	<0.01	143.8	-24.2	<0.01	32.4	-24.8	<0.01	154.7	-30.1	<0.01
BALK	104.5			47.3			117.9			24.3			114.9		

LD - Littoral-Dinaric donkey; NA - North-Adriatic donkey; IST - Istrian donkey (Ivankovic et al., 2000); ALB - Albanian donkey population (Papa and Kume, 2012); CAT - Catalonian donkey breed (Folch and Jordana, 1997); BALK - Domestic Balkan donkey.

Discussion

In this study the Balkan donkey breed was characterized through the morphometric investigation. The obtained values were compared between donkeys classified into group A (≤3 years of age) and group B (>3 years of age). Animals disclosed high homogeneity of body values (5.08% ≤ CV ≤ 24.8%) in both age groups although three different geographical populations of donkeys have been investigated. This high level of homogeneity points out to the uniformity of morphometric characteristics of the Balkan donkey breed. Significant morphometric differences in body length, head length, chest circumference and body weight were found between age groups. Since the body weight was calculated using body length and chest circumference, changes in these parameters obviously generated the change in weight. Variations in chest circumference and body length were expected due to the processes of growth and lipogenesis which follow increase in foals' weight and reproductive status of investigated animals. Comparison of the selected morphological parameters revealed significant differences among the Balkan donkey and other donkey breeds (Catalonian, Croatian and Albanian). Cluster analysis indicated morphological similarity between Balkan donkey and Albanian donkeys. This could be attributed to close geographic proximity and the overlap of their habitats. Additional molecular characterization of both breeds is needed to get insight in their molecular genetics diversity.

Conclusion

The results of morphometric characterization of the endangered Balkan donkey breed showed consistency of the obtained values within the breed and differences when compared to other previously described donkey breeds. According to the normal distribution of the body measures determined and significant morphological differences in wither height, chest depth, body length, chest width and chest circumference among the all above mentioned breeds, recorded values for Balkan donkey could be taken as referent. The information gained through the morphometric analysis provides a basis for development of the breed standard and an introduction for genetic research of donkey populations in Serbia, which is currently underway in our laboratory. The obtained results will provide useful information in order to preserve donkeys as animal genetic resource (FAO, 2011).

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References

1. Allendorf FW and Luikart G 2007. Conservation and the Genetics of Populations. Blackwell Publishing, Oxford, UK.

2. Beja-Pereira A, England PR, Ferrand N, Jordan S, Bakhiet AO, Abdalla MA, Mashkour M, Jordana J, Taberlet P and Luikart G 2004. African origins of the domestic donkey. Science 304, 1781-1781.
3. Folch P and Jordana J 1997. Characterization, reference ranges and the influence of gender on morphological parameters of the endangered Catalonian donkey breed. Journal of Equine Veterinary Science 17, 102-111.
4. Ivankovic A, Caput P, Mioc B and Pavic V 2000. The phenotype Features of Donkeys in Croatia. Agriculturae Conspectus Scientificus 65, 99-105.
5. Kugler W, Grunenfelder HP and Broxham E 2008. Donkey breeds in Europe. Monitoring institute for rare breeds and seeds in Europe. St. Gallen, Switzerland.
6. Official Gazette of Republic of Serbia, No. 38/10.
7. Papa L and Kume K 2012. The results of identification and characterisation of the donkey population in Albania. Agriculture and Forestry 58,125-134.
8. Pejić N 1996. Konj (*Equus caballus*). Faculty of Agriculture Novi Sad, Novi Sad, Serbia.
9. Rischkowsky B and Pilling D (ed.) 2007. The State of the World's Animal Genetic Resources for Food and Agriculture. Food and Agriculture Organization, Rome, Italy.
10. Trailovic R, Ivanov S, Dimitrijevic V and Trailovic D 2011. Some morphological and health characteristics of domestic Balkan donkey in Stara planina. In Proceedings of 2nd International Fair of Horse Breeding, 30 September - 2 October 2011, Novi Sad, Serbia, pp. 180-187.
11. Vucicevic M, Drljacic A, Francuski J, Ivanov S, Trailovic R and Trailovic D 2011. Estimation of physiological values of haematological parameters in Domestic Balkan donkey before and after the grazing period. In Proceedings of 2nd International Fair of Horse Breeding, 30 September - 2 October 2011, Novi Sad, Serbia, pp. 194-200.

Original paper

MILKING CHARACTERISTICS OF ISTRIAN AND LITTORAL DINARIC DONKEY BREEDS

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Abstract

Istrian and Littoral Dinaric donkey are autochthonous breeds which habitat primarily in the Mediterranean part of Croatia. During the second half of the 20th century they have lost primary function as working animals, which led to breeds suppression. Nowadays these two breeds are in the status of endangered ones. One of the possibilities for their economic re-affirmation is launching the program for production and processing donkey's milk. The aim of the study was to determine the possibility of milk production of Istrian and Littoral Dinaric jennies in terms of quantity and chemical composition. Istrian jennies produce 745.4 mL/milking while Littoral Dinaric produce 317.8 mL/milking. Milk form Littoral Dinaric jennies contained a significantly higher proportion of milk fat and proteins. There were no significant differences in the content of lactose, dry matter, number of somatic cells and microorganisms in milk between these two breeds. Considering the potential for milk production and milk quality, we believe that both breeds are suitable for integration in the programs for milk production, thus enabling for both population to be economically sustainable. It is necessary to develop production technology and markets, with special emphasis considering quality of donkey milk and safety of consumers.

Key words: *autochthonous breeds, chemical composition, donkey milk, utilisation*

Introduction

Conservation of autochthonous breeds of domestic animals in recent decades has been imposed as one of the important demands in global and national animal production. Safety of food production in the future may considerably depend on cumulative adaptability preserved in autochthonous, today often neglected breeds. Based on past experience, analysis and assumptions for development of environment for food production FAO set a new strategic guidelines for the protection of animal genetic resources in the Global Plan of Action for Animal Genetic Resources, accepted in Interlaken in 2007 (FAO, 2007).

Donkeys are one of the important species of domestic animals, particularly adapted to areas with small amount of rainfalls and scarce vegetation. Such areas are now found in the Mediterranean countries of Europe, Middle East, Asia and Africa. Today 193 donkey breeds are registered, of which 29% breeds in Europe, 24% breeds in Near East, 17% breeds in Asia and Pacific, 14% breeds in Africa, 13% in Latin America and the

Caribbean, and 3% breeds in the area of North America (FAO, 2014). Preserved genetic diversity of donkeys in Europe reflects the efforts of preserving all existing breeds as well as more complete inventory of the donkey breeds in the field. In Croatia in the area of the Mediterranean and sub-Mediterranean climate zones three autochthonous donkey breeds are preserved, Littoral Dinaric, North-Adriatic and Istrian donkey. In the past these breeds played an important role in the cultivation of small agricultural lands, transport of goods and people, especially in the Dinaric and insular areas in Croatia.

Littoral Dinaric donkey has the largest population size, mainly distributed in central and southern Mediterranean part of Croatia, although it can be found in other areas. Because of small body frame and small body weight it belongs to a group of small donkeys in the world. On the contrary, Istrian donkey is a breed with larger body frame and larger body mass. It is assumed that in the origin of Istrian donkey some Italian breeds were involved, while breeds with smaller body frame, mostly from Southeast Europe, participated in shaping of Littoral Dinaric donkey. Ivanković et al. (2000) report that Littoral Dinaric donkey has withers height 97 cm and body weight 93 kg, while the Istrian donkey is considerably more developed, with withers height of 124 cm and body weight of 218 kg.

Social and economic changes that took place during the second half of the 20thcentury, especially agriculture industrialization and depopulation of rural areas, caused the reduction in demand for working animals like donkeys. Their number during mentioned period was notably reduced. If we consider reproductive aspect, all breeds were neglected (due to reduced necessity for young animals). In the middle nineties of the 20thcentury the interest for donkey breeds as genetic heritage was initiated and breed inventory was made. In terms of breeds preserving, relatively small population size and poor reproductive activity of the population were troubling. In recent years some efforts are taken to find new models of economic utilization of donkeys in order to save the breed from extinction, to improve reproduction, to preserve the identity of the region and to improve food offer. Milk production of autochthonous donkey breeds is one of the favorable opportunities because it is based on regular reproduction. Although in Croatia tradition of donkey milk production does not exist, in times of insufficient medical care and protection, it was often used in small quantities as a *functional food* (drug) in the rehabilitation of various health disorders, particularly in the treatment of diseases of the respiratory system. Such traditional knowledge is still present in the population, and is often used as functional food in the treatment of some diseases of the immune, circulatory, respiratory, and other systems nowadays. Due to the market demand a small number of donkey farms for milk production were established in Croatia. Such farms try to achieve economic sustainability, but they also increase chances for long-term conservation of autochthonous breeds. Since there is very little knowledge about the potential of autochthonous donkey breeds in milk production, the aim of this research was to determine milk yield and chemical milk composition of Littoral Dinaric and Istrian breed.

Materials and methods

The study included 28 jennies from three farms (Pula, Imotski, Ivanić Grad), of which eight were Istrian, and 18 jennies from Littoral Dinaric breed. Jennies were kept in the whole day free housing system. Meal was primary pasture and concentrate in the amount of about one kg/day (a mixture of corn and barley; 50:50). Each jenny was subjected to two control manual milking during June and July in 2013. Three hours before milking foals were separated (physically) from the mothers to avoid milk suckling. After milking, the amount of milk was measured and milk samples for chemical and hygienic analysis

were taken. The samples were immediately frozen at -20°C and stored till chemical analysis was performed. Chemical composition is determined by infrared spectrophotometry according to HRN ISO 9622:2001. With fluoro-opto-electronic method according to HRN EN ISO 13366-2:2007/Ispr.1: 2007 the number of somatic cells account (SCC) is determined. Quantitative determination of the number of microorganisms in the milk (BCC) is done using epifluorescent flow cytometry method, according to IDF 161A:1995. The results were processed using the GLM procedures of the SAS statistical package (SAS Institute, 1999).

Results and discussion

Research has shown that the Istrian jennies produce significantly higher amount of milk ($p<0.001$) compared to Littoral Dinaric (745.38 vs. 317.83 mL/milking). This result is expected if we consider relation between the size of the body frame and body mass (218 vs. 93kg) partly. Given the amount of milk produced in one milking, we can conclude that their potential is beneficial, especially of Istrian jennies. Salimei and Fantuz (2012) observed (based on several studies: Alalbiso et al., 2009; Chiofalo et al., 2004; Guo et al., 2007; Ivanković et al., 2009) significant variability in the amount of milk at hand milking (466±260 mL/milking), in relation to the machine milking (772±148 mL/milking). Introduction of machine milking in the regular procedure is acceptable to farmers after organisation of market conditions considering donkey's milk, and establishing continuity of demand. Taking into account that jennies in the regular milking procedure could be milked four times per day, the amount of milk of mentioned breeds would be sufficient for its sustainable production. Average values and chemical composition of donkey's milk are shown in Table 1.

Table 1. *Chemical composition and hygienic parameters of Istrian and Littoral-Dinaric donkey milk*

Traits	Istrian donkey		Littoral-Dinaric donkey		Significance level
	Mean	St.dev.	Mean	St.dev.	
Milk yield (mL/milking)	745.38	*302.23*	317.83	*154.81*	***
Dry matter (%)	8.44	*0.321*	8.55	*0.570*	ns
Lactose (%)	5.92	*0.305*	5.85	*0.419*	ns
Fat (%)	0.42	*0.112*	0.59	*0.151*	**
Protein (%)	1.47	*0.194*	1.63	*0.248*	*
SCC	10.600	*2.200*	9.500	*1.900*	ns
BCC	2.600	*1.250*	6.240	*2.460*	ns

*** P<0.001; ** P<0.01; * P<0.05; ns – not significant

Milk dry matter and lactose in researched donkey breeds did not differ significantly. Furthermore, significant differences in the number of SCC and BCC in the milk between breeds are not observed. Milk of Littoral-Dinaric jennies contain higher amount of milk fat content (P<0.01) and milk protein (P<0.05) compared to the milk of Istrian jennies (0.59 vs. 0.42%, 1.63. vs. 1.47%). Compared to previous studies (Guo et al., 2007; Piccione et al., 2008; Alalbiso et al., 2009; Chiofalo et al., 2004; Ivanković et al., 2009) observed content of milk fat is much lower, while content of milk protein does not differ significantly. The number of SCC and BCC is very small, which is in agreement with previous studies. This can be explained by frequent milking or frequent suckling of foals, which prevents longer retention of milk in the udder as well as short teats canal of udder.

In addition, donkey milk contains certain substances that inhibit the growth of microorganisms (lysosyme, lactoferrin), which prevents their significant growth in the udder. The amount of lysozyme is almost twenty times higher, while the amount of lactoferrin is almost three times higher in jennie's than in cow's milk (Salimei and Fantuz, 2012).

There was a significant negative correlation between milk yield and fat content ($P<0.05$), i.e. milk yield and protein content ($P<0.01$). Knowing the physiology of the synthesis and milk releasing the observed correlation is understandable, as well as observed positive correlation between milk fat and milk protein content ($P<0.01$; Table 2).

Table 2. *Correlation coefficients of donkey milk composition components*

	Dry matter	Lactose	Fat	Protein	SCC	BCC
Milk yield	-0.196	-0.022	-0.361*	-0.419**	0.564**	-0.324
Dry matter		0.875**	0.455**	0.673**	0.072	0.063
Lactose			0.169	0.239	0.150	0.052
Fat				0.603**	0.171	0.451*
Protein					-0.131	0.051
SCC						0.761**

** $P<0.01$; * $P<0.05$

Positive correlation between milk yield and SCC as well as SCC and BCC microorganisms is observed ($P<0.01$). This indicate that the majority of milk yield is actually synthesized and deposited in mammary cisterns and canals, and only small portion of milk is synthesized during the second phase of the milking.

Conclusion

This research indicates favorable potential of Littoral Dinaric, and especially Istrian jennies for milk production. The interest of the farmers is determined by market conditions, i.e. by the continuity of demand for donkey milk. There is the need to establish processing units that could process donkey milk into nutritional products or cosmetic products. Since the production technologies were undeveloped, it is necessary to improve them and introduce a machine milking as standard procedure on larger dairy farms. Development of such programs, with richer offer of different dishes raises the sustainability of endangered donkey breeds, enriches the tourist offer and maintains the overall biodiversity of the area. It is necessary to adopt health norms for milk safety and continue to research the effects of donkey milk on consumer's health.

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References

1. Alabiso A, Giosuè C, Alicata ML, Mazza F and Iannolino G 2009. The effects of different milking intervals and milking times per day in jennet milk production. Animal 3, 543-547.

2. Chiofalo B, Azzara V, Piccolo D, Liotta L and Chiofalo L 2004. The chemical and physical parameters of the ragusanaass's milk during lactation. In: Proceedings of 6th congress new findings in equine practices, Campobasso, Italy, pp. 77-84.
3. FAO 2007. Global Plan of Action for Animal Genetic Resources and the Interlaken Declaration. Commission on Genetic Resources for Food and Agriculture, Rim.
4. FAO 2014. (DAD-IS;http://dad.fao.org/).
5. Guo HY, Pang K, Zhang XY, Zhao L, Chen SW, Dong ML and Ren FZ 2007. Composition, physio-chemical properties, nitrogen fraction distribution, and aminoacid profile of donkey milk. Journal of Dairy Science 90, 1635-1643.
6. Ivanković A, Caput P, Mioč B and Pavić V. 2000. Fenotipske značajke magaraca u Hrvatskoj. Agriculturae Conspectus Scientificus 65, 99-105.
7. Ivanković A, Ramljak J, Štulina I, Antunac N, Bašić I, Kelava N and Konjačić M 2009. Characteristics of the lactation, chemical composition and milk hygiene quality of the Littoral-Dinaricass. Mljekarstvo 59, 107-113.
8. Piccione G, Fazio F, Caola G and Refinetti R 2008. Daily rhythmic ityin nutrient content of asinine milk. Livestock Science 116, 323–327.
9. Salimei E and Fantuz F 2012. Equid milk for human consumption. International Dairy Journal 24, 130-142.
10. SAS 1999. SAS Version 8. SAS Institute Inc., Cary, NC.

Original paper

ESTIMATION OF THE VARIANCE COMPONENTS OF THE SOW LITTER SIZE TRAITS USING REML METHOD - REPEATABILITY MODEL

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Abstract

Variance components for sow litter size traits were estimated using the REML method. Number of live born piglets (NBA), number of still born piglets (NSB), number of total born piglets (NTB) and number of weaned piglets (NW) were treated as traits which repeated several times during sow lifetime - repeatability model. Results of the fertility of Swedish Landrace sows realized on three pig farms in the Republic of Serbia were presented in four data sets DS1 (farm 1), DS2 (farm 2), DS3 (farm 3) and DS23 (farms 2 and 3 together). Fixed part of the model for litter size traits at farrowing (NBA, NSB and NTB) included parity, mating season as year-month interaction, litter genotype and weaning to conception interval as class effects. The age at farrowing was modelled as a quadratic regression nested within parity, whereas preceding lactation length was included as linear regression. In case of NW the model included parity, weaning season as year-month interaction, number of piglets in litter subsequent to crossfostering and litter genotype as class effects. The age at farrowing was included into the model in the same way as in case of previous traits. Random part of the model was the same for all analysed traits and represented as effect of common environment in litter where sows had been born, permanent effect of environment in sows' litters and direct additive genetic effect. Heritability of NBA varied between 0.050 (DS2) and 0.076 (DS3), NSB between 0.004 (DS3) and 0.027 (DS2), NTB between 0.065 (DS2) and 0.073 (DS3) and of NW between 0.010 (DS2) and 0.028 (DS1). Share of permanent environment of sow in phenotypic variance was higher than share of litter effect and mostly lower than share of direct genetic effect.

Key words: *heritability, litter size traits, pigs, variance components*

Introduction

In order to achieve maximum accuracy in evaluation of breeding value of sows for litter size traits, it is necessary to estimate as accurately as possible their variance components. For that purpose, it is necessary to determine the most optimal mixed model for analysis. That means to include all class, regression and random effects which influence variation of

mentioned traits. Determining of these effects is based on knowledge of the biology of pig fertility.

The litter size traits, at least most of them, are low hereditary traits which impedes their improving through selection. On the other hand, direct additive genetic effect is the the most common source of variation in relation to other variance components (random effects). Another problem related with improvement of litter size traits through selection is fact that those traits can be under negative maternal effect, and they can only be measured in sows after first farrowing which effects prolonging of the generation interval. Also, undesirable correlation between litter size and some important carcass quality traits which for a long time have been in the center of attention in pig selection (i.e. back fat thickness) is well known. Mentioned litter size traits are expressed several times during a lifetime of breeding females. It can cause dilemma whether these traits should be approached as traits which repeat several times during lifetime (repeatability model) as suggested by Logar (2000) or as specific traits (multi trait model) as presented by Tholen et al. (1998).

Residual Maximum Likelihood method (REML) is mostly applied for estimation of variance components of production traits in pigs which enables the most objective estimation of parameters using information deriving from the matrix of animal relationship.

The objective of this study was to estimate variance components of litter size traits, based on reproduction performance realized on three pig farms in the Republic of Serbia, as follows: number of live born piglets (NBA), number of still born piglets (NSB), number of total born piglets (NTB) and number of weaned piglets (NW) for the purpose of selecting traits which would be included into aggregate genotype in estimation of the breeding value of sows. This is extremely important for building of modern selection system in pig production. Radojković et al. (2012) stated that despite the great genetic progress in the last two decades in terms of production traits of pigs, biological limits have not been reached. Traits are still characterized by significant phenotypic and genetic variability, which, in addition to heredity, variable in different traits, provides the basis for the expectation that conventional selection will enable continuous improvement of production performance of pigs for significant number of years to come.

Materials and methods

Litter records of Swedish Landrace sows (the largest pig breed population in Serbia) collected over a period of 13 years on three pig farms (farm 1, 2 and 3) in the Republic of Serbia were analysed in this study. Data sets from mentioned farms have been marked as DS1 (farm 1), DS2 (farm 2), DS3 (farm 3) and as DS23 when analysed data included a combination of fertility records from farms 2 and 3. In Table 1 the structure of analysed data and used pedigree files per farm is presented.

Average number of litters per sow, as well as average number of selected sows per litter, was approximately similar on all farms, indicating rather equal structure of analysed data sets.

Pedigree files were done for three generations. Share of ancestors in total number of animals in pedigree file was the lowest in case of DS1 (14.39%), and highest in case of DS3 (32.86%). Only in DS3 is the number of base animals greater than 15%, this pulls the base animal percentage in the combined dataset.

Table 1. *Structure of data sets and pedigree files (second part of table)*

Parameter	**DS1**	**DS2**	**DS3**	**DS23**
No. of litters	11014	6757	8452	15209
No. of sows	2803	1826	2235	4061
Average no. of litters per sow	3.93	3.70	3.78	3.74
No. of sires	192	127	157	204
No. of litters from which sows are selected	2022	1344	1617	2636
Average no. of sows per common litter	1.39	1.36	1.38	1.54
Total no. of animals in pedigree file	3274	2559	3329	5023
No. of animals with records	2803	1826	2235	4061
No. of ancestors	471	733	1094	962
No. of base animals (percentage, %)	330 (10.07%)	374 (14.61%)	728 (21.87%)	808 (16.09%)

Based on presented number of sires, averagely selected sows per litter and number of ancestors in pedigree, the presence of strong genetic relation between farms 2 and 3 is noticeable since significant number of Swedish Landrace boars was used on both farms. This was reason why variance components of analysed traits were also determined based on data from both farms together, as well as separately for each farm.

In Table 2 the number of analysed data, average values and phenotypic variability of litter size traits in analysed DS are presented.

Table 2. *Descriptive statistics for litter size traits according to analysed data sets*

Trait	*Data set*	*No. of records*	$\overline{X}$	*SD*	*min*	*max*	*CV*
Number of live	DS1	11014	9.13	2.66	0	19	29.14
born piglets	DS2	6757	9.76	2.78	0	20	28.49
(NBA)	DS3	8452	9.76	2.89	0	20	29.66
	DS23	15209	9.76	2.84	0	20	29.15
Number of still	DS1	11014	0.77	1.21	0	14	156.78
born piglets	DS2	6757	0.68	1.05	0	11	154.31
(NSB)	DS3	8452	0.52	0.96	0	15	182.51
	DS23	15209	0.59	1.00	0	15	168.83
Number of total	DS1	11014	9.90	2.84	1	20	28.69
born piglets	DS2	6757	10.44	2.90	1	20	27.78
(NTB)	DS3	8452	10.29	2.96	1	20	28.75
	DS23	15209	10.36	2.93	1	20	28.33
Number of	DS1	11014	7.83	2.38	0	14	30.45
weaned piglets	DS2	6757	8.68	1.83	0	13	21.09
(NW)	DS3	8452	8.53	2.36	0	13	27.71
	DS23	15209	8.60	2.14	0	13	24.94

Analysed traits of litter size showed no significant differences between farms, in regard to average, or parameters of phenotypic variability. Only the average NW in DS1 was lower compared to the other farms.

Variance components of studied traits and share of single variance components in the phenotypic were estimated using the method of Residual Maximum Likelihood - REML. Application of REML method for estimation of dispersion parameters is necessary due to fact that sample representing the basis for estimation is not random, but is a population under selection. During determination of variance parameters using REML method analysed traits were treated as traits which repeat several times during a production lifetime (repeatability model). In such treatment of the litter size traits, a confounding problem involving maternal effect can occur but it is successfully solved by inclusion of the litter in which animals were born as a random effect in the model (Andersen, 1998; Logar, 2000; and Lukovic et al., 2004).

Special method of data preparation enabled simultaneous application of two different models used for analysis of the variability of litter size traits at birth (NBA, NSB and NTB) in litters of primiparous and older sows. This approach is a consequence of the fact that in the litters of primiparous sows two very important effects are not expressed, duration of previous lactation and period from previous weaning to conception.

In preparation of data, in reference to the effect of duration of previous lactation, real values of this parameter (in litters of older sows) were substituted with deviation from the average value for duration of lactation on farm (real value of the parameter minus average value), and in primiparous sows, for this parameter value 0 was included. When duration of lactation is included into the model as linear regression effect, it means that in primiparous sows this effect is not present, whereas for other parities, the effect of this factor on other traits of litter size at birth is normally analysed.

The effect of duration of period from weaning to conception on size of subsequent litter was studied as a fixed effect with categorical classes. According to previous findings relating to specific expression of this effect on litter size traits at birth, as well as distribution of frequency of this parameter, duration of this period was divided into 10 classes, as suggested by Lukovic et al. (2003), in the way presented in Table 3.

Table 3. *Classes of duration of the period from weaning to conception*

Class	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Duration (days)	<4	4	5	6	7	8	9	10-23	24-32	>32

In the results pertaining to fertility realized in the first farrowing, for duration of period from weaning to conception, it was necessary to include any value besides those presented in Table 3. In that case, in analysis of variance of studied traits for fertility results for first farrowing, forming of unit vector occurs, since the same values appear. The model practically ignores this effect (since in this case there is no variability – it is not possible to calculate variance, i.e. it is equal 0) in primiparous sows, whereas in case of results of realized fertility in latter parities this effect is taken into consideration without any disturbances.

In scalar notation, used models for estimation of variance components of litter size traits at birth (NBA, NSB and NTB) by REML method are presented in equation (1):

$$\begin{bmatrix} y_{1ijklmno} \\ y_{2ijklmno} \end{bmatrix} = \mu + P_i + S_j + LG_k + b_{1i}(x_{ijklmno} - \bar{x}) + b_{2i}(x_{ijklmno} - \bar{x})^2 + \\ + \begin{bmatrix} 0 \\ WC_l + b_3(z_{ijklmno} - \bar{z}) \end{bmatrix} + l_m + p_{ijklmno} + a_{ijklmno} + e_{ijklmno} \quad (1)$$

where $y_{qijklmno}$ represents NBA, NSB or NTB in litters of primiparous sows ($y = 1$) or older sows ($y = 2$). Class effects in the models were: parity (P_i), mating season as interaction of year and month (S_j), litter genotype (LG_k) and interval from weaning to conception (WC_l). Age of the sow at farrowing was modelled as square regression effect nested between parities ($x_{ijklmno}$), whereas duration of previous lactation was included as a linear regression effect (z_{ijklmo}). Random part of the models is presented through the effect of common environment in the litter where sows were born (l_m), permanent effect of the environment in sows' litters ($p_{ijklmno}$) and direct additive genetic effect ($a_{ijklmno}$). Previous analyses showed a negligible estimate for the maternal effect which was reason for exclusion of this effect from the models. This effect was probably not expressed because on every farm equalization of litters was done by crossfostering of piglets.

Model used for estimation of variance components of NW is presented in equation (2):

$$y_{ijklmno} = \mu + P_i + S_j + LG_k + R_l + b_{1i}(x_{ijklmno} - \bar{x}) + b_{2i}(x_{ijklmno} - \bar{x})^2 + \\ + l_m + p_{ijklmno} + a_{ijklmno} + e_{ijklmno} \quad (2)$$

where $y_{ijklmno}$ represents NW. Indications of the effects have the same meaning as in equation (1) except S_j which in this case is the effect of weaning season as an interaction between year and month. The model also included as a fixed effect, the number of reared piglets in litter (R_l), i.e. number of piglets in litter after equalization. The purpose for inclusion of this effect into the model was to diminish the effect of equalization procedure on variability of NW, as well as on objectivity of calculated parameters of dispersion. The statistical significance of systematic influences which have been included in the models and coefficients of determination were shown in the paper presented by Radojković et al. (2007).

For estimation of phenotypic and genetic variances of analysed traits by REML method, as well as establishing the percentage share of individual variance components in phenotypic variance, was used program package "VCE-5" (Kovac et al., 2002). Previously fixed part of the model was analysed using procedures of the program package "SAS/STAT" (SAS Inst. Inc., 2001).

Results and discussion

The shares of individual variance components in the phenotypic variance for NBA are presented in Table 4.

As presented in equations (1 and 2), direct additive genetic effect of the animal, effect of common litter environment and effect of permanent environment which sows provide to

their litters are included as random effects within mixed model used for estimation of parameters using REML method in all traits.

Table 4. *Estimation of the share of individual variance components in the phenotypic variance for number of live born piglets (NBA)*

Data set	$\hat{\sigma}^2_{Ph}$ *	$\hat{h}^2 \pm se_{(\hat{h}^2)}$	$\hat{l}^2 \pm se_{(\hat{l}^2)}$	$\hat{p}^2 \pm se_{(\hat{p}^2)}$
DS1	6.389	**0.055** ± 0.004	0.027 ± 0.005	0.038 ± 0.006
DS2	7.224	**0.050** ± 0.004	0.018 ± 0.004	0.053 ± 0.007
DS3	7.744	**0.076** ± 0.008	0.012 ± 0.004	0.058 ± 0.007
DS23	7.584	**0.064** ± 0.008	0.028 ± 0.005	0.048 ± 0.007

* - $\hat{\sigma}^2_{Ph}$: estimates of phenotypic variance; $\hat{h}^2$: estimates of the heritability; $\hat{l}^2$: estimates of the common litter effect; $\hat{p}^2$: estimates of the effect of permanent environment

Values of heritability coefficients varied from 0.050 (DS2) to 0.076 (DS3) and it can be stated that they were rather uniform in studied DS. Presented interval of values of heritability coefficients obtained in this research is very close to values shown by Kim (2001); Noguera et al. (2002); Chen et al. (2003); Stella et al. (2003); Vukovic (2003); and Holm et al. (2004). Higher values of studied parameter were presented by Logar (2000); Hannenberg et al. (2001); Peskovicova et al. (2002); Lukovic et al. (2004); Holm et al. (2005); Radojković et al. (2005); Lukovic et al. (2007); Ibanez-Escriche et al. (2010); Pandey and Singh (2010); and Wolf (2010).

The effect of litter in which sows were born explained from 1.2 % (DS3) to 2.8 % (DS23) of total variability of studied trait. Although the share in total variability of NBA explained by this effect was small, in certain cases (DS1) it was almost 50 % of direct additive genetic effect, which justified its inclusion in the model. Results obtained in this research are in accordance with results presented by Logar (2000); Lukovic et al. (2004) and Lukovic et al. (2007).

Share of the effect of sow permanent environment in total variability of NBA varied in interval from 0.038 (DS1) to 0.058 (DS3). Part of phenotypic variance explained with this effect was considerably high, in case of DS2 even higher than direct additive genetic effect. In concordance to results obtained in this research are results relating to influence of this effect on variation of studied trait presented by Logar (2000); Vukovic (2003); Stella et al. (2003); Wolf et al. (2005); Lukovic et al. (2004); Lukovic et al. (2007) and Wolf (2010). Somewhat higher values were presented by Hanenberg et al. (2001); Damgaard et al. (2003); Chen et al. (2003) and Barbosa et al. (2010), whereas Peskovicova et al. (2002) established lower value.

Estimation of the share of individual variance components in total variability of NSB established based on different data sources is presented in Table 5.

Heritability of studied trait varied within 0.004 (DS3) and 0.027 (DS2), and it was in accordance with results obtained in application of this method by Kim (2001) and Vukovic (2003), whereas Hannenberg et al. (2001) established slightly higher value for the studied parameter.

The effect of common litter environment explained a very small part of total variability of studied trait in the interval from 0.4 % (DS3) to 2.0 % (DS1), whereas the effect of

permanent environment of sows explained a slightly higher share which varied from 1.5 % (DS1) to 3.1 % (DS2) of total NSB variability.

Table 5. *Estimation of share of variance components in the phenotypic variance for number of still born piglets (NSB)*

Data set	$\hat{\sigma}^2_{Ph}$ *	$\hat{h}^2 \pm se_{(\hat{h}^2)}$	$\hat{l}^2 \pm se_{(\hat{l}^2)}$	$\hat{p}^2 \pm se_{(\hat{p}^2)}$
DS1	1.378	**0.025** ± 0.003	0.020 ± 0.003	0.015 ± 0.004
DS2	1.070	**0.027** ± 0.005	0.006 ± 0.003	0.031 ± 0.005
DS3	0.884	**0.004** ± 0.003	0.004 ± 0.003	0.025 ± 0.006
DS23	0.967	**0.016** ± 0.003	0.004 ± 0.002	0.028 ± 0.004

* - $\hat{\sigma}^2_{Ph}$: estimates of phenotypic variance; $\hat{h}^2$: estimates of the heritability; $\hat{l}^2$: estimates of the common litter effect; $\hat{p}^2$: estimates of the effect of permanent environment

In Table 6 the share of individual variance components in the phenotypic variance of NTB is presented.

Direct additive genetic effect explained from 6.5% (DS2) to 7.3% (DS3) of total variability of studied trait which are lower values compared to results presented by Logar (2000); Marios et al. (2000); Kim (2001); Hannenberg et al. (2001); Serenius et al. (2003); Vukovic (2003); Wolf et al. (2008); Barbosa et al. (2010) and Schneider et al. (2012).

The effect of common litter environment was lower than the direct additive genetic effect and its share in total variability of NTB varied in the interval from 0.015 (DS3) to 0.034 (DS23), which is in accordance with results presented by Logar (2000); Marios et al. (2000) and Bolet et al. (2001).

Table 6. *Estimation of the share of individual variance components in the phenotypic variance for number of total born piglets (NTB)*

Data set	$\hat{\sigma}^2_{Ph}$ *	$\hat{h}^2 \pm se_{(\hat{h}^2)}$	$\hat{l}^2 \pm se_{(\hat{l}^2)}$	$\hat{p}^2 \pm se_{(\hat{p}^2)}$
DS1	7.293	**0.066** ± 0.007	0.032 ± 0.005	0.040 ± 0.005
DS2	7.846	**0.065** ± 0.011	0.025 ± 0.009	0.030 ± 0.011
DS3	8.022	**0.073** ± 0.007	0.015 ± 0.007	0.065 ± 0.011
DS23	8.009	**0.067** ± 0.008	0.034 ± 0.005	0.041 ± 0.005

* - $\hat{\sigma}^2_{Ph}$: estimates of phenotypic variance; $\hat{h}^2$: estimates of the heritability; $\hat{l}^2$: estimates of the common litter effect; $\hat{p}^2$: estimates of the effect of permanent environment

Share of permanent effect of sow in the phenotypic variance of studied trait was lower than additive effect, but higher than the effect of litter, and varied in the interval from 0.030 (DS2) to 0.065 (DS3). Similar results in regard to share of this effect in total variability of NTB were obtained by Logar (2000); Kim (2000) and Wolf (2010). Contrary to mentioned authors Hannenberg et al. (2001); Damgaard et al. (2003) and Barbosa et al. (2010) established higher share of this effect compared to the values interval presented in this research, whereas the value of this parameter presented by Vukovic (2003) was lower.

Phenotypic variances, heritability as well as individual shares of other random effects in total variability of NW established based on analysed data sets are presented in Table 7.

Table 7. *Estimation of the share of individual variance components in the phenotypic variance for number of weaned/ reared piglets (NW)*

Data set	$\hat{\sigma}^2_{Ph}$ *	$\hat{h}^2 \pm se_{(\hat{h}^2)}$	$\hat{l}^2 \pm se_{(\hat{l}^2)}$	$\hat{p}^2 \pm se_{(\hat{p}^2)}$
DS1	2.384	**0.028** ± 0.005	0.007 ± 0.003	0.019 ± 0.006
DS2	1.593	**0.010** ± 0.004	0.002 ± 0.002	0.041 ± 0.006
DS3	1.739	**0.013** ± 0.005	0.002 ± 0.002	0.018 ± 0.006
DS23	1.674	**0.011** ± 0.003	0.012 ± 0.005	0.017 ± 0.004

* - $\hat{\sigma}^2_{Ph}$: estimates of phenotypic variance; $\hat{h}^2$: estimates of the heritability; $\hat{l}^2$: estimates of the common litter effect; $\hat{p}^2$: estimates of the effect of permanent environment

Heritabilities of studied trait were extremely low and varied in the interval from 0.010 (DS2) to 0.028 (DS1) what is in accordance with results obtained by Radojković et al. (2011). Obtained values of this parameter were lower than values established using this method procedure on data sets proportional in regard to scope of data used in the analysis by Kim (2001); Chen et al. (2003); Vukovic (2003); Radojković et al. (2005) and Pandey and Singh (2010). Even though the effect of number of reared piglets in litter after equalization (R_l) was included in applied model for genetic analysis of this trait, such attempt did not enable overcoming of the problem caused by equalization in analysis. With this model, in practice, the genetic variability of the ability of sow to rear certain number of piglets was estimated, and not the number of reared piglets in the narrow sense of the term.

The effect of litter explained only from 0.2 % (DS2 and DS3) to 1.2 % (DS23) of total variability.

Share of permanent effect of sows in total variability of NW was, with the exception of DS1, higher than direct additive effect and varied in the interval from 0.017 (DS23) to 0.041 (DS2), which is in concordance with the result presented by Chen et al. (2003). Slightly higher share of this effect in phenotypic variance of studied trait was established by Kim (2001), whereas Vukovic (2003) established lower value.

Conclusion

Share of additive genetic component of heritability in total phenotypic variability was the highest of all analysed variance components of litter size traits at farrowing (NBA, NSB and NTB), whereas in case of NW it was lower than the effect of permanent environment of sow which she provides to litters. Presented values of heritability varied within the limits of values which in application of REML method were also established by other authors except for NW whose deviance may be the consequence of litter equalization. Share of permanent environment of sow was higher than the share of litter (except in NSB, DS1) and mainly lower than share of direct genetic effect (except in cases: NBA, DS2; NSB, DS2, DS3 and DS23; NW, DS2, DS3, DS23). Share of phenotypic variability of studied traits which was explained by the effect of litter and permanent environment was within the limits of values presented in literature.

Based on share of individual variance components in the phenotypic variability, objectivity of estimated dispersion parameters as well as objectivity in determination of phenotypic values of analysed traits, it can be emphasized that NBA or NTB are most acceptable traits for inclusion into aggregate genotype in estimation of sows' breeding value.

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References

1. Andersen S 1998. The national Danish pig breeding program. In: International workshop Introduction of BLUP animal model in pigs. Praga-Uhrineves, 1998-09-03/05, 9.
2. Barbosa L, Lopes PS, Regazzi AJ, Torres RA, Santana MLJ, Veroneze R 2010. Estimation of variance components, genetic parameters and genetic trends for litter size of swines. Revista Brasileira de Zootecnia 39, 10, 2155-2159.
3. Bolet G, Bidanel JP, Ollivier L 2001. Sellection for litter size in pigs. II. Efficiency of closed and open selection lines. Genet. Sel. Evol. 33, 515-528.
4. Chen P, Baas TJ, Mabry JW, Koehler KJ, Dekkers JCM 2003. Genetic parameters and trends for litter traits in U.S. Yorkshire, Duroc, hampshire and Landrace pigs. Journal of Animal Science 81, 46-53.
5. Damgaard LH, Rydhmer L, Lovendahl P, Grandinson K 2003. Genetic parameters for within-litter variation in piglet birth weight and change in within-litter variation during suckling. Journal of Animal Science 81, 3, 604-610.
6. Hanenberg EHAT, Knol EF, Merks JWM 2001. Estimates of genetic parameters in different parities in Dutch Landrace pigs. Livestock Production Science 69, 179-186.
7. Holm B, Bakken M, Vangen O, Rekaya R 2004. Genetic analysis of litter size, parturition length, and birth assistance requirements in primiparous sows using a joint linear-threshold animal model. Journal of Animal Science 82, 9, 2528-2533.
8. Holm B, Bakken M, Vangen O, Rekaya R 2005. Genetic analysis of age at first service, return rate, litter size, and weaning-to-first service interval of gilts and sows. Journal of Animal Science 83, 1, 41-48.
9. Ibanez-Escriche N, Lopez de Maturana E, Noguera JL, Varona L 2010. An application of change-point recursive models to the relationship between litter size and number of stillborns in pigs. Journal of Animal Science 88, 11, 3493-3503.
10. Kim HJ 2001. Genetic parameters for productive and reproductive traits of sows in multiplier farms. Doctoral Dissertation. Gottingen, Faculty of Agricultural Sciences, Georg-August-University of Gottingen, 68 pp.
11. Kovac M, Groeneveld E, Garcia-Cortes LA 2002. VCE-5, a package for the estimation of dispersion parameters. In: 7th World congress on Genetic Applied to Livestock Production, Vol. 33, 741-742. Montpellier, 19-23 aug. 2002. INRA 30: 3-10.
12. Logar B 2000. Breeding value for litter size in pigs in population with different genetic groups. Master's thesis. Ljubljana, University of Ljubljana, Biotechnical Faculty, Zootechnical Department, 96 pp. [In Slovenian]

13. Lukovic Z, Gorjanc G, Malovrh S, Kovac M 2003. Estimation of genetic parameters for number of piglets born alive using random regression model. In: Proceedings of Genetica 2003. Ljubljana: Genetics Society of Slovenia, Bled, 2003-05-31/06-04, Javornik B., Luthar Z. (Eds.), 243-245.

14. Lukovic Z, Malovrh S, Gorjanc G, Kovac M 2004. A random regression model in analysis of litter size in pigs. South African Journal of Animal Science 34, (4), 241-248.

15. Lukovic Z, Uremovic M, Konjacic M, Uremovic Z, Vincek D 2007. Genetic Parameters for Litter Size in Pigs Using a Random Regression Model. Asian-australasian journal of animal sciences (1011-2367), 20 , 2, 160-165.

16. Marios D, Brisbane JR, Laforest JP 2000. Accounting for lactation lenght and weanong-to-conception interval in genetic evaluation for litter size in swine. Journal of Animal Science 78, 1796-1810.

17. Noguera JL, Varona L, Babot D, Estany J 2002. Multivariate analysis of litter size for multiple parities with production traits in pigs: I. Bayesian variance component estimation. . Journal of Animal Science 80, 10, 2540-2547.

18. Pandey A and Singh SK 2010. Estimates of heritability (h2) along with standard error of growth traits and reproductive traits in Landrace desi their cross-brads and data pooled over all genetic group pigs. International Journal of Genomics and Proteomics 1, 1, 9-11.

19. Peskovicova D, Wolf J, Groeneveld E, Wolfova M 2002. Simultaneous estimation of the covariance structure of traits from field test, station test and litter recording in pigs. Livestock Production Science 77, 155-165.

20. Radojković D, Petrović M, Mijatović M 2005. Estimation of genetic variability of fertility traits of pigs. 8th International Symposium "Modern Trends in Livestock Production", Belgrade – Zemun, Serbia and Montenegro, 5. – 8.10.2005.. Biotechnology in Animal Husbandry 21, 5-6, b. 2, 93-97.

21. Radojković D, Petrović M, Mijatović M, Radović Č 2007. Fixed part of the model for breeding value estimation in pigs based on litter size. 2nd International Congress on Animal Husbandry "New Perspectives and Challenges of Sustainable Livestock Farming", Belgrade - Zemun, Serbia, October 03-05, 2007. Biotechnology in Animal Husbandry 23, 5-6, b. 2, 429-436.

22. Radojković D, Petrović M, Mijatović M, Radović Č, Popovac M 2011. The effect of scope of analyzed data on the value of the heritability coefficient of litter size traits in pigs. 3rd International Congress "New Perspectives and Challenges of Sustainable Livestock Production" Belgrade, Republic of Serbia, 5 – 7th October, 2011. Biotechnology in animal husbandry, 27, 3, b. 2, pp. 777 – 783.

23. Radojković D, Petrović M, Radović Č 2012. Current and future chalenges of selection of pigs. Proceedings of The First International Symposium on Animal Science, November 8-10th, Belgrade, Serbia, pp. 195-206.

24. SAS Inst. Inc. (2001): The SAS System for Windows, Release 8.02. Cary, NC

25. Serenius T, Sevon-Aimonen M L, Mantysaari E 2003. Effect of service sire and validity of repeatability model in litter size and farrowing interval of Finish Landrace and Large White populations. Livestock Production Science 81, 213-222.

26. Schneider JF, Rempel LA, Rohrer GA 2012. Genome-wide association study of swine farrowing traits. Part I: Genetic and genomic parameter estimates. Journal of Animal Science 90, 10, 3353-3359.

27. Stella A, Stalder KJ, Saxton AM, Boettcher PJ 2003. Estimation of variances for gametic effects on litter size in Yorkshire and Landrace sows. Journal of Animal Science 81, 2171-2178.

28. Tholen E, Stork FJ, Trappman W, Schllander K 1998. Information management and BLUP in the pig breeding herdbook societies of North-Rhine Westfalia. In: International workshop Introduction of BLUP animal model in pigs, Praga-Uhrineves, 1998-09-03/05, 9.

29. Vukovic V 2003. Estimation of genetic parameters for most important reproductive and productive traits of pigs. Doctoral Dissertation. Skopje, University " St. Cyril and Methodius " Faculty of Agriculture, 115 pp. [In Macedonian]

30. Wolf J, Žakova E, Groeneveld E 2005. Genetic parameters for a joint genetic evaluation of production and reproduction traits in pigs. Czech Journal of Animal Science 50, 3, 96-103.

31. Wolf J, Žakova E, Groeneveld E 2008. Within-litter variation of birth weight in hyperprolific Czech Large White sows and its relation to litter size traits, stillborn piglets and losses until weaning. Livestock Science 115, 195–205.

32. Wolf J 2010. Heritabilities and genetic correlations for litter size and semen traits in Czech Large White and Landrace pigs. Journal of Animal Science 88, 9, 2893-2903.

Original paper

THE ASSESSMENT OF BREEDING VALUE OF FIRST FARROWED SOWS BY THE METHOD OF SELECTION INDICES

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Abstract

The goal of this research paper was to assess the breeding value of first farrowed Swedish Landrace sows by the means of selection indices method. The traits on the basis of which the breeding value of animals was assessed are following: daily liveweight gain, average thickness of collected back fat measured at five sites and number of liveborn piglets in the first litter. The liveweight gain and carcass quality traits determined at the end of performance test were corrected for the body mass of 100kg by the method of basic indexes and following mean values were determined: for corrected daily liveweight gain (KZDP) 499.92g/day and for corrected average collected backfat thickness (KSL) 20.01mm. The first farrowed sows on average produced 8.09 liveborn piglets in the litter. Studying the effect of the gilts` birth year and season on KZDP and KSL it was determined that the gilts` birth year and season had no statistically significant influence ($P>0.05$) on KZDP variation but they had a statistically significant effect on KSL ($P<0.01$). The year and the season of farrowing and the class of backfat thickness in performance test did not display any statistically significant effect ($P>0.05$) on BZPL, while the KZDP class and the age at first farrowing had a statistically significant effect on the variability of these trait ($P<0.05$; $P<0.01$). All studied traits varied statistically significantly ($P<0.01$) under the impact of the gilts` sire or dam. Heritability coefficients were: $h^2= 0.402$ for KZDP, $h^2= 0.261$ for KSL and $h^2= 0.177$ for BZPL. The relation between KZDP and KSL was of a medium strength both at phenotype and genetic levels ($r_{ph}=0.491$; $r_g=0.411$), while the relation of these traits with BZPL did not exist, except for the genetic relationship between KSL and KZDP which was of a medium strength ($r_g=0.252$). Three equations for the selection indexes were constructed among which as the most optimal was chosen the one which includes all three traits (KZDP, KSL and BZPL) and whose correlation coefficent of selection index and aggregate genotype was $r_{IAG} = 0.5473$.

Key words: *backfat thickness, daily liveweight gain, heritability, piglets, selection index*

Introduction

The assessment of animal breeding value represents a very delicate procedure which should consider a number of traits of which some have better and some have poorer values and on the basis thereon to make a conclusion and choose or remove the animal from further breeding (Popovac et al., 2014). One of those procedures is the method of selection

indexes. The method of selection indexes has found its special application in pig breeding in the countries where the pig breeding is not at the very high level and where the conditions for the use of BLAP AM-method on which the assessment of swine breeding value is based in the countries with developed livestock productions have not been created yet. A practical application of this method is relatively simple because when the equation for the assessment of breeding value is constructed it means the simple exchange of determined phenotype values of the traits of animals whose breeding value is being assessed and as the result we obtain the assessed animal breeding value expressed in index points (Radojković et al., 2010). The major characteristic of this metodological procedure is that it can compensate for the values of traits and produce, as a final result of the assessment of breeding value, the number of index points on whose basis we rank the animals whose breeding value is being assessed. This methodological procedure is particularly good in the conditions in which the traits have a high heritability and where the impact of environment factors is low (Sellier et al., 2000).

Taking into account the fact that liveweight gain and carcass quality traits in pigs display from medium to high heritability (Brkić et al., 2001; Imboonta et al., 2007; Hoque and Suzuki, 2008; Saintilan et al., 2012), and the fact that reproductive traits, although they have low heritability (Damgaard et al., 2003; Luković et al., 2004; Radojković et al., 2012) influence in the same degree a comprehensive estimation of sows breeding value, the equation of selection index for the assessment of breeding value of sows has been constructed on the basis of their daily liveweight gain and average backfat thickness measured at the end of performance test and on the number of liveborn piglets in the first litter.

Material and methods

A set research goal was to construct the equation of selection indexes that served to assess the breeding value of the farm-raised Swedish Landrace sows breed on the basis of their productive results obtained in performance test and during the first farrowing. Data set on which this was performed contained the information for 1020 sows born in 5 consecutive years and farrowed in 6 consecutive years. The sows were the ascendants of forty-one sires, where the minimum number of daughters per one sire was 10 for the reason of obtaining the accuracy in calculating the genetic parameters whilst the each sire on average produced 24.88 daughters included in the analysis.

The traits included in the analysis were: corected daily liveweight gain at the end of test (**KZDP**), corrected average collected backfat thickness measured on 5 sites on the backs at the end of test (**KSL)** and number of liveborn piglets in the first litter (**BZPL**).

The correction of the liveweight gain and carcass quality traits was performed for the body mass of gilts of 100kg, by the method of basic indexes based on regression analysis.

Statistical processing of data included the establishing of descriptive statistical indicators and measures of variation, then examination of the variability of traits on phenotype and genetic level and phenotype and genetic relationship of these traits. Descriptive statistical processing of data was done by the use of programme package STATISTICA, *Version 5.0.* Variability of traits on phenotype and genetic level, heritability and interrelationship of the same traits and parameters necessary (variances and covariances) for the construction of selection indexes were calculated by the method of the least squares by means of programme packages LSMLMW – Harvey (1990) and SAS, 9.1.3.(2007).

Several mixed models were used on whose basis we studied the variability of the traits of liveweight gain and carcass quality traits and reproductive traits;

$$\textbf{Model 1: } Y_{ijk} = \mu + G_i + S_j + o_{ijk} + e_{ijk}$$

Where: Y_{ijk} – is the manifestation of the trait (KZDP, KSL), μ – population general average, G_i – fixed effect of the gilts` birth year, $S_j$ – fixed effect of the gilts` birth season, o_{ijk} – random effect of the gilt`s sire, e_{ijk} – random effect of non-determined factors.

$$\textbf{Model 2: } Y_{ijklm} = \mu + G_i + S_j + P_k + D_l + b\,(X - \bar{X}) + o_{ijklm} + e_{ijklm}$$

Where: Y_{ijklm} – is the trait manifestation (BZPL), μ – population general average, G_i – fixed effect of the sows` first farrowing year, S_j – fixed effect of the season of the first farrowing of sows, P_k – fixed effect of the class of the corrected daily liveweight gain at the end of test, D_l – fixed effect of the class of corrected collected backfat thickness at the end of test, $b\,(X - \bar{X})$ – linear regression effect of the age of sows at the first farrowing, o_{ijklm} – random effect of sows` sire, e_{ijkil} – random effect of non-determined factors.

$$\textbf{Model 3: } Y_{ijk} = \mu + G_i + S_j + o_{ijk} + e_{ijk}$$

Where: Y_{ijkl} – is the trait manifestation (KZDP, KSL, BZPL), μ – population general average, G_i – fixed effect of the year of birth of gilt-sow, S_j – fixed effect of the season of birth of gilt-sow, o_{ijk} – random effect of the sire of gilt-sow, e_{ijk} – random effect of non-determined factors.

Model 3 is constructed in such a way so as to include only those factors which displayed statistically significant effect on some of the studied traits and which could influence both groups of traits, in order to calculate the heritability and relationship of the liveweight gain trait and reproductive traits that served for the construction of selection index.

Heritability of studied traits was calculated by the method of interclass correlation of half-sibs on father´s side. Heritability equation can be expressed in a following way:

$$h^2 = (4\sigma_s^2) / (\sigma_s^2 + \sigma_e^2)$$

Where: h^2 – is a heritability coefficient (heritability), σ_s^2 – intersire variance, σ_e^2 – intra-sire (error) variance.

Correlation coefficients on phenotype and genetic levels were calculated on the basis of following equation:

$$r_{XY} = (Cov_{XY}) / \sqrt{(\sigma_X^2 + \sigma_Y^2)}$$

Where: r_{XY} – is the correlation coefficient between the X and Y traits, Cov_{XY} – covariance between X and Y traits, σ_X^2 – variance of the X trait, σ_Y^2 – phenotype variance of the Y trait.

The strength of the interrelation of traits was determined on the basis of Roemer-Orphal classification (Latinović, 1996). Statistical significance of correlation coefficents was determined on the basis of the tables of statistical significance produced by Snedekor and Cochran (1980).

Breeding value of first farrowed sows assessed by the method of selection indexes can be expressed by following equation:

$$I = b_1 (X_1 - \bar{X}_1) + b_2 (X_2 - \bar{X}_2) + \ldots\ldots + b_n (X_n - \bar{X}_n)$$

Where: I – is a relative animal breeding value estimated by selection index (value of selection index determined for each animal), b_i – partial coefficients of multiple regression

for each trait included in selection index, $(X_i - \bar{X}_i)$ – difference between the phenotype value of the individual trait and population average.

For calculating the partial regression coefficients (b) we used matrix equation from which the following equation stems out:

$$P * b = G * v$$

$$b = P^{-1} * G * v$$

Where: b – is the vector of the solutions of the partial regression coefficients for each trait (X), P^{-1} – inverse phenotype matrix formed of variances and covariances of the traits included in selection index, G – genetic matrix formed of variances and covariances of the traits included in selection index, v – vector of relative economic value of the traits included in selection index.

The accuracy of assessed breeding value by the method of selection indexes is expressed by the correlation coefficent between selection index and agreggate genotype of each individual. Higher value of this correlation coefficent increases also the accuracy of the assessment of the animal breeding value by the means of selection indexes. Agreggate genotype can be explained by following equation:

$$AG = v_1 * h^2_1 * X_1 + v_2 * h^2_2 * X_2 + ... + v_i * h^2_i * X_i$$

Where: AG – is an agreggate genotype, v_i – economic value of the trait (X_i), h^2_i – trait heritability coefficient (X_i), X_i – trait phenotype value of each individual.

Correlation coefficient of selection index and agreggate genotype is calculated by means of following equation:

$$r_{IAG} = \sigma_I / \sigma_{AG}$$

Where: r_{IAG} – is a correlation coefficient between selection index and agreggate genotype, σ_I – standard deviation of selection index, σ_{AG} – standard deviation of agreggate genotype.

For calculating the economic value of the traits included in selection index we used the methodology used by Popovac et al. (2014), where the traits economic value is presented as a ratio of cost fluctuations per trait unit when the traits have been changed for the set selection goal. The set selection goal was to improve the traits for 10% out of which we obtained following economic values of the traits:

BZPL:	1,
KZDP:	0.02334,
KZLSL:	-0.58348.

Results and discussion

Table 1 shows the average values and variability of corrected daily liveweight gain and corrected collected backfat thickness in peformance tested gilts of Swedish Landrace breed.

The average value of corrected daily liveweight gain at the end of test in examined gilts was 499.92 g/day, what is in harmony with the results obtained by Mijatović et al. (2009), taking into account that they determined a somewhat higher value of corrected daily liveweight gain of 502 g/day. Vidović et al. (2012), determined a significantly higher value of this trait in Swedish Landrace gilts which was 670 g/day. Corrected thickness of

collected backfat thickness at the end of performance test in tested gilts on average was 20.01 mm, what are somewhat lower values in relation to the values (22.30 mm) determined by Radivojević, (1992) in his research.

Table 1. *Average value and variability for KZDP and KSL*

Trait	N	$\bar{X}$	SD	Min	Max	CV	μ ± se
Corrected daily liveweight gain (g) **KDP**	1020	499.92	74.50	317.0	705.0	14.90	496.19±2.68
Corrected thickness of collected backfat thickness (mm) **KSL**	1020	20.01	3.76	10.3	34.1	18.79	19.97±0.13

The years and the seasons of birth and the sires of gilts are included in the model as factors which helped to explain the existing variability of KZDP and KSL at the end of direct test. Statistical significance of these factors and F_{exp} values per studied years and seasons of birth and the gilts` sires are shown in Table 2.

Table 2. *Statistical significance and F_{exp} values for KZDP and KSL per years, seasons of birth and gilts sires*

Traits	Effects			R^2
	Birth season	Birth year	Sire	
KZDP F_{exp}	1.668^{ns}	0.133^{ns}	3.339 **	0.158
KSL F_{exp}	7.804**	7.960**	2.562 **	0.187

^{ns}-no statistical significance, **-P<0.01; R^2- model determination coefficient

The results of the variance analysis displayed in Table 2 show that the year and the season of birth of gilts did not express (P>0.05) a significant effect on KZDP, while KSL statistically significantly (P<0.01) varied under the influence of the year and the season of birth of gilts. The research also showed a statistically significant (P<0.01) effect of sire of gilts on variability of studied traits of liveweight gain and carcass quality.

The research by Gogić et al. (2012) and Radović et al. (2012) show statistically significant variation in daily liveweight gain and the backfat thickness under the effect of gilts` birth year. Significant effect of the season of gilts` birth on KZDP and KSL established Vuković (1998) in his research. On the other hand, Popovac et al. (2014) did not determine a significant variation of the liveweight gain trait and carcass quality trait under the influence of the year and season of gilts` birth, while the same authors established that the sire had statistically highly significant effect on daily liveweight gain and backfat thickness in performance tested gilts. The results of research presented in this paper are in harmony with the research of Mijatović et al. (2009) and Vidović et al. (2012), who determined a statistically significant variation of daily livewight gain under the influence of gilts` sire. Variability of backfat thickness measured on different sites and of daily liveweight gain in performance tested gilts under the influence of sire was determined also by Brkić (2002) in his research.

Average value and variability of the number of liveborns in litter (BZPL) in first farrowed sows of Swedish Landrace included in this trial are shown in Table 3.

Table 3. *Average value and variability for BZPL*

Trait	N	$\bar{X}$	SD	Min	Max	CV	μ ± se
Number of liveborn in first farrowed litter **(BZPL)**	1020	8.09	2.41	1	14	29.79	7.91±0.27

The first farrowed sows on average had 8.09 liveborns in litter, what are the values lower or in concordance with the values for this trait in comparison with the records found in literature. Radojković (2007) determined the value of this parameter of fertility in the interval from 8.08 to 9.14 liveborn piglets in litter on different farms. Average values of BZPL determined in different studies are as follows: Radojković (2000) 8.20, Brkić (2002) 9.63, Damgaard et al. (2003) 10.60 and Bečkova and Vaclavkova (2008) 10.28 liveborn piglets in the first farrowed litter.

Statistical significance of the factors and F_{exp} values for BZPL per studied years and seasons of sows farrowing, the class of daily liveweight gain and the class of backfat thickness, regression effect at first farrowing and sows` sire are shown in Table 4.

Table 4. *Statistical significance and F_{exp} values for BZPL per years and seasons of farrowing, classes of KZDP and KSL, age at first farrowing and sows sires*

Traits	Effects						R^2
	Year of farrowing	Season of farrowing	Class KZDP	Class KSL	Age at first farrowing	Sire	
BZPL F_{exp}	2.096^{NZ}	0.178^{NZ}	2.717*	0.788^{NZ}	41.346**	1.991**	0.143

^{ns}- P>0.05; **-P<0.01; *-P<0.05; R^2- model determination coefficient.

The results displayed in Table 4 show that the year and season of the sows` first farrowing did not exhibit (P>0.05) statistically significant effect on BZPL.

F values shown in Table 4 suggest that the class KZDP affects statistically significantly (P<0.05) BZPL, but they show no statistically significant (P>0.05) effect of KSL class on the same trait. The classes are formed (6 classes) in such a way that the values that have one standard deviation more or less in relation to the average make one class. Excluding the classes 1 and 6 for KZDP due to a relatively small number of animals which are within these classes there was a trend of increasing the number of liveborn piglets with the increase of the KZDP class.

Besides mentioned fixed effects on BZPL the regression effect of age at first farrowing of sows on this trait was also examined and it was established that BZPL statistically significantly (P<0.01) varies depending on the age of sow in the moment of first farrowing. Average age of the first farrowed sows at farrowing was 343.68 days, where with the increase of the age at first farrowing for 1 day the litter increased by 0.02 liveborn piglets as well.

Analyzing the effect of sows` sires on BZPL during its first farrowing it was found out that the sire exhibits statistically significant (P<0.01) effect on BZPL.

Studying the phenotype variability of traits of fertility of sows of Swedish Landrace breed Radojković et al. (2007) reports that the year and season of farrowing display no statistically significant effect on BZPL and other traits of the size of litter at first farrowing where the results of these studies are in harmony with the results of previously mentioned

authors. Significant effect of the year and season of farrowing on BZPL determined Petrović et al. (1998) and Sobczynska et al. (2007) in their research.

The results of this research are consistent with the research of Čehov and Tvrdon (2008) and Mijatović et al. (2009) who established statistically significant variation of BZPL depending on the class of daily liveweight gain obtained by tested Swedish Landrace gilts, the authors also state that the sows with highest daily liveweight gain also had the greatest number of liveborn piglets in litter.

The results obtained in this paper confirm the results of Radojković (2007) that age at first farrowing exhibits statistically significant effect on first farrowed BZPL.

Analysing the effect of sires of first farrowed sows on BZPL in their first litter in the available literature we found some results which also indicate to the significant effect of the sire on this trait (Brkić, 2002; Sobczynska et al., 2007; Radojković et al., 2007), what was shown also in this research.

Table 5 shows the heritability values (h^2) for KZDP, KSL and BZPL traits obtained by the use of different mixed models on whose basis we have studied variability, heritability and relationship between these traits.

Table 5. *Values of heritability coefficients and heritability error*

Trait	Model 1		Model 2		Model 3	
	h^2	**SE h^2**	**h^2**	**SE h^2**	**h^2**	**SE h^2**
KZDP	0.377	0.106	-	-	0.402	0.110
KSL	0.260	0.087	-	-	0.261	0.087
BZPL	-	-	0.172	0.072	0.177	0.072

h^2-heritability coefficient; **SE h^2**-heritability error

In Table 5 we can see that the heritability values obtained by the use of different models of the same traits are equalised. Heritability coefficients (h^2=0.377; 0.402) for KZDP show mean heritability of this trait and give possibility of successful selection-improving work when the improvement of this trait is in question. Heritability value of h^2=0.260; 0.261 for KSL depending on the model applied, indicate also to the mean heritability of this trait. Contrary to the traits of liveweight gain and carcass quality the reproductive traits have low heritability what is shown also by heritability coefficients obtained for BZPL of h^2=0.172; 0.177.

The results of this research are very similar to the results of Vuković et al. (2007) and Szynder-Nedza et al. (2010), who determined the heritability of gilts` daily liveweight gain of h^2=0.270 or h^2=0.390 allocating this trait into the group of mean heritability value. Low heritability of h^2=0.140 for KZDP in gilts at the end of test in their research using the REML method was established by Malovrh and Kovač (1999) defining this trait as a low heritability trait.

Backfat thickness has a medium heritability what is shown by the research of Vuković et al. (2007) and Urankarova et al. (2012), where the authors state that phenotype manifestation of this trait depends 38% or 28% upon genetic factors. High heritability of backfat thickness of h^2=0.610 at the end of test was determined in the research by Imboonta et al. (2007). All showed values of heritability obtained in this research for KSL are lower in relation to the values found in literature.

Heritability values for BZPL determined in this research are higher in relation to the heritability obtained by the means of different methods in the studies by: Damgaard et al. (2003) h^2=0.120, Luković et al. (2004) h^2=0.102 and Radojković et al. (2012) h^2=0.064.

In Table 6 the values of the coefficients of phenotype correlations are shown above the diagonal line while the values of coefficients of genetic correlations are shown under the diagonal line.

Table 6. *Coefficients of phenotype and genetic correlations between KZDP, KSL and BZPL*

Trait	KZDP	KSL	BZPL
KZDP	-	0.491**	0.087**
KSL	0.411**	-	0.039^{ns}
BZPL	0.092**	0.252**	-

[1)]Correlation coefficient for 5 and 1% safety (d.f.=1000) is 0. 062 and 0.081.

Relationship between KZDP and KSL is statistically significant and of medium strength nearing close to a strong traits relationship. Coefficient of phenotype correlation of r_{ph}=0.491 shows that in the individuals that have higher daily liveweight gains the share of adipose tissue in total gain increases as well. On the other hand, positive value of genetic correlation coefficient of r_g=0.411 warns us that one-sided selection for high daily liveweight gains could lead to the aggravation of the quality of carcass in the pig populations in which this kind of selection is being applied.

Relationship between KZDP and BZPL is statistically significant, but there is correlation neither at phenotype nor at genetic level (r_{ph}= 0.087; r_g=0.092).

Correlation coefficient (r_{ph}=0.039) between KSL and BZPL at the phenotype level is statistically insignificant and shows that there is no relationship of these traits. Genetic relation (r_g=0.252) is statistically significant and of medium strength.

Hicks et al. (1998) in their research on the relationship between the daily liveweight gain and backfat thickness at the end of test determined a negative very low or almost no relationship both at phenotype and genetic level (r_{ph}=-0.14; r_g=-0.08), taking into account that in their calculations they included the results of measuring the boars that finished the performance test. Results obtained by Nguyen and McPhee (2005) for the relationship between daily liveweight gain and backfat thickness show a moderate negative coefficient of genetic correlation of r_g=-0.250 indicating that in the individuals selected for higher daily gains and meatiness the backfat thickness decreased at the same time. Relationship between the traits displayed in this paper is both at the phenotype and genetic level significantly stronger with a positive pre-sign what is not in harmony with the results obtained by previous two groups of authors. The value obtained for genetic correlation coefficient is very close to the value of genetic correlation coefficient (r_g=-0.437) determined by Brkić et al. (2001) where they determined negative pre-sign of this coefficient indicating the possibility of improving one trait so that in indirect way the value of other trait improves as well.

Obtained results are in harmony with the results obtained by Vuković (2003), who also did not establish any phenotype correlation between KZDP and KSL on one hand and BZPL on the other, taking into account that he determined lower value of correlation coefficient of negative pre-sign of r_{ph}=-0.006 between KZDP and BZPL. Absence of relationship on genetic level between backfat thickness at the end of test and the number of liveborn piglets in the first litter was determined by Holm et al. (2004) showing coefficient of

genetic correlation of r_g=-0.000, but the results shown in this paper are not in harmony with the results of the above mentioned group of authors.

On the basis of parameters determined by the analyses the several mixed selection models were constructed where on the basis of the value of correlation coefficient of selection index and agreggate genotype on one hand and selection strategy on the other we chose the best equation of selection index for the assessment of breeding value of sows after the first farrowing. Table 7 shows the equations of selection indexes and correlation coefficent of index and agreggate genotype (r_{IAG}).

Table 7. *Equations of selection indexes and correlation coefficient of index for assessment of breeding value of first farrowed sows and agreggate genotype (r_{IAG})*

Selection index	r_{IAG}
$SI_1 = 0.2072^a (x_1 - 8.09) + 0.0099^b (x_2 - 499.92) - 0.1471^c (x_3 - 20.01)$	0.5473
$SI_2 = 0.1684^a (x_1 - 8.09) + 0.0097^b (x_2 - 499.92)$	0.5492
$SI_3 = 0.2140^a (x_1 - 809) - 0.1824^c (x_3 - 20.01)$	0.5158

a – values of partial regression coefficients for BZPL; **b** – values of partial regression coefficients for KZDP; **c** – values of partial regression coefficients for KSL; $\mathbf{x_i}$ – phenotype values of traits of each individual.

The accuracy of constructed mixed selection indexes measured by correlation coefficents r_{IAG} ranged from r_{IAG} = 0.5158 in index 3 to r_{IAG} = 0.5492 in index 2. Approximate values of correlation coefficients of different indexes give possibility to choose selection index which includes a greater number of traits to which we gave a similar importance in this research while at the same time the assessed breeding value of individuals do not loose the accuracy. In line with this as the most optimal selection index for assessing the breeding value of first farrowed sows we chose SI_1 which includes three traits and they are BZPL, KZDP and KSL.

Obtained values for the correlation coefficients for index and agreggate genotype are lower in relation to the values determined by Brkić (2002), who obtained the indicators of accuracy of mixed selection indexes in the interval of r_{IAG} = 0.572 to r_{IAG} = 0.640. The author included a number of traits of gain and reproductive traits and obtained as the most accurate index for the assessment of sows breeding value the one that included following traits: age at the end of test, daily liveweight gain, meatiness, number of total born piglets, the litter mass at 28th day and index of piglets mass at 28th day. Observing the accuracy (from r_{IAG} = 0.231 to r_{IAG} = 0.405) of selection indexes constructed by Radojković et al. (2009) for the assessment of sows breeding value on the basis of their reproductive indicators and accuracy (from r_{IAG} = 0.318 to r_{IAG} = 0.821) of selection indexes for assessment of gilts breeding value displayed by Brkić (2002) it can be concluded that the accuracy of mixed selection indexes is somewhere in the middle between the accuracy of these two groups of indexes. The reason for lower accuracy of selection indexes for assessment of animal breeding value on the basis of their reproductive performances could be that these traits have a low heritability what negatively affects the accuracy of obtained estimation of index so all this influenced the assessment of animal breeding value on the basis of the liveweight gain and carcass quality traits to have medium to high heritability by means of selection indexes which found a broad application in practice (Cleveland and See, 2006). What is evident when we speak about the liveweight gain trait and carcass quality trait included in the model for the assessment of swine breeding value is that the daily liveweight gain and backfat thickness are the traits that have a primary place in

selection in this kind of animals (Suzuki et al., 2005; Kralik et al., 2007; Popovac et al., 2014).

Conclusion

Analysing metodological procedure for the construction of selection indices we arrived at the conclusion that this is a relatively simple method by which we can assess animal genetic potential and select breeding animals in the production conditions in which it is not possible to use more complex selection methods. It should also be pointed out that selection indices involving liveweight gain and carcass quality traits as well as reproductive traits represent a good method applied to assess sow breeding value since it provides a unique numerical value as sublimation of positive and negative values of these two groups of equally important traits in sow selection.

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References

1. Bečková R and Václavková E 2008. The effect of age at the first mating on the longevity of Czech Landrace and Czech Large White sows. Reserch in Pig Breeding 2, 2, 1-5.
2. Brkić N, Gajić Ž and Pušić M 2001. The genetic and phenotypic variability and relationship of some growth and slaughter characteristics of the performance tested gilts. Genetika 33, 1-2, 1-10.
3. Brkić N. 2002. Evaluation of breeding value of sows by integrated selection indeks. PhD, University of Belgrade, faculty of Agriculture.
4. Cleveland E and See T 2006. Selection Programs for Seedstock Producers. Improvement Federation Factsheet. www. Pork.org/filelibrary/Factsheets/PIGFactsheets/NEWfactSheets/06-02-02g.pdf
5. Čehova M and Tvrdon Z 2008. An influence of different growth ability of Czech Large White gilts and Landrace gilts on their reproductive performance. Czech Journal of Animal Science 47, 8, 319–327.
6. Damgaard HL, Rydhmer L, Lovendahl P and Grandinson K 2003. Genetic parameters for within-litter variation in piglet birth weight and change in within-litter variation during suckling. Journal of Animal Science 81, 3, 604-610.
7. Gogić M, Petrović M, Živković B, Radović Č, Radojković D, Parunović N and Marinkov G 2012. The effect of various factors on traits of performance tested gilts. Biotechnology in Animal Husbandry 28, 2, 313-322.
8. Harvey WR 1990. User's guide for LSMLMW and MIXMDL, PC-2 Version.
9. Hicks C, Satoh M, Ishii K, Kuroki S, Fujiwara T and Furukawa T 1998. Effect of sex on estimates of genetic parameters for daily gain and ultrasonic backfat thickness in swine. Asian-Australian Journal of Animal Science 12, 5, 677-681.
10. Hoque A and Suzuki K 2008. Genetic parameters for production traits and measures of residual feed intake Duroc and Landrace pig. Animal Science Journal 79, 543-549.

11. Holm B, Bakken M, Klemetsdal G and Vangen O 2004. Genetic correlations between reproduction and production traits in swine. Journal of Animal Science 82, 12, 3458-3463.

12. Imboonta N, Rydhmer L and Tumwasorn S 2007. Genetic parameters for reproduction and production traits of Landrace sows in Thailand. Journal of Animal Science 85, 1, 53-59.

13. Kralik G, Kušec G, Kralik D and Margeta V 2007. Svinjogojstvo. Poljoprivredni fakultet Sveučilišta Josipa Juraja Štrosmajera u Osjeku, Agronomski fakultet Sveučilišta u Mostaru.

14. Latinović D 1996. Populaciona genetika i oplemenjivanje domaćih životinja (praktikum). Univerzitet u Beogradu.

15. Luković Z, Malovrh Š, Gorjanc G and Kovač M. 2004. A random regression model in analysis of litter size in pigs. South African Journal of Animal Science 34, 4, 242-248.

16. Malovrh Š and Kvač M 1999. Genetic correlations between test station and on-farm performance for backfat thickness and daily gain. Acta Agraria Kaposváriensis 3, 2, 145-154.

17. Mijatović M, Petrović M, Radojković D, Pušić M and Radović Č 2009. Influence of performance test traits of gilts on variability of their reproductive performance as primiparous sows. Biotechnology in Animal Husbandry 25, 5-6, 825-831.

18. Nguyen HN and McPhee PC 2005. Genetic parameters and responses of performance and body composition traits in pigs selected for high and low growth rate on fixed ration over a set time. Genet. Sel. Evol. 37, 199-213.

19. Petrović M, Teodorović M, Radojković D and Kosovac O 1998: Determinacija faktora koji utiču na variranje reproduktivnih osobina krmača. Savremena poljoprivreda 47, 5-6, 121-126.

20. Popovac M, Petrović M, Radojković D, Stanojević D, Miletić M and Perišić P 2014. The assessment of genetic potential in performance tested gilts by means selection indexes method. Genetika 46, 1, 95-104.

21. Radivojević R 1992. Association of the direct test with production traits of sows of the swedish landrace breed. PhD. University of Novi Sad, faculty of Agriculture.

22. Radojković D 2000. Estimation of breeding value of sows and effects of direct and indirect selection. MSc, University of Belgrade, faculty of Agriculture.

23. Radojković D 2007. Variability of fertility traits and breeding value estimation of pigs. PhD, University of Belgrade faculty of Agriculture.

24. Radojković D, Petrović M, Radović Č, Parunović N, Popovac M and Gogić M 2012. Heritability and correlation of litter traits in pigs determined by REML method. Biotechnology in Animal Husbandry 28, 4, 818-823.

25. Radojković D, Petrović M, Mijatović M and Radović Č 2007. Fixed part of the model for breeding value estimation in pigs based on litter size. Biotechnology in Animal Husbandry 23, 5-6, 429-436.

26. Radojković D, Petrović M, Mijatović M and Radović Č 2009. The effect of scope of analyzed data on accuracy of selection indices for evaluation of sow breeding value. Biotechnology in Animal Husbandry 25, 5-6, 429-436.

27. Radojković D, Petrović M, Mijatović M and Radović Č 2010. Metodologija za procenu priplodne vrednosti svinja na osnovu osobina plodnosti primenom selekcijskih indeksa. Biotechnology in Animal Husbandry 26, spec.issue, 113-121.

28. Radović Č, Petrović M, Parunović N, Brkić N, Živković B, Gogić M and Stanišić N 2012. The effect of genotype and year on traits of performance tested gilts. Biotechnology in Animal Husbandry 28, 3, 463-468.

29. Saintilan R, Sellier P, Billon Y and Gilbert H 2012. Genetic correlations between males, females and castrates for residual intake, feed conversion ratio, growth rate and carcass composition traits Large White growing pigs. Journal of Animal Breeding and Genetic 129, 103-106.

30. SAS 2007. SAS Version 9. 1. 3, SAS Institute Inc. Cary, NC, USA.

31. Sellier P, Le Roy P, Fouilloux NM, Gruand J and Bonneau M 2000. Responses to restricted index selection and genetic parameters for fat androstenone level and sexual maturity status of young boars. Livestock Production Science 63, 265–274.

32. Snedekor WG and Cochran GW 1980. Statistical Methods.iowa State University.

33. Sobczynska M, Blicharski T, Korwin-Kossakowska A and Kamyczek M 2007. Materinal effects on farrowing and pre-weaning traits in Line 990 pigs. Animal Science Papers and Reports 25, 3, 173-182.

34. StatSoft Inc. 1995. STATISTICA for Windows, Version 5.0.

35. Suzuki K, Kadowaki H, Shibata T, Uchida H and Nishida A 2005. Selection for daily gain, loin-eye area, backfat, thickness and intramuscular fat based on desired gains over seven generations of Duroc pigs. Livestock Production Science, 97, 193-202.

36. Szyndler-Nędza M, Tyra M and Różycki M 2010. Coefficients of heritability for fattening and slaughter traits included in a modified performance testing method. Ann. Anim. Sci. 10, 2, 117-125.

37. Urankar J, Flisar T, Kovač M and Malovrh Š 2012. Effect of group size on breeding value accuracy in gilts. 20th Int. Symp. "Animal Science Days", Kranjska gora, Slovenia, Sept. 19th−21st, 2012. Acta argiculturae Slovenica, Supplement 3, 187–191.

38. Vidović V, Krnjajić J, Lukač D, Višnjić V and Stupar M 2012. Growth intensity of the fertile breed gilts in the nucleus pig farm. Biotechnology in Animal Husbandry 28, 4, 787-796.

39. Vuković V 1998. Variability and connection between production and reproduction traits in swines with different genotypes. MSc, University of Belgrade, faculty of Agriculture.

40. Vuković V 2003. Estimation of genetic parameters for most important reproductive and productive traits of pigs. PhD, University St. Cyril and Methodius, faculty of Agriculture Skopje.

41. Vuković V, Andonov S, Kovač M, Kocevski D and Malovrh S 2007. Estimation of genetic parameters for number of teats and traits from performance test of gilts. 3rd Joint Meeting of the Network of Universities and Research Institutions of Animal Science of the South Eastern European Countries, Thessaloniki 10-12 February 2007.

Review paper

ANATOLIAN WATER BUFFALO HUSBANDRY IN TURKEY

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Abstract

Anatolian Water Buffalo is found mostly in the half northwestern of Turkey including north part of the middle Anatolia. It is more common along the coast of the Black Sea. It is also found in Eastern Anatolia. From a taxonomical point of view it is classified as 'Mediterranean' type. In the past, buffalo farming has been an important production source for Turkey (1 117 000 heads assessed in 1971), while currently the population size is of only 117,591 heads. In Turkey most farmers keep 1-2 buffaloes for family consumption and this system is very widespread in villages while farms with around 100 heads are located near to the big cities. In spite of the popular indifference, farming of this species has survived in order to promote productive systems in agreement with sustainable rural development and trend to revalue autochthonous genetic types. There are *in situ* and *ex situ* conservation programmes and incentive premiums in order to stop the reducing tendency of population.

Nationwide water buffalo improvement program has also initiated so-called community based herd improvement program.

Anatolian Water Buffalo is reared for triple aptitude: meat, milk and draught. Most meat is especially used for making sausages. Concerning milk production, research is evidencing that controlled farming and feeding conditions may significantly improve performances. Milk is also employed to make a very popular traditional product, known as 'lüle kaymağı'and yogurt production.

Key words: *Anatolian Water Buffalo husbandry herd improvement program*

Introduction

Anatolian Water Buffaloes breed morphologican characteristics is registred by the National committee for breed registration (Anonymus, 2004). According to this source Anatolian Water Buffalo is generally small structured with easy temperament and having generally long horne. Color of horne and nail of legs are black and coat colour varies from dark black to light black or brown. Body coat is covered with long hairs. Young animals have black hairs till 1-1.5 years and it becomes reddish black colour after weaning (Anonymus, 2004). According to the Turkish Statistic Institute data (www.turkstat.gov.tr) 100,000 water buffalo slaughtered in 1991 (buffalo population of 360150 head) producing 8803 metric ton buffalo meat. Average carcass weight estimates 110 kg. It is estimated that 250,000 heads of buffalo population exist in Turkey. Adult body weight of water buffalo raised in Turkey is approximately 400 kg. Average lactation yield and length of buffaloes of Turkey are 1 ton and 250 day respectively. Average age at first insemination is 22-24 month. Average open day period is three months. First heat cyclus is observed generally after 3 month of calving. They are raised in a coastal

area of North Anatolia, especially Samsun and north part of central Anatolia relatively close to coastal area such as Tokat, Trakya region (Istanbul) that has the biggest number of buffalo population of Turkey. In addition the eastern part of Turkey such as Muş, Kars, Sivas has also Water buffalo populations. Afyon in western Anatolia and Diyarbakır in South Eastern Anotolia has also Buffalo population. Average rate of live weight gain is 400 gram in young period up to 1 years of age. Buffaloes are raised as the form of family operation 3-5 head of farm size. They are used in forest area also for their pulling power. The buffaloes used as draft animals are castrated in 12 years of age. They can be used up to 12 years of age as a pulling power. Most important reason for raising Buffaloes are their milk and meat and especially cream or (fat) characteristics which is favourable for consumer. Buffalo for meat is favourable for garlic flavoured sausage. Buffalo cream is favourable as for additives to the famous Turkish deserts. They are also raised as meat production with average size of 50 head of herd in the region close to big cities. Average withers height of adults of male is 140 cm. Average fat content and total solid content of milk in first lactation is 8% and 16 % respectively.

According to FAO (2008) data, there are about 185.29 million domesticated buffaloes raised in the 42 countries of which 179.75 million(97%) are in Asia among five world continents. However, there are about 158 million buffaloes left in the world. During the last 10 years the world Buffalo production increased by 1.49 % annualy.

Also, in Turkey the buffalo population has declined dramatically over the last decades. The total population according to the Turkish Statistic Institute was 117,591 heads in 2013 (www.turkstat.gov.tr). Former buffaloes have been used as draft animals for centuries. Also they have been used for source of meat and milk products. Than buffalo number was decreased, because of increasing demand for cattle breeding and increasing technology in agriculture.

In spite of the popular indifference, farming of this species has survived in order to promote productive systems in agreement with sustainable rural development and trend to revalue autochthonous genetic types. In this context, beginning from 1963 some provincial, regional and national research institutes have established programmes aimed to revalue and improve reproductive and productive potentiality of Anatolian water buffalo. In 1989 improvement plans have been established by crossing Anatolian buffalo with “Murrah” buffalo imported from Bulgaria.

Concerning milk production, research is evidencing that controlled farming and feeding conditions may significantly improve performances. Indeed, in comparison with buffaloes reared in village conditions, buffaloes under experimental conditions show more favourable values for mean lactation period (245 d *vs* 250 d), maximum and minimum milk yield (1.715 *vs* 1.603 litres and 442 *vs* 186 litres, respectively) and mean fat milk percentage (8% *vs* 7 %). Milk is also employed to make a very popular traditional product, known as ‘lülekaymağı.

Buffalo for draught purpose is limited to tree stump hauling in forest area when mechanical equipments may not be used. In Turkey most farmers keep 1-2 buffaloes for family consumption and this system is very widespread in villages while farms with around 100 heads are located near to the big cities.

Concerning housing system, most farmers own 1-2 buffaloes as source of animal protein for family consumption and this system, very widespread in villages, is based on pasture resource exploitation; on the contrary, big herds (around 100 heads) are located near the big cities and buffaloes in this case receive concentrate supplemented feed (maize, wheat, barley, cottonseed and sugar beet by-products) when available. Although artificial insemination forms cornerstone of buffalo improvement program yet at present it represent only very rare occasion mostly for research purposes.

Artificial insemination is not applied in villages while it is widely used in experimental farms, such as Afyon and Bandırma district of Turkey and at the Department of Animal Science of Mustafa Kemal University (Sekerden,O., 2008). The programme is currently in progress at Zootechnics Institute of Bandirma district in the Turkish province of Balikesir employing artificial insemination and breeding programmes. The larger farms with 40-50 females maintain their own males and the villages usually share a common male. Natural mating sires within the herd is a common practice. Live weight is about 450-500 and 700-800 kg in the adult female and male respectively. The water buffalo population and the amount of production from water buffalo in Turkey are also decreasing. There is only one water buffalo breed called Anatolian water buffalo in Turkey. The water buffalo *in-situ* conservation program was conducted in Balıkesir province of western Turkey. The number of water buffalo population decreased from 117,8000 (1970) to 847,268 (2010) and increased by 117,591 in 2013 again. The latest attempt regarding Conservation of animal genetic resources establishing Water buffalo breeding organization of Turkey which started in 2008 and central water buffalo breeder association established in 2011. Nationwide water buffalo improvement program was initiated under the name of community based improvement program. So far 14 provinces and 2873 farms with total of 16082 adult water buffalo individuals were included in the nationwide improvement program. Recently this kind of support put on implementation that water buffalo breeding stock breeder who is a member of breeder union and joins the nationwide herd improvement program can receive 250 Euro per head as incentive premium. The aim of this project is to create superior breeding stock. Other water buffalo breeders rather than be included in improvement program also receive support as premium but lower (150 Euro) than included improvement program. Due to opening door for subsidizing the support to the farmer this activity wıll help very much stopping the tendency for decreasıng the number of water buffalos in Turkey .

Table 1. *Means and standard errors (SE) for certain carcass quality characteristics of male and female Anatolian Water Buffalos*

Characteristics	Male		Female		Sig.
	Mean	SE	Mean	SE	
Hot carcass weight, kg	325.40	2.65	288.20	5.99	***
Carcass length, cm	127.07	1.00	132.16	1.93	*
Chest depth, cm	45.37	0.43	46.26	0.60	NS
Leg length, cm	72.62	1.30	67.87	0.84	**
Leg width, cm	29.96	0.65	28.99	0.61	NS
Conformation score	5.20	0.42	5.50	0.40	NS
Fatness score	7.70	0.47	7.30	0.68	NS
Backfat thickness, cm	19.84	1.69	19.77	2.06	NS
Fat colour parameters					
Lightness (L*)	64.00	1.04	60.03	0.96	*
Redness (a*)	6.14	0.49	7.01	0.89	NS
Yellowness (b*)	7.03	0.63	7.08	0.59	NS
pH_u	5.49	0.01	5.44	0.01	***

NS= not significant (P>0.05)
*P<0.05; **P<0.01; ***P<0.001

The water buffalos in Turkey are named as Anatolian Water Buffalos and they are among Mediterranean Water Buffalos which are subgroup of river buffalos (Soysal et al., 2005). They are mostly bred in Samsun and Sinop in North Anatolia sea shore; in Çorum and Amasya in Middle Anatolia; in Afyon and Balıkesir in Inner West Anatolia; in Sivas and Muş in East Anatolia and in Diyarbakır in Southeast Anatolia (Soysal, 2009). In Turkey water buffalos are particularly bred for milk production and they are slaughtered for meat production after they finish their productive age. The cream produced from Anatolian Water Buffalo milk is a popular product which is consumed together with many local desserts (Soysal et al., 2005). In some regions, Anatolian Water Buffalo milk is also used for cheese production. Anatolian Water Buffalo meat is consumed as fresh or in meat products like Turkish style fermented sausage, pastrami and salami. In the recent years there has been a rise in the production for meat only. Anatolian Water Buffalo meat is more commonly used in Turkish sausage as it decreases the fermentation duration and is believed to give taste. In some regions in Turkey, Anatolian Water Buffalo breeding is a traditional production model which has great importance in the economy and culture of its breeders. In recent years strong emphasis was placed on the management of farm animal genetic researches in general and also on the water buffalo husbandry in particular. İn general, the efforts related to conservation and sustainable utilisation of the farm animal genetic resources are coordinated and supported financially and technically by the General Directorate of Agricultural Research and policy of the Ministry of Food, Agriculture and Livestock.

According to the A. Yılmaz et al. (2011) certain carcass and meat quality characteristics of Anatolian Water Buffalos were given in the following tables

Table 2. *Least-square means for meat quality characteristics of Anatolian Water Buffalos due to gender and aging duration*

Characteristics	Gender (G)		Aging Duration (AD)		SEM	Significance		
	Male	Female	7-day	21-day		G	AD	G × AD
WHC[a], %	9.81	9.98	10.57	9.23	0.284	NS	*	NS
Cooking loss, %	27.40	27.93	28.84	26.49	0.336	NS	**	NS
Shear force, kg	3.26	3.18	3.54	2.90	0.095	NS	**	NS
Colour parameters at 1 h								
Lightness $(L^*)^{1h}$	39.33	36.49	37.35	38.48	0.257	***	*	NS
Redness $(a^*)^{1h}$	21.78	22.98	21.29	23.47	0.281	*	***	NS
Yellowness $(b^*)^{1h}$	7.48	7.56	7.30	7.74	0.189	NS	NS	NS
Colour parameters at 24 h								
Lightness $(L^*)^{24h}$	40.87	38.23	38.82	40.29	0.215	***	**	NS
Redness $(a^*)^{24h}$	23.99	25.89	25.30	24.58	0.262	***	NS	NS
Yellowness $(b^*)^{24h}$	7.84	7.91	8.90	6.85	0.198	NS	***	NS

[a] WHC=water holding capacity NS= not significant (P>0.05). *P<0.05; **P<0.01; ***P<0.001

Table 3. *Means and standard errors (SE) for sensory characteristics of male and female Anatolian Water Buffalos*

Characteristics	Male		Female		Sig.
	Mean	SE	Mean	SE	
Odour intensity	4.54	0.12	4.53	0.12	NS
Tenderness	4.67	0.12	4.68	0.12	NS
Juiciness	4.29	0.12	4.23	0.12	NS
Flavour intensity	4.87	0.12	4.96	0.12	NS
Flavour quality	4.79	0.12	4.79	0.12	NS
Overall acceptability	4.66	0.12	4.69	0.12	NS

NS= not significant (P>0.05)

There is only one water buffalo breed called Anatolian Water Buffalo in Turkey. The water buffalo *in- situ* conservation program was conducted in Balıkesir province of western Turkey. There is also *ex- situ* conservation program for Anatolian Water Buffalo breed in Turkey at Bandırma Animal Research Institute in Turkey. The latest attempt regarding Conservation of animal genetic resources establishing Water buffalo breeding organization of Turkey in 2008. *In-situ and ex-situ* conservation and incentive premium support programs are carried out to stop the declaring the population number.

Due to opening door for subsidizing the support to the farmer this activity will help very much stopping the tendency for declaring the number of water buffalo in Turkey. Recently (2009) this kind of support put on implementation that water buffalo breeding stock breeder who is a member of breeder union can receive 150 Euro per head as incentive premium.

Table 4. *Several characteristics of Anatolian Water Buffalo raised in Turkey*

Parameters	**Maximum**	**Minimum**	**Sources**
Lactation Yield (kg)	1070.5±279.9	709.6±23.0	Şekerden et al (2000b) Uslu, N.T. (1970b)
Lactation Length (day)	269.2±70.0	222.0±44.2	Şekerden et al (2000a) Şekerden et al (2000b)
Fat (%)	8.1±0.205	6.6±0.68	Kök, S., (1996) Şekerden et al (2000a)
Adult Body Weight	518.6±17.2	411.0±9.07	İlarslan et al (1983) Uslu N.T, (1970a)
Calving Interval	434.3±57.1	365.2±17.5	Şekerden et al (2000a) İlarslan et al (1983)
Age at first insemination (day)	679.7±210.9		Şekerden et al (2000a)
Age at first calving (day)	1313.2±234.8	964.1±3.94	Şekerden et al (2000b) İlarslan et al (1983)
Birth Weight (Male)	34.3±1.20	26.7±0.52	Uslu N.T; (1970b)
Birth Weight (Female)	31.6±0.90	22.1±0.48	Alaçam et al. (1992) Uslu N.T., (1970b)
Service Period	112.45	70.8	İlarslan et al (1983) Şekerden et al (2000b)
Gestation Length (day)	326.5±5.8 (artificial insemination)	317.0±51.5 (natural insemination)	İzgi and Asker, (1989) İzgi and Asker, (1989)

Daily Live Weight Gain (gr) (0-3 Month)	(Male) 0.483	(Female) 0.456	Şekerden et al. (2000c)
Daily Live Weight Gain (gr) (3-6 Month)	(Male) 0.305	(Female) 0.294	Şekerden et al. (2000c)
Daily Live Weight Gain (gr) (6-9 Month)	(Female)	(Male)	Şekerden et al. (2000c)
Male		0.314	
Female	0.357		
Daily Live Weight Gain (gr) (9-12 Month)	(Male) 0.504	(Female) 0.360	Şekerden et al. (2000c)
Fat Content of Milk	8.1	6.1	Kök, S. (1996) Soysal and Kök (1997)
Total Solid Matter of Milk	17.7 (3. Lactation)	15.3 (1. Lactation)	Şekerden et al.(2000b)
Ash % of Milk	0.830	0.743	Şekerden et al.(2000a) Şekerden et al.(2000b)
Water of Milk	82.3		Kök, S. (1996)
Protein % of Milk	4.6	4.2	Şekerden et al. (2000a) (Soysal and Kök, 1997) Kök S. (1996)
Casein % of Milk	3.4 (3. Lactation)	3.0 (1. Lactation)	Şekerden et al.(2000b)

Genetic constitution of population

In order to reveal the genetic constitution of Anatolian water buffalo several molecular genetic diversity studies have been carried out. The genetic variation and relationship among six Turkish water buffalo populations typical of different regions were assessed using a set of twenty-six heterologous (bovine) microsatellite markers. Between 7 and 17 different alleles were identified per microsatellite in a total of 254 alleles. The average number of alleles across all loci in all the analyzed populations was found to be 12.57. The expected mean heterozygosity (H_E) per population was between 0.5 and 0.58. The overall polymorphic information (PIC) value was between 0.33 and 0.86. Significant departures from Hardy-Weinberg equilibrium were observed for 44 locus-population combinations. Population differentiation was analyzed by estimation of the F_{ST} index (values ranging from 0.053 to 0.123) among populations. The PCA analysis identified three clusters: the Merzifon and Danamandira populations represented one cluster each, and the Afyon, Coskun, Pazar and Turhal formed a single cluster. The assignment of individuals to their source populations performed using the Bayesian clustering approach implemented in STRUCTURE 2.2 software evidenced a high differentiation of Merzifon and Danamandira populations as well. The results of this study could be useful for the conservation strategies of the Turkish buffalo (Soysal et al., 2008)

Another molecular genetic study for indigenous water buffalo population to Anatolia was characterised with 11 cattle autosomal microsatellite loci. A set of 4 cattle microsatellite loci was found to be polymorphic in the Anatolian buffalo genome. Genotyping of these polymorphic microsatellite loci revealed alleles ranging from 3 to 9. The observed heterozygosity ranged from 0.550 to 0.775 and the expected heterozygosity ranged from 0.494 to 0.815. The F_{IS} value changed from 0.101 to 0.205. This result shows that Anatolian water buffalo population samples seemed to be in Hardy-Weinberg expectation (Soysal et al., 2007).

Table 5. *Mean, standard deviation and comparison between males and females ([1]), distinctly for class of age, of somatic traits measured on buffaloes raised in Danamandra village, of Silivri district of İstanbul Province of Turkey*

	<1 Year		1 ≥Years <2		2 ≥Years <4		≥ 4 Years	
	N	$X \pm S_{\bar{X}}$	N	$X \pm S_{\bar{X}}$	N	$X \pm S_{\bar{X}}$	*N*	$X \pm S_{\bar{X}}$
1.Withers height								
Male	28	87.46±12.01	3	131.33^{a} ±5.02	11	126.54±9.59	1	141.30
Female	22	90.95±10.74	5	105.00^{b}±13,64	0	-	32	134.15±5.32
2. Rump height								
Male	28	93.28±12,22	3	137.33^{a} ±9,28	11	128.09±7,50	1	147.50
Female	22	95.81±10.88	5	109.20^{b}±12,79	0	-	32	132.87±7,35
3.Tail base height								
Male	28	86.00±10.90	3	126.00±3,98	11	117.70±6,33	1	136.500
Female	22	89.22±11.44	5	103.8±14,65	0	-	32	122.34±6.28
4. Body length								
Male	28	75.82±16.67	3	116.00^{a} ±9.53	11	127.09±11.44	1	142.50
Female	22	74.27±9.62	5	96.40^{b} ±22.74	0	-	32	138.56±8.26
5. Chest width								
Male	28	20.07±4.39	3	29.33±1.14	11	32.63±5.27	1	39.30
Female	22	20.36±4.83	5	22.60±4.72	0	-	32	33.78±4.92

([1]) Different letter means significant difference for P<0.05

	≤1 Year		1> Years ≤2		2> Years ≤3		3 >Years ≤4		Adult	
	N	$X \pm S_{\bar{X}}$	*N*	$X \pm S_{\bar{X}}$	*N*	$X \pm S_{\bar{X}}$	*N*	$X \pm S_{\bar{X}}$	*N*	$X \pm S_{\bar{X}}$
1.Withers height										
Male	4	110.75±9,93	*18*	122.1±8.29	*13*	129.38^{A} ±4,68	*6*	132.83±8.03	*35*	138.23±7.22
Female	*6*	99.00±11.15	*10*	122.7±8.09	*12*	122.92^{B} ±5,07	*8*	127.13±5.24	*91*	133.14±6.76
2. Rump height										
Male	*4*	112.00±10.29	*18*	122,78±8.40	*13*	129.85^{a} ±3.55	*6*	133.00±9.4	*35*	135.71±7.06
Female	*6*	103.60±11.69	*10*	125.5±7.60	*12*	125.58^{b} ±6.34	*8*	125.88±4.85	*91*	132.57±7.20
3. Body length										
Male	*4*	118.25^{a}±6.85	*18*	123.17±8.11	13	137.85^{A} ±8.00	*6*	145.67^{a} ±10.21	*35*	145.09±7.88
Female	*6*	102.2^{b}±10.47	*10*	121.9±8.07	12	127.42^{B}±10.06	*8*	132.00^{b} ±4.78	*91*	142.43±8.77
4. Chest depth										
Male	*4*	29.50^{B} ±3.00	*18*	59.67±5.19	13	64.96^{A} ±1.56	6	74.67^{A} ±5.24	35	77.2±8.45
Female	*6*	45.20^{A} ±8.70	*10*	61.70±7.10	12	64.92^{B} ±3.20	8	67.38^{B} ±2.92	91	71.1±4.85

([1]) Different letter means significant difference for P<0.05 (small letter) or P<0.01 (capital letter)

Morphometric characteristics of Water Buffalo population of Turkey

An investigatıon done by Soysal et al. (2007) in which 76 males and 127 females of the Istanbul district and 32 males and 70 females raised in Danamandra village of Silivri district were measured. On each buffalo, withers height, rump height, body length, chest depth and chest width were determined. The results showed a significant difference between males and females starting from 12 months in buffaloes of Danamandra village and from 3 years of age in animals of Istanbul district.

References

1. Anonymus 2004. Registration of Anatolian Water Buffaloe Annexe 6; Registreted at 20/04/2004 by National Animal Breed Registratıon commity and release on Official Gazette of the Turkish Rebublic dated 12/12/2004 by the regulation numbered 2004/39 and listed native animal genetic resources www.resmigazete.gov.tr
2. FAO 2008. Food and Agricultural Organization of The United Nation (FAO). Rome, 2008 (www.fao.org/stat,2008*)*
3. İlarslan M, Karabulut A, Aşkın A, İzgi N 1983. Yerli Mandalarda Vücut Yapısı, Döl ve Süt Verimi Üzerine Araştırmalar. Zirai Araş. Enst. Yay. No:14 Afyon
4. İzgi AN, Asker R 1989. Çeşitli Çevre Şartlarının Mandaların Doğum Ağırlığı Üzerine Etkisi. Mandacılık Araş. Enst. Yayın No:18 Afyon.
5. Kök S 1996. Marmara ve Karadeniz Bölgesinin Çeşitli İllerindeki manda Populasyonlarının Kimi Morfolojik ve genetik Özellikleri Üzerinde Bir Araştırma. Trakya Üniv. Fen Bilimleri Enst. Doktora Tezi.
6. Şekerden Ö, Tapkı İ 2000a. Mustafa Kemal Üniversitesi Manda Sürüsü Süt ve Döl Verim Özellikleri. Atatürk Üniversitesi Zir. Fak. Dergisi .
7. Şekerden Ö, Kebapçı M 2000b. Afyon Kocatepe Tarımsal Araştırma Enstitüsü Anadolu Mandalarında Laktasyon Süt Verim Ve Bileşiminin Laktasyon Dönemlerine Göre Değişimi. Süt ve Döl Verim Özellikleri. Atatürk Üniversitesi Zir. Fak. Dergisi (Basımda).
8. Şekerden Ö, Kebapçı M, Kopar A 2000c. Kocatepe tarımsal Araştırma Enstitüsü Anadolu Manda Sürüsünün Kan Serumu Tf Tipleri, Tf tipleri için Genetik Yapısı ve Büyüme Performansı , Tf Tipleri ve Büyüme Özelliği Arasındaki İlişkiler. Atatürk Üniv. Ziraat Fakültesi Derg.
9. Sekerden O 2008. İmprovement of Productive and reproductive efficiency of Anatolian Buffaloes through the use of artificial insemination with Italian Buffalo semen 7th RBI global conferance on conservation of animal genetic resources Hanoi, Wietnam 14-18 Sept 2008 Conference proceeding book.
10. Soysal Mİ, Gürcan EK, Tuna YT 2004. A research on Several Body measurement of Water Buffaloein Danamandra willage of Silivri Districht of Province of İstanbul of Turkiye .7th world water Buffaloe congres PSASA Sci. Annual Meeteing Oct.20-23 Manila -Philiphines
11. Soysal Mİ, Ozkan E, Kök S, Tuna YT, Gurcan EK 2005. Genetic charaecterization of İndigenous Anatolian Water Buffaoloe using microsratellite DNA marker.Journal of Tekirdağ Agric.Faculty number 3 Volume 2
12. Soysal Mİ, Tuna YT, Gurcan EK 2005. An investigation of Water buffaloe breed in Danamandra Willage of Silivri Districht of Province of İstanbul of TurkiyeJournal of Faculty of Agriculture volume 2 Number 1
13. Soysal Mİ, Tuna YT, Gurcan EK, Ozkan E, Kok S, Castellino N, Cobanoglu O, Barone CM 2007. Anatolian Water Buffaloe Husbanndry of Turkey.A preliminary Result of somatic characterization prooceding of 8th world buffaloe congres İtaly Oct 19-21,2007;İtalian journ.of Anim.Sci. vol 6
14. Soysal Mİ, Ozkan E, Gargani M, Pariset L, Valentini A 2008. Genetic variatiıon of Turkish Warer Buffaloe populatıon 7th RBI Global Conferance on the Conservation of Animal Genetic Resources Hanoı-Wietnam 14-18 Sept.2008.
15. Soysal MI 2009. Productionof water Buffalo and Their product (in Turkish) ISBN;978 9944 5405 37
16. Turkstat 2013. Turkish Statictic Institute (www.turkstat.gov.tr,201

17. Uslu NT 1970a. Afyon Bölgesi Mandalarının Çeşitli Özellikleri ve Köy şartlarında Süt Verimleri Üzerinde Mukayeseli Araştırmalar. (Doktora Tezi) Birlik Matbaası, Bornova (1970).
18. Yılmaz A, Ekiz B, Soysal Mİ, Yılmaz İ, Yalcin H 2011. Certain carcac and meat quality Characteristic of Anatolian Water Buffalo.VIII RBI Global conference on conservation of animal Genetic Resources Tekirdag Turkiye 4-8 October 2011.

Original paper

THE EFFECT OF THE USE OF SEXED SEMEN ON REPRODUCTIVE TRAITS AND SEX RATIO OF BLACK AND WHITE CALVES

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Abstract

Sperm sexing is a relatively new biotechnological procedure which offers us the possibility to choose the offspring sex. This method is widely used today in the insemination of the dairy cattle breeds and it is based on the difference in total DNA quantity between X and Y chromosome spermatozoa. In this research paper we have analysed the fertility, sex ratio and characteristics of female Black and White breed calves inseminated by the sexed sperm produced by 6 bulls on 6 commercial farms of the PK Belgrade. Conception rate was 44% with average gestation length of 274.9 days. Average calf birth weight was 36.75 kg. The stillbirths and twinning rates were 7.16 and 1.13%, respectively. Artificial insemination by sexed sperm assessed by χ^2 test highly significantly ($P<0.01$) alters the sex ratio between female and male calves (85:15%). By means of GL model we have assessed the effects of farm, insemination season, sex of the calves and inseminating bull on gestation length and calf birth weight. All mentioned factors have manifested statistical significance ($P<0.01$) on studied traits, except for the inseminating bull trait which did not statistically significantly affect ($P>0.05$) gestation length. Regardless the low conception rate, the application of sexed sperm in dairy cattle breeding is very significant since it enables the breeders to use the best replacement female animals what may result in the improvement of genetic basis of breeding stock.

Key words: *Black and White breed, fertility traits, sex relationship, sexed sperm*

Introduction

Artificial insemination of dairy cattle by sexed semen facilitates faster genetic improvement. By the application of sexed semen we can, in the first place, produce a greater number of descendants of desired sex in shorter time period in relation to a conventional artificial insemination (Seidel, 2002; De Vries et al., 2008). So far a several methods for separating X and Y spermatozoa have been developed but most often used method recently has been the method of flow cytometry which provides a satisfying degree of separating and is based on difference in total DNA quantity between spermatozoa with X and Y chromosomes.

The research shows that the fertility with sexed sperm is lower in relation to the fertility obtained by use of conventional non-sexed sperm (Seidel, 1999; Tubman et al., 2004; Norman et al., 2010; DeJarnette et al., 2011; Healy et al., 2013). Thus, Seidel et al. (2002) reported that in Holstein heifers the pregnancy rate ranged from 21 to 35% for sexed sperm compared with 58% for conventional. Lower fertility is caused by the damages occurring on spermatozoa during sorting of sperm. The process with spermatozoa during sorting is very invasive; they are placed under high pressure and great speeds and abruptly halted thereupon, causing the damage of the spermatozoa by such physical forces. High pressure within the system has been associated with decreased fertility (Seidel et al., 2003). For this reason it is recommended that sexed sperm is to be used primarily in heifers that show distinct signs of estrus (Foote, 2010). The heifers are the most fertile part of the herd and they are not burdened by the production, and that fact is of crucial importance in the use of sexed sperm.

There was no increase in calf abnormalities that have been reported to date with sexed sperm relative to controls. There is a possibility that early embryonic mortality may be increased slightly with sexed sperm (Seidel et al., 1999; Tubman et al., 2004; Healy et al., 2013).

The aim of this paper was to determine the conception rate, gestation length, calf birth weight, sex ratio, calf vigor, neonatal death rate and calf twinning rate after insemination of Black and White breed heifers by sexed semen.

Materials and methods

Study Design, Animals, and Housing

Data for the analysis was collected, from January 2012 until January 2014, from the total of 1205 inseminations in 530 heifers of Black and White breed raised on 6 commercial farms of the Agricultural Corporation of Belgrade. The conditions on all farms were uniformed and there were minimal differences between the heifers regarding the feeding and care conditions.

The heifers were grouped according to height and weight, and fed mixed rations to meet all or part of their nutritional requirements according to pasture availability. All of the heifers enrolled in the breeding program were grouped together and, as such, minimal differences exist between these heifers.

Reproductive Management

Artificial insemination was performed once daily, on the same morning as estrus detection. Insemination was carried out by multiple AI technicians using sexed semen straws collected from 6 dairy sires, provided by CRI (Wisconsin, USA) and sorted by flow cytometry. Sperm viability and semen quality were not evaluated before insemination. In most cases, sex-sorted straws were used only for the first or second breeding and heifers returning to estrus were inseminated with conventional semen for subsequent breedings.

Data and Statistical Analysis

Data available for analysis included: heifers identity, date of insemination, date of successful conception, date of calving (birth), identity of the calf sire, type of sperm (sexed or non-sexed), calf sex, calf birth weight, calf vigor (1-calf with inborn anomalies; 2-poorly developed and avital calf; 3- moderately developed and vital calf; 4-5- well developed calf, vital and in type), incidence of twinning and stillbirths. Gestation length was calculated as the

difference of the date of successful conception and the date of calving. All stillborn calves were recorded, but their sex was not determined. During the trial no anatomic anomaly was perceived in calves. Both fertility traits and characteristics for total of 530 calves obtained by the application of sexed semen were analyzed. After the records obtained on different farms have been completed and edited the establishing of average values, standard deviation and frequency of studied traits took place.

By means of GLM procedures of the Statistical Analysis System (SAS Institute, 2013), the assessment of the effect of farm, insemination season, calf sex and inseminating sires on gestation length and calf birth weight was carried out.

The following GL model was used:

$$Y_{ijklm} = \mu + F_j + S_k + P_l + O_m + e_{jklm}$$

In which:

Y_{ijklm} – is the phenotypic manifestation of studied trait,

μ-general average of population

F_j-fixed effect of the j farm (j=1...6)

S_k- fixed effect of the k calving season (k=1...4)

P_l- fixed effect of the calf sex (l=1,2)

O_m- fixed effect of the l sire (m=1...6)

e_{jklm}- random error.

χ^2 test, within the same SAS programme package (SAS Institute, 2013) was used to study the calf sex ratio in both types of artificial inseminations.

Results and disscusion

Table 1 shows the mean values for the conception rate, gestation length, calf birth weight, calf vigor, neonatal death rate and calf twinning rate obtained after the insemination of heifers by sexed semen.

Table 1. *Average values of studied traits*

Traits	n=530
Conception,%	44
Gestation length, days Mean ±SD	274,92±9,04
Birth weight, kg Mean ±SD	36,75±2,0
Vitality of calves[a]	4,12
Stillbirth, %	7,16
Twinning, %	1,13

[a]1-calf with inborn anomalies; 2- poorly developed and avital calf;
3- moderately developed and vital calf; 4-5- well developed calf, vital and in type

The effect and statistical significance of farm, season of insemination, calf sex and inseminating sire on gestation length are shown in Table 2.

Table 2. *Analysis of variance for gestation length*

Source	DF	Mean square	*F*-value	Significance
Farm	5	611.241966	8.54	<.0001***
Season	3	253.087765	3.53	0.0147*
Calf sex	2	615.512068	8.60	<.0001***
Sire	5	72.581875	1.01	0.4304 ^{NS}
Error	510	71.59860		

DF- Degrees of Freedom; MS- mean square; F-value; P- probability; N.S; non-significant, * P<0.05, **P<0.01; ***P<0.001

From the results displayed in the table we can observe that the farm and calf sex have a very high significant effect (P<0.001) on gestation length whereas inseminating sire does not statistically significantly affect mentioned trait. Similarly to a previous table, Table 3 displays the significance of studied factors on calf birth weight.

Table 3. Analysis of variance for birth weight

Source	DF	Mean square	*F*-value	Significance
Farm	5	35.6066081	14.34	<.0001***
Season	3	8.8772715	3.57	0.0140*
Calf sex	2	151.4725914	60.99	<.0001***
Sire	5	12.2940260	4.95	<.0001***
Error	510	2.483482		

DF- Degrees of Freedom; MS- mean square; F-value; P- probability; N.S; non-significant, * P<0.05, **P<0.01; ***P<0.001

The farm, calf sex and inseminating sire have a very highly significant (P<0.001) effect on birth weight. The season significantly (P<0.01) affected a given trait.

Table 4. Mean values of gestation length and calf birth weight observed per sex

Sex	Traits	
	Gestation length, days	Birth weight, kg
Female	274,73	36,61
n	425	425
Male	276,23	37,74
n	75	75
P	<.0001***	<.0001***

P- probability; N.S; non-significant, * P<0.05, **P<0.01; ***P<0.001

Table 4 shows mean values of gestation length and calf birth weight observed per sex. The calf sex statistically very highly significantly (P<0.001) affects the observed traits.

The sex ratio and statistical significance determined by χ^2 test are shown in Table 5. Artificial insemination by sexed semen very highly significantly (P<0.001) alters the calf sex ratio (85:15).

Table 5. *The sex ratio of calves and χ^2 value*

	Sex ratio		χ^2	P
	Female, %	Male, %		
Sexed semen, n	85	15	245,00	<0.001***
	500	500		

χ^2-value; P- probability; NS non-significant, * P<0.05, **P<0.01; ***P<0.001

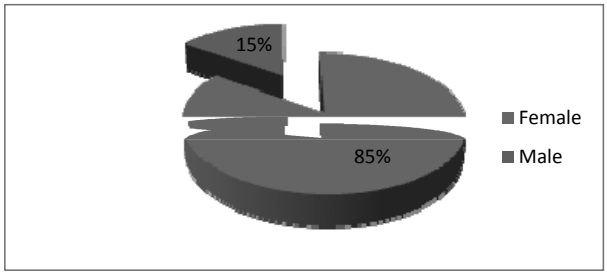

Figure 1. The sex ratio of calves

Many studies show that conception rate as a consequence of the application of the sexed sperm is highly variable, and that it is lower in relation to conventional sperm. Obtained rate of conception in this paper was in harmony with the rate of conception obtained by the heifers that were artificially inseminated by sexed sperm in other countries and ranged from 39 to 57% (Cerchiaro et al., 2007; DeJarnette et al., 2009). The determined rate of conception for sexed sperm was higher than that reported by Weigel (2004), Bodmer et al. (2005) and Healy et al., 2013.

A low rate of conception realized by sexed sperm has most probably been provoked by damage of spermatozoa during sorting process and by the decreased concentration of spermatozoa in the applied doses (Seidel et al., 1999; Bodmer, 2005; Garner and Seidel, 2008).

Conception rate for sexed semen in this study was significantly higher than the same rate established for Holstein heifers by Healy et al. (2013) and is in line with majority of published reports in which it amounts even up to 75% (Cerchiaro et al., 2007; DeJarnette et al, 2009; Norman et al., 2010). At the same time, in a number of studies, we can notice that the highest conception rates were recorded in moderate climate conditions to which studied region also belongs.

Improved efficacy of the sorting can in the future increase the rate of conception and thus contribute to wider use of sexed sperm in dairy cattle breeding.

Gestation length represent the trait whose duration is characteristic for every species of domestic animals and depends on greater number of factors. Significant differences in the gestation length depending on the parents breed, sires in particular, have been confirmed by many authors (O'Ferrall et al., 1990; Cundiff et al., 1986; Gregory et al., 1997). The results obtained for gestation length were in harmony with several studies (DeJarnette et al, 2009; Norman et al. 2010) which report that the gestation length in heifers is statistically sifgnificantly influenced by the season of insemination, inseminating sire, twinning frequency and calf sex. Male sex prolongs the gestation length what is reflected in higher body mass at birth in relation to the female calves.

Type of insemination, in the present study, did not statistically significantly (P= 0.4734) shorten or prolong gestation length what is in harmony with the research by Tubman et al., 2004 and Healy et al., 2013.

Studying the calf birth weight is significant for a number of reasons, among the others, a great calving mass of calves is one of the major causes of difficult calvings, and later causes the death of calves which can occur immediately after the birth. Calf birth weight is influenced by different genetic and non-genetic effects, such as sex, body mass and the age of dam, sire, calving in order, calving season, breed, gestation length, duration of a dry period (Nelsen et al., 1984; Cundiff et al. 1986). The results obtained by Tubman et al., 2004, correspond to the results obtained in the present study meaning that in addition to the sex, season of insemination and inseminating sire the type of artificial insemination also has a statistically significant ($P<0.01$) effect on calf birth weight.

Calf vigor was evaluated for all live born calves by the marks from 1 to 5. The evaluation is great deal subjective, therefore due to such scoring and partly due to differences originating from dams a statistically significant difference ($P<0.01$) was confirmed between the calves produced by sexed and those produced by non-sexed semen. The results obtained are not in harmony with the results of Tubman et al., 2004, who proved that there is no significant difference in the results for calf vigor depending on the type of insemination and sex.

Many farmers throughout the world today face the constant increase in the calf mortality rate, what highly unfavorably affects the economic value of milk production (Meyer et al., 2000; Steinbock et al., 2003; Zadeh et al., 2008). The rates of single stillborn calves in our research produced by heifers inseminated by sexed semen are lower than the rates obtained by Norman et al., 2010. Healy et al. (2013) report that the rate of stillborns is influenced by following factors: sex, twinning, gestation length, AI technician, semen type, the age of dam and the season of insemination. DeJarnette et al., 2009, in their study suggest that increased rate of stillborns produced by heifers inseminated by sexed semen occurs as a consequence of the process of sorting which damages sperm and leads to reduced vigor of foetus and ultimately to stillbirth.

The rates of the twinning for Holstein heifers and White and Black heifers in previous studies ranged from 0.76% to 1.3% (Mee, 1991; Zadeh et al., 2008; Norman et al, 2010).

The twinning rate in this study was higher than expected one for the heifers of dairy breeds and statistically depended ($P<0.01$) on the type of semen. The results obtained are consistent with the values reported by Healy et al., 2013, who, contrary to the results obtained in this

study, point out that the type of semen had no statistically significant effect (P>0.05) on twinning rate but that it affected the sex of twins. The use of sexed semen (Table 5) increases significantly the frequency of the pairs of female twins at the expense of the male ones (Tubman et al., 2004; Norman et al, 2010; Healy et al., 2013).

The inheritance of the sex is the consequence of the random pairing of gamets in the proces of insemination and therefore, it is likely to expect an almost equal sex ratio (1:1) in cattle offspring. By the application of sexed sperm, heifers will deliver female calves in about 90% cases instead of 49% what is an average frequency when we use the sperm which has no been sexed (Seidel, 2003; Cerchiaro et al, 2007; DeJarnette et al., 2009).

The relation of sexes accomplished in this study which is obtained by sexed sperm was acceptable and similar to that reported by Bodmer et al. (2005) and Healy et al. (2013). However, it was somewhat lower than majority of reports in literature, which reported that by the application of sexed sperm we can obtain about 90% female calves (Cerchiaro et al, 2007; DeJarnette et al., 2009; Norman et al, 2010). The realised relation of sexes can be considered to be the consequence of reduced accuracy in sorting due to increased rapidity (Seidel, 2003). The relation of sexes in the respective research was statistically significantly (P<0.01) altered by the application of sexed sperm.

The obtained sex ratio for conventional insemination was not in line with a majority of published results stating that about 50 to 52% male calves are being born (Tubman et al., 2004; Zadeh et al., 2008; DeJarnette et al., 2009). However, similarly to our study, Norman et al. (2010) determined 48.5% birth of male calves. Type of insemination, which involves also the inseminating bull, had a significant effect on sex ratio (Norman et al., 2010; Healy et al., 2013).

Conclusion

The results obtained in this study are consistent with a majority of studies which compared the fertility traits, sex ratio and calf characteristics realised by artificial insemination with sexed semen. It can be said that the application of sexed sperm does not lead to the increase in the abnormalities and that it does not affect in a negative way the studied calf characteristics. Sexed sperm enables the breeders to use only the best replacement heifers in the herd what will result in improvement of the genetic base of breeding stock. Sexed sperm should be used only for the insemination of sound heifers on the farms with good organisation of reproduction because otherwise the fertility can additionaly decrease. Regardless all limitations it has, the use of sexed sperm is very significant because in this way we can obtain the animals of desired sex and superior hereditary traits.

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References

1. Bodmer M, Janett F, Hässig M, Daas Nd, Reichert P and Thun R 2005. Fertility in heifers and cows after low dose insemination with sex-sorted and non-sorted sperm under field conditions. Theriogenology 64,1647–1655.
2. Cerchiaro I, Cassandro M, Dal Zotto R, Carnier P and Gallo L 2007. A field study on fertility and purity of sex-sorted cattle sperm**.** J. Dairy Sci. 90, 2538–2542.
3. Cundiff LV and MacNeil MD 1986. Between and within-breed genetic analysis of calving traits and survival to weaning in beef cattle. J. of Animal Sci. 63, 1, 27-33.
4. De Vries A, Overton M, Fetrow J, Leslie K, Eicker S and Rogers G 2008. Exploring the impact of sexed semen on the structure of the dairy industry. J. Dairy Sci. 91, 847–856.
5. DeJarnette JM, Nebel RL and Marshall CE 2009. Evaluating the success of sex-sorted semen in US dairy herds from on farm records. Theriogenology 71, 49–58.
6. DeJarnette JM, Leach MA, Nebel RL, Marshall CE, McCleary CR and Moreno JF 2011. Effects of sex-sorting and sperm dosage on conception rates of Holstein heifers: Is comparable fertility of sex-sorted and conventional semen plausible J. Dairy Sci. 94 ,3477–3483.
7. Foote RH 2010. The history of artificial insemination: Selected notes and notables. J. Anim. Sci, 80, 1-10.
8. Garner DL and Seidel GE 2008. History of commercializing sexed semen for cattle. Theriogenology 69, 886–895.
9. Gregory KE, Bennet GL, Van Vleck LD, Echternkamp S and Cundiff LV 1997. Genetic and anviromental parameters for ovulatin rate, twining rate, and weigth in a cattle population for twining. J. Anim Sci. 75, 1213-1222.
10. Healy AA**,** House JK and Thomson PC 2013. Artificial insemination field data on the use of sexed and conventional semen in nulliparous Holstein heifers. J. Dairy Sci. 96, 1905-14.
11. Mee JF 1991. Factors affecting the spontaneous twining rate and the effect of twinning on calving problems in nine Irish dairy herds. Ir.Vet J. 44, 14-20.
12. Meyer CL, Berger PJ and Koehler KJ 2000. Interactions among factors affecting stillbirths in Holstein Cattle in the United States. J. Dairy Sci. 83, 2657–2663.
13. Nelsen TC, Short RE, Urick JJ and Reynolds WL 1984. Genetic variance components of birth weight in a herd of unselected cattle. J. Anim. Sci. 59, 1459-1466.
14. Norman HD, Hutchison JL and Miller RH 2010. Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. J. Dairy Sci. 93, 3880–3890.
15. O′Ferrall GJM and Ryan M. 1990. Beef crossing: effects of genotype of the foetus on the performance of dairy cows. Irish Journal of Agricultural Reserch. 29, 101-108.
16. SAS 2013. SAS Version 9.1.3, SAS Institute Inc. Cary, NC, USA.
17. Seidel GE, Schenk JL, Herickhoff LA, Doyle SP, Brink Z and Green RD 1999. Insemination of heifers with sexed sperm. Theriogenology 52, 1407–1420.
18. Seidel GE 2007. Overview of sexing sperm. Theriogenology 68, 443–446.
19. Seidel GE 2003. Sexing mammalian sperm—Intertwining of commerce, technology, and biology. Anim. Reprod. Sci. 79, 145–156.

20. Seidel GE Jr 2002. Sexing sperm for beef and dairy cattle breeding. Pages 281–286 in Factors Affecting Calf Crop: Biotechnology of Reproduction. M. J. Fields, R. S. Sand, and J. V. Yelich, ed. CRC Press, Boca Raton, FL.

21. Seidel GE Jr. and Garner DL 2002. Current status of sexing mammalian spermatozoa. Reproduction 124, 733–743.

22. Steinbock LA, Näsholm AB, Berglund K, Johansson K. and Philipsson J. 2003. Genetic Effects on Stillbirth and Calving Difficulty in Swedish Holsteins at First and Second Calving. J. Dairy Sci. 86, 2228-2235.

23. Tubman LM, Brink Z, Suh TK and Seidel GE 2004. Characteristics of calves produced with sperm sexed by flow cytometry cell sorting. J. Anim Sci. 82, 1029-36.

24. Weigel KA 2004. Exploring the role of sexed semen in dairy production systems. J. Dairy Sci. 87(Suppl. 13), 120–130.

25. Zadeh GH, Javaremi AN, Miraei-Ashtiani SR and Kohram H 2008. An Observational Analysis of Twin Births, Calf Stillbirth, Calf Sex Ratio, and Abortion in Iranian Holsteins. J. Dairy Sci. 91,4198-4205.

Abstract

THE INFLUENCE OF DHI MILK SAMPLE QUALITY ON THE RELIABILITY OF LABORATORY ANALYSIS

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Abstract

DHI (Dairy Herd Improvement) testing - Cow productivity control implies the determination of milk quality and quantity, during a certain period of time. In accordance to the breeding program for cattle, milk recording in the Republic of Serbia is conducted according to the internationally recognized AT_4 method. This method is recognized by the International Committee for Animal Recording - ICAR, as the reference method. Advantages of measuring individual cow samples through DHIA testing include the ability to monitor individual cow performance, to calculate and monitor average group performance and to monitor variation among individuals or groups of cows, or changes within individuals or groups of cows over time. The DHI samples must be properly sampled, preserved, labeled and stored.

The aim of this paper is to highlight the importance of proper sampling of raw milk preservation, labeling, storage and transport to the laboratory, in order to obtain reliable test results.

This paper presents the experience regarding the work of the Laboratory for milk quality control, at the Agricultural Faculty, University of Novi Sad. Raw milk samples were obtained from farms in Vojvodina, by the AT_4 method. Milk samples are collected by DHI supervisors. Samples were collected in 40 ml bottles, containing potassium dichromate ($K_2Cr_2O_7$) as preservative and kept under refrigeration at temperature not exceeding 4°C but without freezing during transportation to the Laboratory for milk quality control, Novi Sad. Analysis of milk samples was performed on Foss's instrument MilkoScan^{TM}FT. The milk samples are heated prior to analysis to a temperature of 40$\pm$2°C and visually controlled. Sample identifications performed based on the bar code label, by an automatic scanner installed on each analyzer. Statistical analysis was performed in STATISTICA 12.0.

In the period from July 2013 to May 2014, the Laboratory analyzed a total of 332992 milk samples. From the overall analyzed samples 0.57% was inadequate for analysis. The largest number of inadequate samples was curdy, in total 1174 samples (61.34%). These samples were not preserved and probably were not stored at appropriate temperature. With no bar code label were 518 samples, 27.06% of the total number of inadequate samples. 162 samples were not supplied with enough amount of milk for analysis what was 8.46% of the total number of inadequate samples. The most of the samples (99.43%) were properly sampled, preserved and stored. The results of regression analysis show that the influence of the months was significant

only in the case of the total number of samples. In other cases the influence of months was not statistically significant. The total number of samples has increased by an average rate of changes of 4.27% and the number of inadequate samples has increased at the rate of 9.21%. In that regard, it can be said that it is necessary to supervise the sampling and training of samplers (DHI specialists) in order to have a high level of samples quality.

Key words*: labeling, preservation, raw milk, sampling, storage*

Original paper

VARIATION OF MILK YIELD AND MILK COMPOSITION OF PIVSKA PRAMENKA SHEEP BREED THROUGH LACTATION

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Abstract

The aim of this paper is to present the research results on the milk traits and variation of milk composition during lactation of Pivska pramenka breed, as one of the most important autochthonous sheep breeds in Montenegro. Investigation was conducted on the family sheep farm located in the municipality of Žabljak, a typical rearing area of Pivska pramenka breed.

The following milk traits were studied: length of lactation, milk yield (daily milk yield – DMY and total milk yield - TMY) and milk composition (Fat, Protein, Lactose, Solid non-fat - SNF and Somatic cells count - SCC).

Based on the data of 68 completed lactations, average length of lactation was 178.5 days and average milk yield 94.1 kg. On the basis of 289 milk samples analysed in 5 consecutive milk recordings, average daily milk yield was 0.549 litres, with 5.71% of fat, 5.75% of protein, 4.47% of lactose, 11.04 % of solid non-fat and $600x10^3$ SCC on average.

Differences in averages for milk composition among test days (days of milk recording) during lactation were significant ($P<0.01$). The largest variation was for milk fat, from 2.95% in the first control to 9.42% in the fifth control, while variation of protein was between 4.5% in the first and 7.4 in the fifth control. The content of lactose exposed decreasing trend during lactation (from 4.7% in first to 3.1% in fifth control).

Key words: *lactation, milk composition, milk yield, Pivska pramenka*

Introduction

Milk is still an important product of sheep sector, in spite of dominance of meat market value. Europe is the main sheep milk producer in the world (2/3 of total production), with the Mediterranean countries (Italy, Spain, France and Greece) as the leading producers (Mioč et al., 2007). Sheep milk is mainly used for production of different types of cheese, then for sour milk products and to a smaller extent is directly consumed as fresh milk (Domaćinović et al., 2008).

Economic importance of sheep milk in the countries of Balkan Peninsula has been constantly decreasing, due to the fact that lamb meat production is in focus of farmers' orientation and

their breeding programs (Petrović et al., 2013). The same trend is present in Montenegrin sheep sector.

During the sixties of 20th century Montenegro had about 600,000 heads of sheep. Since that period, intensive industrialization led to a drastic reduction of the total sheep population. Only during the last 20 years sheep population decreased by 53%, from 480,000 heads in 1991 to 207,000 heads in 2012.

Sheep production is characterized by semi extensive system of rearing, focused on utilisation of grassland and pasture areas. Regarding breed structure, autochthonous breeds (Pramenka group of breeds) prevail with a number of breeds which are invaluable from the aspect of preservation of farm animal genetic resources (Markovic et al., 2008). Two autochthonous breeds are dominant: Pivska or Jezeropivska (approx. 20% in total) and Sjenička (approx. 25% in total). Breeds like Bardoka, Ljaba, Sora or Zetska Žuja are less numbered, and all together participate by 15% in the total. Share of crossbreds in the total population is very high, about 40% (Marković et al., 2011).

These autochthonous sheep breeds are for triple-purpose (milk, meat and wool). Since wool is nowadays practically without economic importance, thus ratio between market value of the main products is estimated as: meat 70-75%, milk 25-30%. This ratio depends on the region, way of production, milking capacities of sheep, as well as on working force available for hand milking (Adzić et al., 2004; Marković et al., 2011).

Production of sheep milk in Montenegro is highly seasonal due to the seasonality in lambing of ewes, usually from February to April. Milking of ewes mainly starts after three month suckling period and lasts until autumn. Milk is usually processed in household into traditional dairy products: local product called skorup or kajmak, different types of cheese, yogurt, jardum, etc.

Raw milk quality is one of the key prerequisites for the amount and quality of dairy products. Yield and composition of milk are influenced by breed, age of ewes, parity, litter size, nutrition, health of animals, environment, stage of lactation, etc. The last one – stage of lactation has a significant effect on milk traits (Pavić et al., 2002; Oravcova et al., 2007; Kuchtik et al., 2008).

Milk production is a function of the stage of lactation, usually presented in number of days in milk. The most common approach in lactation curve modelling is to fit suitable function of time, $y = f(t)$, to test-day records (Komprej et al., 2012). Two types of lactation curves can be found in sheep to describe the effect of stage of lactation on milk traits. The first one is common to the intensive production systems. Daily milk yield (DMY) is increasing from lambing to the peak of lactation appearing three to five weeks ahead. After that daily milk yield is gradually decreasing toward the end of lactation with different persistence, depending on rearing conditions and breed. The second type, known as an atypical lactation curve, appears in ewes kept in poor environment or in the extensive production systems where daily milk yield decrease continually from the start toward the end of lactation.

Most of the previous studies of milk traits of sheep in Montenegro were focused on total milk yield and lactation length measured individually, while milk composition (solids, butterfat, and milk protein) were determined only on the group milk samples, but not on milk records of individual ewes. The aim of this paper is to present the results of investigation of variation of

daily milk yield and milk composition during lactation of Pivska pramenka breed on the basis of test day records.

Materials and methods

Investigation was conducted on the family sheep farm located at village Javorje, Žabljak Municipality. It is at the foot of the Mountain Durmitor, at an altitude of 1500 meters, what is typical rearing area of Pivska pramenka breed. Milk is mostly used for production of well-known traditional dairy products ”skorup” and cheese “prljo”. This breed is well adapted to the conditions of a cold mountain climate. Feeding of sheep is primarily based on pasture, while during wintertime the hay is the main feed, supplemented by grain during lambing season. During summer season (from May to end of September) the farmers usually remove flocks from the permanent settlements to mountain pastures.

The investigations were carried out during 2013. Due to a very long and cold winters lambing season starts in the second part of March and ends in the middle of April. Suckling period, when lambs are permanently with their mothers, lasted 2.5 months. One of the characteristics of sheep sector in this region is growing of lambs at the pasture until the age of 6-7 months. Due to the lack of enough workers, after the weaning the lambs stay with their mothers during the day as well, thus having possibility to suck ewes. Lambs are separated only during the night and ewes are milked regularly only in the morning.

Milk recording was done in accordance with the ICAR guidance at five consecutive terms, starting in April. First two recordings were done during suckling period, and the rest during the period of milking of ewes. Milk yield was determined by measuring cylinder graduated at 20 ml. Individual samples of milk for chemical analyses were taken from each of the recorded animal, thus total of 289 milk samples was collected and delivered to the lab for analyses.

Chemical composition of milk was analysed in the Dairy Laboratory of Biotechnical Faculty, in the following way: fat (F), protein (P), lactose (L) and solid non-fat (SNF) by the IC spectrophotometry (IDF 141C: 2000), somatic cells – 10^3/ml (SSC) by flow cytometry METI EN ISO 13366-2-2011 (IDF 148-2) using MilcoScan 120FT.

All data were analysed by using one-way analysis of variance (ANOVA) in the statistical package Statistica 12.

$$Yijkl = \mu + Ci + eij$$

where:

μ = overall mean

Ci = fixed effect of test day (i = 1, …, 5)

eij = random residual error.

Results and discussion

The obtained results on 68 ewes of Pivska pramenka breed (Table 1) show that the average milk yield was 94.11 kg during 178.5 milking days on average. Very wide range of variation in TMY, from 50 kg to 200 kg, indicates that this breed has a genetic potential for higher milk

production. Milk yield in this research is slightly lower than the previous one for this breed of sheep (Ljumović and Adžić 1987; Mitić 1987), while similar to the results obtained by Adzic et al. (1992).

Pivska pramenka has similar milk yield to Sjenička breed, but higher than the other breeds from the Pramenka group: Zetska Žuja and Ljaba from Montenegro, Pirotska from Serbia and Vlasicka from Bosnia and Herzegovina (Ljumović and Adzić, 1987; Mitić, 1987; Petrović et al., 2002 and 2006). However, autochthonous breeds from Croatia produced higher milk quantity in the lactation (Pandek et al., 2005).

Average daily milk yield (DMY) and milk components: fat, protein, lactose, SNF – solid non-fat and somatic cells count – SCC were determined on 289 individual measurements and on the same number of milk samples taken in five consecutive visits during milking.

Average daily milk yield was 0.549 l. It was the highest at first recording (0.720 l), then it has been constantly decreasing till the last (fifth) test day (0.280 l), Table 2. All differences in milk yield among test days (days of recording) were significant ($P<0.01$), except for the difference between the first and second recording which was not significant ($P>0.05$).

Table 1. *Statistical parameters for milk traits of Pivska pramenka breed*

Parameter	N	$\bar{X}$	SD	CV (%)	Range
Lactation length, days	68	178.53	24.11	13.50	126.0 – 230.0
TMY - Lactation milk yield, *l*	68	94.11	25.41	27.00	50.20 –198.90
DMY - daily milk yield, *l*	289	0.549	0.24	43.7	0.10 – 2.40
Fat, %	289	5.71	2.55	44.6	1.85 – 12.42
Protein, %	289	5.75	1.18	20.51	3.79 – 10.36
Lactose, %	289	4.47	0.67	14.99	1.86 – 5.46
SNF, %	289	11.69	0.82	7.02	9.00 – 14.72
SCC, 10^3	278	600.52	1085.50	180.70	37 - 5311

Fat is the milk component with the highest variation through the lactation. Average fat content was 5.71%, and contrary to the milk yield it has had significant increase from 2.95% in the first up to 9.42% in the fifth recording. The differences among the test days were significant ($P<0.01$).

Variation of fat content through lactation and the effect of stage of lactation on fat content are in accordance with the results of Pavić et al. (2002); Kuchtik et al. (2008); Komprej et al. (2012). However, the average fat content in milk is lower than in majority of the results of other researchers. One of the reasons for this may be incomplete milking; thus the last milk is retained.

Protein, lactose and SNF are milk components with much smaller range of variation than milk fat. Similar to fat content, protein has been increasing from the first recording (4.51%) up to the fifth (7.36%), while lactose content exposed the opposite trend, but at smaller scale, from 4.72% in the first to 3.11% in the fifth recording, Graph 1.

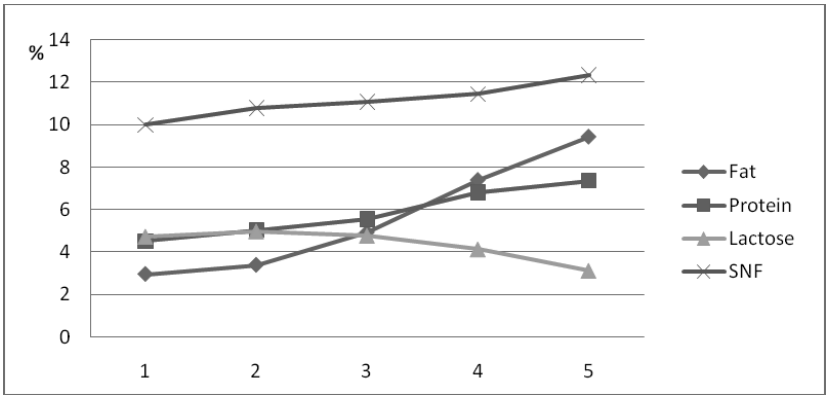
Table 2. *Variation of daily milk yield (DMY) and chemical composition of sheep milk in different stages of lactation*

Parameter	I	II	III	IV	V
Daily milk yield, *l*	0.72 ± 0.04^{a}	0.67 ± 0.03^{a}	0.58 ± 0.02^{b}	0.420 ± 0.02^{c}	0.28 ± 0.02^{d}
Fat, %	2.95 ± 0.09^{a}	3.38 ± 0.09^{a}	4.91 ± 0.12^{b}	7.38 ± 0.20^{c}	9.42 ± 0.34^{d}
Protein, %	4.51 ± 0.51^{a}	5.03 ± 0.04^{b}	5.54 ± 0.06^{c}	6.80 ± 0.10^{d}	7.36 ± 0.19^{e}
Lactose, %	4.72 ± 0.02^{a}	4.98 ± 0.03^{b}	4.77 ± 0.02^{a}	4.12 ± 0.06^{c}	3.11 ± 0.10^{d}
SNF, %	9.99 ± 0.06^{a}	10.78 ± 0.05^{b}	11.07 ± 0.06^{b}	11.44 ± 0.2^{c}	12.32 ± 0.16^{d}
SCC, 10^3/ml	563 ± 302^{ab}	206 ± 41.2^{b}	342 ± 75.4^{b}	1113 ± 284^{ac}	2067 ± 819^{d}

^{a,b,c,d} - different letter in subscript means statistical significant differences ($P<0.05$)

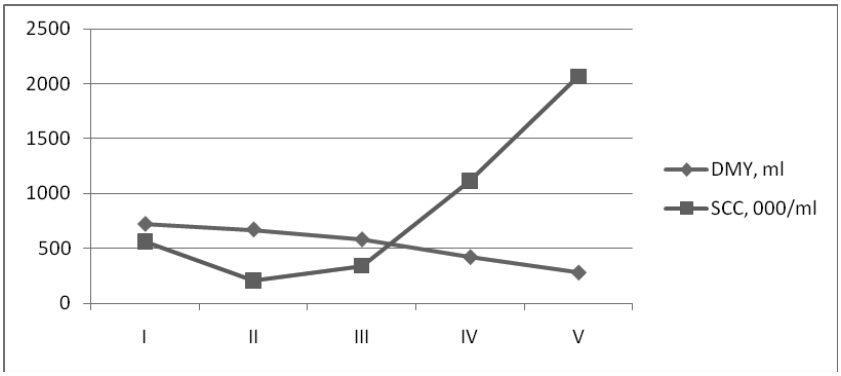
Similar to protein, SNF had a mild growing trend from the first (9.99%) to the fifth recording (12.32%). Stage of lactation had a significant influence on SNF content, thus all differences among test days (recordings) were significant ($P<0.01$), except for the difference among the second and third test day which was not significant ($P>0.05$).

Somatic cell count - SCC in sheep milk is important as parameter of udder health status (mastitis indicator) as well as for the processing of milk (indicator of quality). Average SCC was 600,520/ml. SCC amounted to 563,000 at the beginning of lactation (first recording) then during the middle of lactation (second and third recording) went down and significantly increased reaching 1,113,000 in the fourth recording and even 2,067,000 cells in the fifth recording.



Graph 1. *Variation of the milk components through lactation*

Increasing of SCC during the second half of lactation, parallel with decreasing of daily milk yield is in accordance with the results of Gaicone et al. (2005). SCC curve goes to the opposite direction in comparison to the shape of DMY curve, Graph 2.



Graph 2. *Variation of DMY and SCC through lactation*

Conclusion

The stage of lactation had a significant effect on the daily milk yield and the content of all studied milk components.

A wide range of milk yield indicates the plasticity of the genome of Pivska pramenka and good capacity for improvement by implementation selection in the existing breed.

Content of fat in milk has been increasing in parallel with the decreasing of milk yield.

From April to October protein and SNF content increased in parallel, but lactose decreased slightly.

The results of milk yield and milk composition of Pivska pramenka sheep breed show that there are still quite a lot of possibilities to improve the milk yield and fat content. Nutrition, maintenance and state of health can contribute to improve the production and to influence a rearing success.

References

1. Adzić N, Marković B 2003. Osnovne morfološke i proizvodne karakteristike zetske zuje. Savremena poljoprivreda 3-4, 373 – 376.
2. Domaćinović M, Antunović Z, Mijić P, Šperanda M, Kralik D, Đidara M, Zmaić K 2008. Proizvodnja mlijeka – sveučilišni priručnik, ISBN 978-953-7243-19-7, Poljoprivredni fakultet Osijek.
3. Giaccone P, Scatassa M L, Todaro M 2005. The influence of somatic cell count on sheep milk composition and cheese-making properties. Ital. Journal of Animal Science 4 (Suppl. 2), 345-347.
4. Grupa autora – Adžić et al. 1992. The influence of genotype on prolificy and other productive traits of sheep under omprove and intensive feeding condition and care in mountainous regions. Final Report of project JFP-685. Agricultural institute Podgorica.
5. Komprej A, Drobnič M, Kompan D 1999. Milk yield and milk traits in Slovenian sheep breeds. Acta Agraria Kaposváriensis 3, 2, 97-106.

6. Kuchtik J, Šustova K, Urban T, Zapletal D 2008. Effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling in East Friesian ewes. Czech J. Anim. Sci. 53, 2, 55–63.
7. Ljumivić M, Adžić N 1988. Mliječnost i prinos vune crnogorskih ekotipova ovaca. Poljoprivredni institute Titograd, Zbornik radova, pp. 217-228.
8. Marković B, Marković M and Adzić N 2007. The farm animal genetic resources of Montenegro, Biotechnology in Animal Husbandry 23, 3-4, 1-9.
9. Marković B, Marković M, Radonjić D, Veljic M 2011. Sustainable sheep and goat production based on local breeds in Montenegro. 8th Global Conference on the conservation of animal genetic resources Tekirdag, Turkey. Book of papers, pp. 413-421.
10. Mioč B, Pavić V, Sušić S 2007. Ovčarstvo. Hrvatska Mljekarska udruga - Zagreb.
11. Mitić N 1987. Ovčarstvo. Naučna knjiga Beograd.
12. Oravcová M, Margetín M, Peškovičová D, Daňo J, Milerski M, Hetényi, Polák P 2007. Factors affecting ewe's milk fat and protein content and relationships between milk yield and milk components, Czech J. Anim. Sci. 52, 7, 189–198.
13. Pandek K, Mioč B, Barać Z, Pavić V, Antunac N, Prpić Z 2005. Mliječnost nekih pasmina ovaca u Hrvatskoj. Mljekarstvo 55, 1, 5-14.
14. Pavić V, Antunac N, Mioč B, Ivanković A, Havrenek J 2002. Influence of stage of lactation on the chemical composition and physical properties of sheep milk. Czech J. Anim. Sci. 47, 2, 80–84.
15. Petrović P M, Ilić Z, Petrović Caro V 2013. Ovčarstvo i kozarstvo. Monografija – ISBN 978-86-82431-70-1, Institut za stočarstvo Zemun.
16. Petrović P M, Žujović M, Negovanović D, Ružić Muslić D, Strsoglavec S 2002. Uticaj paragenetskih faktora na osobine mlečnosti ovaca. Biotehnologija u stočarstvu 18, 1-2, 23-27.

Abstract

THE EVALUATION OF MILK PRODUCTION FOR THE TIGAIE BREED WHICH IS RAISED IN DIFFERENT TRADITIONAL PARTS OF ROMANIA

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Abstract

The purposes of the research was to assess the real potential of the Tigaie breed for milk production and the identification of the improvement ways of production. The biological material used for evaluation was represented by growing herds which were exploited in three Romanian counties in which the growing of this breed is an action with old traditions. The quantitative milk production was appreciated based on monthly check-ups, the used method was the official one used in Romania and in the UE, named the A_4 method. During the lactation more successive check-ups were planned, for equal periods of 30 days, and for determining the total milk production obtained for each period, the Fleishmann method was used

The obtained results after applying successive reviews indicate the existence of obvious differences between the lots. These differences are significant statisticaly for $p<5\%$, the only distinguished exception being of + 0.54 l obtained between Vrancea and Vaslui, which is unimportant for the considered significant limit. The completion of the research and the statistical processing of the dates indicate the fact that the Tigaie breed must be subjected to a more extensive program for milk production which would make it more efficient.

Key words: *Romanian sheep, sheep for milk production (milk sheep), Tigaie*

Original paper

EGG QUALITY CHARACTERISTICS IN AUTOCHTHONOUS GENOTYPES OF CHICKENS RAISED ON MACEDONIAN RURAL FARMS

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Abstract

Egg quality characteristics of three different autochthonous chicken phenotypes (phenotype having ashy-silvery-brown leghorn laced plumage color, phenotype having light brown spangled plumage color and phenotype having black or blue plumage color) were examined. These types of chickens are mainly raised as backyard rural area flocks in the villages of R. Macedonia. The data were collected through first systematic approaches in phenotypical identification and characterisation of these autochthonous genotypes realized recently. Three small flocks of old hens collected from different villages and placed in control pens were established. No data about number of eggs/hen/year is available for the established flocks. Further investigations are needed to record productive (number of egg produced) and reproductive data. The plan was that additional, yearly production records be collected from the flock produced as offspring from established flock of old hens collected from different villages and placed in control pens. Set of 90 eggs was collected (30 eggs from each plumage phenotype) and basic egg quality parameters measured. Egg size (weight) was 50.71g for brown laced plumage phenotype, 60.48g for blue plumage phenotype and 52.6 g for light brown spangled plumage phenotype. Egg shell strength was 3965, 3628 and 3924 g/cm^2 for brown laced, blue and brown spangled plumage phenotype, respectively. Light brown spangled plumage phenotype had the highest value of yolk color (10.29) and Hough Units (76.63) compared to brown laced (10.08 and 75.27) and blue (9.09 and 71.65) plumage phenotype.

Key words: *autochthonous, egg quality, genotypes, poultry, rural*

Introduction

Global trends have been oriented toward identification, characterization, protecting and conservation of the animal genetic resources worldwide. Chickens are part of these activities and contribute toward world's food security, safety and sustainability. Many reports have been published on the characteristics of different native genotypes of chickens in order to offer small, but valuable contribution to these global efforts for protecting the genetic poultry resources.

Tharrington et al. (1999), analyzing historical Leghorn strains conclude that genetic selection has resulted in decreased yolk percentage due to increased egg size, meaning that selection improved albumen size but the overall egg quality has been maintained. Zanon et al. (2006) in his study examined the egg characteristics of autochthonous Modenese and Romagnolo breeds and report light and small eggs produced by them but these eggs had higher contents of yolk protein than commercial eggs. General findings of the study of Krawczyk (2009) was that native breed hens kept for many years in closed populations where no selection process was practiced express noticeable differences in some egg quality traits that can change according to egg production level and layer age but not in a pattern noticed in the commercial hybrid hens selected for high production. Ceccobelli (2013) has summarized and described the most important traits for several breeds (Albanian, Serbian, Spanish and Italian) in PhD thesis focusing on molecular tools in analyzing genetic diversity of Mediterranean autochthonous chicken breeds.

Analysing data on the quality of eggs of different genotypes has shown a relatively poor quality of eggs laid by Naked Neck, namely, lower weight, lower quality and poorer inner shell quality. The difference in the quality of the eggs was also identified between autochthonous breeds (Pavlovski et al., 2012).

Macedonia is a small country in Balkan Peninsula with about two million inhabitants in which rural population represents 41% of these two million. Poultry industry officially records about 2.2 million chickens, with three level tiers structure. Egg production covers domestic market demands but poultry meat production covers only 4-5% of the market needs. Overall average egg production at all three levels is 152 eggs/hen/year. First level of production is organized on 10-15 big enterprise farms with average capacity 80-100000 layers where around 1 million layers are kept in cages reaching production level of over 310 eggs/year. Second level is represented by medium and small family owned farms covering 200 000 layers. Third level comprises all other (around one million) chickens that are backyard poultry with average production of 80-100 eggs/hen/year kept for the household needs. In the third level many fancy breeders exist that keep different breeds rarely taking in consideration breeding structure and keeping herdbooks for pedigree breeding purposes (Ministry of Agriculture, Forestry and Water Economy (MAFWE), 2007; 2008). At the moment no national livestock gene bank is established in the country (Ministry of Agriculture, Forestry and Water Economy (MAFWE), 2010). Such gene bank could serve as a main point for conservation, protection, monitoring and inventory of livestock biodiversity. First systematic activities were realized in 2011, aiming to monitor the happenings in the "third (rural) level" of poultry industry. The idea was to investigate if there are remains of archaic, native or indigenous genotype of chickens that used to be there before the era of industrial poultry production. These efforts were part of the state goals toward protection and conservation of the genetic diversity in the area of domestic animals and poultry according to the acquired obligation stated in the "Law for Livestock". Additionally, noticeable efforts are made through different international organisations for protecting and conservation of the genetic resources in agriculture in general and poultry species as a part of it. Activities in this direction could help establishing protected flocks of rare breeds and genotypes that exist among rural areas in the country. The information presented is the result of these and follow-up, ongoing activities in the area of preservation of the indigenous chicken genotypes.

Material and methods

Recently, three small flocks of old hens collected from different villages and placed in control pens have been established through the support obtained from GEF project enabling first systematic approaches in phenotypical identification and characterisation of these autochthonous genotypes. The eggs were collected from these pens (pen of hens having ashy-silvery-brown leghorn laced plumage color, pen of hens having light brown spangled plumage color and pen of hens having black or blue plumage color). Set of 30 eggs from each plumage phenotype was collected and basic egg quality parameters measured. No data about number of eggs/hen/year are available for the established flocks. Analyses of the external (egg mass - g, egg shell strength - g/cm2) and internal (yolk color – La Roche ladder 1-15, albumen quality – Hough units) egg quality parameters were performed in the laboratory for control of the marketing quality of eggs in the Institute for Animal Biotechnology of the Faculty of Agricultural Sciences and Food using automated machine Egg multi tester EMT 5200, for measuring the internal quality and Eggshell Force Gauge for measuring the egg strength (Robotmation Co. Ltd., Tokyo, Japan).

Results and discussion

Morphological characteristics

Using the Generic data collection format for phenotypic characterization of chickens from Annex 3 of the Draft guidelines on phenotypic characterization, determined by Intergovernmental technical working group on animal genetic resources for food and agriculture of the Commission on genetic resources for food and agriculture of FAO (2012), three distinct phenotypes were determined and small flocks of hens with similar phenotype were established for further reproduction and monitoring:

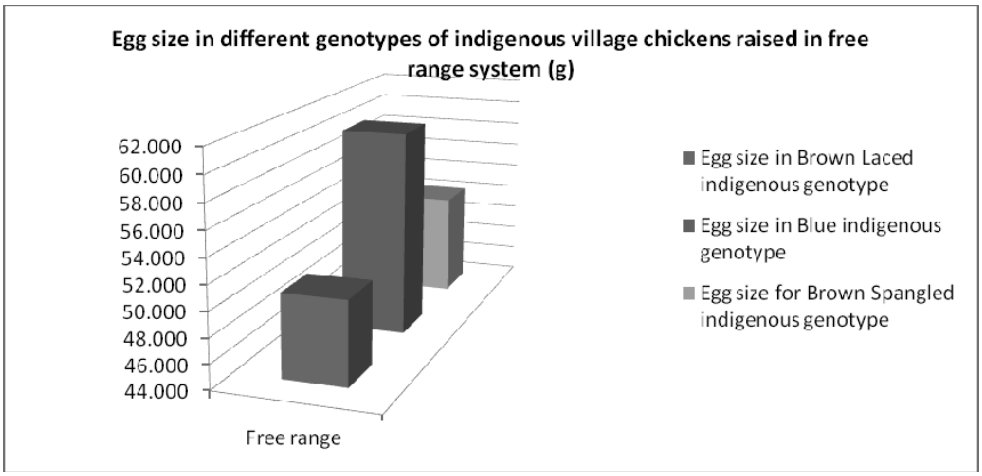
- Phenotype having ashy-silvery-brown leghorn laced plumage color;
- Phenotype having light brown spangled plumage color and
- Phenotype having black or blue plumage color.

Egg quality characteristics

Table 1. *Egg quality characteristics of different indigenous genotypes raised in village area of Macedonia as backyard chickens*

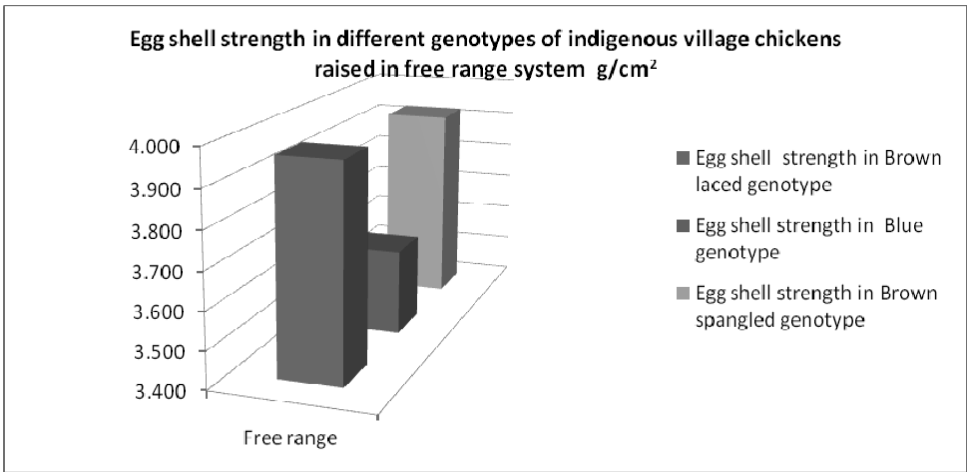
Parameters	Brown laced	Blue	Brown spangled	Average
Egg weight (g)	50.71	60.48	52.26	54.48
Egg shell strength g/cm^2	3965	3628	3924	3839
Yolk color – La Roche points (1-15)	10.08	9.09	10.29	9.82
Internal quality - albumen quality (Hough Units)	75.27	71.65	76.63	74.52

Egg production level is still not recorded, but the plan is to establish a control flock from each of the phenotypes in the next year and to follow their productivity for the year. Estimates are that 100-120 eggs are laid yearly in all three varieties but it hardly depends on the feed used and feeding practice. Sample of 30 eggs from each of the flocks of all three phenotypes was included in this analysis. Results (Table 1.) present average values for egg size (weight) and other egg characteristics (shell strength, yolk color and Hough units). Egg weight was 50.71g, 60.48g and 52.6g for brown laced plumage phenotype, blue plumage phenotype and brown spangled plumage phenotype, respectively. Eggshell strength figures were 3965, 3628 and 3924, and yolk color 10.08, 9.09 and 10.29 for brown laced plumage phenotype, blue plumage phenotype and brown spangled plumage phenotype, respectively. Hens from brown spangled plumage phenotype produce eggs with best albumen quality (76.63 Hough Units) in comparison with brown laced plumage (75.27) and blue plumage phenotype (71.65).



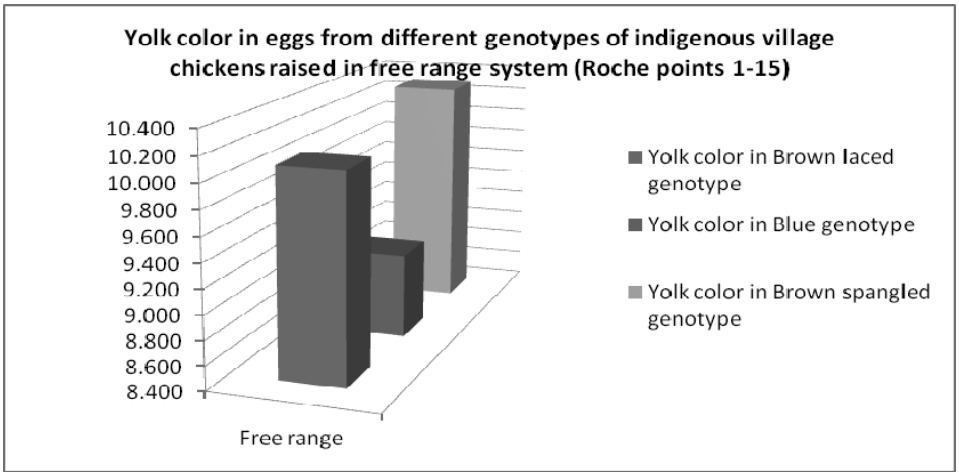
Graph 1. *Egg size of different indigenous genotypes raised in village area of Macedonia as backyard chickens*

The average egg size (Graph 1) was quite high (54.48 g) compared to some other results of research on indigenous eggs. The indigenous eggs are mostly small in size with less weight when compared with other types of chicken eggs (Shabbir et al., 2013). The larger size of eggs especially in the Blue genotype was due also to the greater age of the chickens. The eggs mass increased with the age of hens, especially from traditional production where egg mass showed continuous increase with the age of hens (Škrbić et al., 2011).



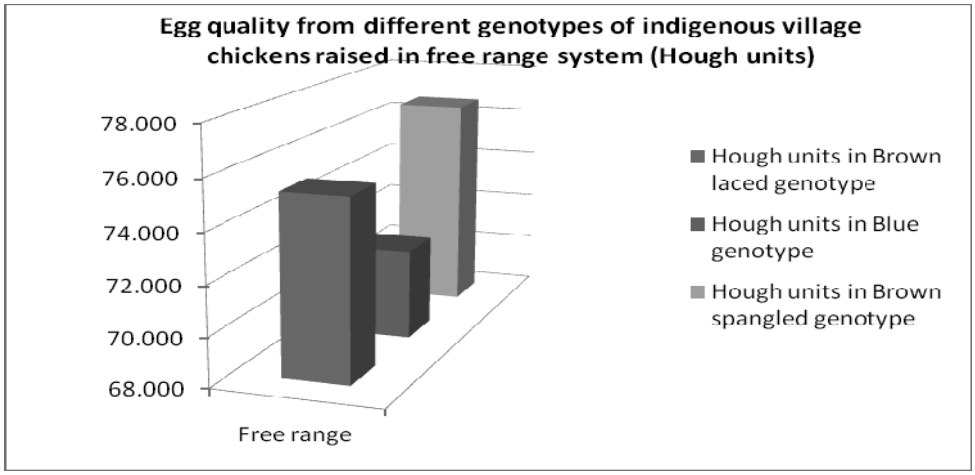
Graph 2. *Egg shell strength in different indigenous genotypes raised in village area of Macedonia as backyard chickens*

Egg shell strength (Graph 2) was 3839 g/cm^2 on average which is a good strength of shell especially in Brown laced genotype that had the smallest mass of eggs and also the best strength of the shell, which indicates a positive correlation between the two parameters. Significant ($P < 0.05$) negative correlation was found between egg shell strength and egg weight (-0.175) indicating that with increasing the egg weight the eggshell strength decreases (Kocevski et al., 2011).



Graph 3. *Yolk color in different indigenous genotypes raised in village area of Macedonia as backyard chickens*

Yolk color (Graph 3) was very good, ranging from 9.09 to 10.29 points in La Roche scale, showing that the trait can be considerably improved when hens are given access to green outdoor areas. Free-range layers consumed plants that were a source of xanthophylls for egg yolk. Van den Brand et al. (2004) and Horsted et al. (2006) observed that plants consumed on free-range have a beneficial effect on yolk color.



Graph 4. *Internal (albumen) quality (Hough Units) in different indigenous genotypes raised in village area of Macedonia as backyard chickens*

The value of Hough units (Graph 4) showed good quality of egg albumen and high freshness, 74.52 average for all genotypes. Eggs from backyard system received the best scores for freshness (albumen height and Haugh units), corroborating the results of similar studies by Kosmidou et al. (2007), Sekeroglu et al. (2008) and Krawczyk (2009). Meanwhile, Rossi (2007) obtained lower Haugh units for the eggs of hens kept under organic production systems compared to caged layers.

Conclusion

Full system for monitoring and recording the local breeds is at the beginning, therefore monitoring of trends and risks in the field of endangered local breeds are scarce. This means that these, first information are indicative and based on scientific research activities and first systematic efforts realized recently.

Presented egg characteristics of the three chicken phenotypes described belong to one "Macedonian" genotype, which is part of the mixed gene pool that exists among village chicken population in Macedonia. Further activities are needed in realization of the conservation program toward stabilization of the phenotypic population and collection of morphometrical, productive and reproductive data for the existing flocks. This will contribute toward definition and characterization of breed or genotype standards for the exterior including body measures, phenotypical, biological, productive and reproductive characteristics.

References

1. Ceccobelli S 2013. Genetic diversity of Mediterranean autochthonous chicken breeds. [Tesi di dottorato], PhD Thesis.
2. FAO 2012. Food and Agriculture indicators, Country: Republic of Macedonia.

3. Horsted K, Hammershoj M, Hermansen JE 2006. Short-term effects on productivity and egg quality in nutrient-restricted versus non-restricted organic layers with access to different forage crops. Acta Agriculturae Scandinavica Section A, 56, 42-54.
4. Kocevski D, Nikolova N, Kuzelov A 2011. The influence of strain and age on some egg quality parameters of commercial laying hens. Biotechnology in Animal Husbandry 27, 4, 1649-1659.
5. Krawczyk J 2009. Effect of layer age and egg production level on changes in quality properties of eggs from hens of conservation breeds and commercial hybrids. Annals of Animal Science 9, 2, 185-193.
6. Kosmidou MD, Fortomaris PD, Sossidou EN, Yannakopoulos, Tserveni-Goussi AS 2007. The effect of an enriched pasture with aromatic plants on some egg quality characteristics of free-range laying hens. Proceedings of the XVIII European Symposium. on the Quality of Poultry Meat and XII European Symposium on the Quality of Eggs and Egg Products. Prague, September 2-5, 73-74.
7. Ministry of Agriculture, Forestry and Water Economy (MAFWE) 2007. National Agricultural and Rural Development Strategy, Skopje R.Macedonia.
8. Ministry of Agriculture, Forestry and Water Economy (MAFWE), 2008. Law for Livestock, Skopje R.Macedonia.
9. Ministry of Agriculture, Forestry and Water Economy (MAFWE), 2010. Bylaw for the way of realization of the monitoring of the biological diversity in the Livestock sector, Skopje R.Macedonia.
10. Pavlovski Z, Skrbic Z and Lukic M 2012. Autochthonous breed of chicken in Serbia: research or development. International Feed Technology Symposium "Feed-to-Food", Novi Sad, Serbia 03-05 October, 2012. Proceedings 127-132.
11. Rossi M 2007. Influence of the laying hen housing systems on table egg characteristics.
12. Proceedings of the XVIII European Symposium. on the Quality of Poultry Meat and XII European Symposium. on the Quality of Eggs and Egg Products. Prague, September 2-5, 49-51.
13. Sekeroglu A, Sarica M, Demir E, Ulutas Z, Tilki M and Saatci M 2008. The effects
14. of housing system and storage length on the quality of eggs produced by two lines of laying hens. Archiv fuer Gefluegelkunde 72, 106-109.
15. Shabbir H, Zulfiqar A, Muhammad NK and Taseer AK 2013. A study on quality traits of chicken eggs collected from different areas of Karachi. Sarhad J. Agric. 29, 2, 255-259.
16. Skrbic Z, Pavlovski Z, Lukic M, Vitorovic D, Petricevic V and Stojanovic Lj 2011. Changes of egg quality properties with the age of layer hens in traditional and conventional production. Biotechnology in Animal Husbandry 27, 3, p 659-667.
17. Tharrington JB, Curtis PA, Jones FT and Anderson KE 1999. Comparison of physical quality and composition of eggs from historic strains of Single Comb White Leghorn chickens. Poult. Sci. 78, 591–594.
18. Van Den Brand H, Parmentier HK and Kemp B 2004. Effects of housing system (outdoor vs. cages) and age of laying hens on egg characteristics. Brit. Poult. Sci. 45, 6, 745-752.
19. Zanon A, Beretti V, Superchi P, Zambini EM and Sabbioni A 2006. Physico – chemical characteristics of eggs from two Italian autochthonous chicken breeds: Modenese and Romagnolo. Proc. XII EPC Congress, Verona 10-14 September, in World Poultry Science Journal, 2 (Supplement), 203.

Original paper

GENETIC AND PHENOTYPIC VARIABILITY FOR RACING PERFORMANCE OF TROTTER HORSE IN SERBIA

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Abstract

Horse race results in studbooks are supposed to give information to manage the selection of trotters based on the analysis of genetic and phenotypic parameters. The objectives of this study was to estimate genetic and phenotypic variability of three racing traits (number of starts, race time and best racing time) of trotter horses in Serbia. The data were obtained from the Trotting Association of Serbia and consisted of 2252 observations. The model included effect of sex, year of birth, season, year of race, distance and race track as fixed effects and sire as random effect. The BLUP sire model was applied to the genetic evaluation of measured traits. Average mean of number of starts, race time and best racing time was 64, 83.15 and 79.28, respectively. Of all tested fixed effects only distance was not statistically significant for number of starts and season for best racing time. However, a statistically highly significant influence of all tested fixed effects on racing time was shown. Heritability estimates were 0.28 for number of starts, 0.19 for racing time and 0.35 for best racing time. The low heritability estimates for number of starts and racing time indicate that selection based on horse phenotypic value induces small genetic change in these traits while middle level of heritability for best racing time indicates that animal's phenotype is a good indicator of genetic merit or breeding value.

Key words: *heritability, racing performance, trotter*

Introduction

Trotter racing is a form of horseracing in which the horses race in a specified gait (trot). Trot is a two-beat gait in which the diagonal limbs move together synchronously (Thiruvenkadan et al., 2009). Horse race results in studbooks are supposed to give information to manage the selection of trotters based on the analysis genetic and phenotypic parameters. Research on the basis of trotting performance was previously done by Minkema (1978), Langlois (1982, 1984a) and Tolley et al. (1985). Later, Ricard et al. (2000) and Thiruvenkadan et al. (2009) also compiled the performance of trotters. In this review, a detailed description of different breeds involved in trotting races has been presented along with description of genetic parameters, breeding evaluation and genetic improvement of the trotters. Trotting performance was mostly measured by time traits, earnings, perfect gaits, number of starts, rank in races and

qualification status. Hintz (1980) reviewed the heritability estimates of trotters and reported that log of earnings is highly heritable (0.41) while time, best time and earnings are moderately heritable (0.34, 0.25 and 0.20, respectively). In order to avoid bias in estimation of genetic parameters the effect of some environmental factors should be considered in the statistical model. Most often these are age, sex, year, racing distance, racetracks, track condition, temperature, class of race, starting method, season etc. According to Štrbac and Trivunović (2013) sex, age, season, racetrack and distance have highly significant effect on race time of trotters. Similar results were obtained by Rohe et al. (2001) and Bugislaus et al. (2006). The objective of this study was to estimate genetic and phenotypic variability of three racing traits (number of starts, race time and best racing time) of trotter horses in Serbia.

Material and methods

Individual race records of trotters in Serbia were used in this analysis. Based on information from the Trotting Association of Serbia, data consisted of 2252 observations. Performance data were used in analysis. These included number of starts, race time and best racing time. The model used contained fixed effect of sex, year of birth, season, year of race, distance, racetrack and sire as random effect. Statistical analysis was conducted using the software Statistica 12. Estimation of genetic parameters was conducted using the LSMLMW (Harvey, 1990), intra-class correlation (half-sibs) method. The statistical model was:

$$Y_{ijklmnop}=\mu+S_i+B_j+Se_k+R_l+D_m+Rt_n+Si_o+e_{ijklmnop},$$

Where:

Y: measured traits,

μ: general average,

S: the effect of sex (i=1..3),

B: the effect of year of birth (j=1...13),

Se: the effect of season (k=1,2),

R: the effect of year of race (l=1...15),

D: the effect of distance (m=1...3),

Rt: the effect of racetrack (n=1...29),

Si: the effect of sire (o=1...8),

e: random error is an error term under usual assumptions for ANOVA (the error distributed normally with mean = 0 and constant variance).

Results and discussion

The means and variability of the three measured traits for the trotter horse in Serbia are shown in Table 1. The average number of starts, racing time (s/km) and best racing time (s/km) were 64.85, 83.15 and 79.25, respectively.

Table 1. *Mean and variability of measured traits*

Traits	N	Mean	SD	CV
Number of starts	2252	64.85	35.23	54.33
Racing time, s/km	2252	83.15	3.44	4.14
Best racing time, s/km	2252	79.28	1.70	2.14

N – number of horses; SD – standard deviation; CV – coefficient of variation

Langlois and Vrijenhoek (2004) stated that the career total number of starts observed in French trotters was 30.3. The number of starts might be regarded as a possible indicator of the soundness of a horse's basic conformation (Thiruvenkadan et al., 2009). It can be considered according to the age or for the whole career. Racing time has frequently been used as a measure of racing performance in trotters. A natural choice to measure a performance trait for a racing horse is to measure its ability to run fast (Thiruvenkadan et al., 2009).

Table 2 shows the effect of sex, year of birth, season, year of race, distance, racetrack and sire on number of race, racing time and best racing time. Of all tested fixed effects only distance was not statistically significant for the number of starts and season for best racing time, while a statistically highly significant influence of all tested fixed effects on racing time was shown.

Table 2. *The effect of studied factors on measured traits*

Traits	Number of race		Racing time		Best racing time	
Source variability	F	P	F	P	F	P
Sex	29.989	0.0000**	21.617	0.0000**	124.868	0.0000**
Year of birth	94.981	0.0000**	59.174	0.0000**	77.036	0.0000**
Season	9.335	0.0023**	22.143	0.0000**	0.005	0.9463^{ns}
Year of race	17.953	0.0000**	39.287	0.0000**	2.742	0.0005**
Distance	0.189	0.8274^{ns}	28.236	0.0000**	6.480	0.0016**
Racetrack	4.922	0.0000**	14.113	0.0000**	3.644	0.0000**
Sire	12.793	0.0000**	8.527	0.0000**	15.620	0.0000**
R^2	*0.578*		*0.419*		*0.498*	

The racing records reflect the speed capacity of a horse when many favourable environmental conditions are met, it is the sum of the effects of many genetic and environmental factors (Arnason, 2001). When trotting performance is measured, the horse's age, sex, birth year, race distance, racetrack, track conditions, prize money, racing season and year are often taken into estimation of breeding value. Age and gender effects are mostly investigated and they will be examined further, as well as the year of racing with the genetic progress. Effect of racing distance on the speed is observable in countries having a wide range of distances in races. Accordingly this effect can bias the estimation of the effect of age because younger horses race on shorter distances (Thiruvenkadan et al., 2009). Racetrack effect results from some physical conditions such as the type of ground (grass being slower than the sand), shape of the curves and evenness and length of the straight lines (Thiruvenkadan et al., 2009). Highly significant effect of all mentioned on trotting horse speed was found by Bugislaus et al. (2006), Rohe et al. (2001), Katona and Distl (1989) and Štrbac and Trivunović (2013). It is

thought that by using individual race information, enviromental factors (rather than just pure genetic factors) will be able to be estimated much more accurately and information from each record will be more appropriately weighted in the final breeding value.

Heritability is one of the most important concepts in animal breeding. There are several working definitions, as heritability is used to help plan breeding programs, determine management strategies, estimate breeding values of individual animals and predict response to selection (Štrbac and Trivunović, 2014). An intensive selection of stallions on the basis of phenotypic racing performance has been practised in many trotter populations for quite a long time. Improvements have been observed in different trotter populations and this is attributed to both genetic and environmental changes. Studies devoted to the estimation of the heritability of the traits linked to racing performance are fundamental for the development of consistent genetic breeding programs. The heritability estimates, genetic and phenotypic correlations are shown in Table 3. Heritability estimates were 0.28 for number of starts, 0.19 for racing time and 0.35 for best racing time. The low heritability estimates for number of starts and racing time indicate that selection based on horse phenotypic value induces small genetic change in these traits while middle level of heritability for best racing time indicates that animal's phenotype is a good indicator of genetic merit or breeding value. There was a negative genetic correlation between time traits and number of starts as was expected because a decrease in the time traits is always related to the improvement in the performance results in races.

Table 3. *Heritability estimates (on the diagonal), genetic (below diagonal) and phenotypic correlation (above diagonal) for measured traits*

Parameters	Number of race	Racing time	Best racing time
Number of race	**0.28**	0.07	0.12
Racing time	-0.55	**0.19**	0.36
Best racing time	-0.40	0.92	**0.35**

Heritability estimates of racing performance range from low to moderate, and traits used in genetic evaluation of trotters are racing time, earnings, percentage of placings, and number of disqualifications in races (e.g., Ojala, 1987; Klemetsdal, 1994; Pösö and Ojala, 1997; Arnason, 1999; Thuneberg Selonen et al., 1999; Bugislaus et al., 2005; Langlois and Blouin, 2007). Klemetsdal (1994) observed that the heritability estimates in Norwegian Trotters for transformed number of starts in 3, 4 and 3 to 6 years of age were 0.02, 0.07 and 0.10, respectively. Arnason et al. (1989) also reported low heritability estimates for number of starts in 3-, 4-, 5- and 6-year-olds and the estimates were 0.07, 0.04, 0.02 and 0.07, respectively, in Swedish Standard bred Trotters. The heritability of the time traits was to the range found in the reviewed bibliography, 0.08–0.50 (Ojala and van Vleck, 1981; Ojala, 1987; Arnason, 1999; Bugislaus et al., 2005; Gomez et al., 2010). The genetic correlations between number of starts and the other traits according to Thiruvenkadan et al. (2009) were generally fairly low.

Conclusion

Selection of stallions on the basis of phenotypic racing performance has been practised in Serbian trotter populations for quite a long time. Knowledge about the genetic and phenotypic

parameters of the trotter horse in Serbia represents an actuality issue if we take into account that such studies are inexistent. In addition, selection and genetic improvement must be based on the analysis of the heritability and average values of racing performance in horse populations used in the race and breeding. The analysis of heritability of the racing performance in the Serbian population of trotters indicates that best racing time has the highest level (0.35). In animal breeding, the knowledge about genetic parameters of the traits that we are interested in is the first prerequisite in establishing a selection programme. Therefore, this traits should be main selection criterion in breeding programme. Because of a high genetic correlation between racing time traits (0.92), direct selection could also result in adequate selection accuracy for racing success for these traits, but modern animal breeding requires dynamic breeding schemes in which it is necessary to use genetic evaluation of breeding animals.

Reference

1. Arnason T 1999. Genetic evaluation of Swedish Standard-bred trotters for racing performance traits and racing status. Journal of Animal Breeding and Genetic 116, 5, 387-398.
2. Arnason T 2001. Trends and asymptotic limits for racing speed in Standardbred trotters. Livestock Production Science 72, 135–145.
3. Bugislaus AE, Roehe R, Willms F and Kalm E 2006. The use of a random regression model to account for change in racing speed of German trotters with increasing age. Journal of Animal Breeding and Genetics 123, 239-246.
4. Bugislaus AE, Röhe R, Willms F and Kalm E 2005. Multivariate genetic analysis to account for preselection and disqualified races in the genetic evaluation of racing performances in German trotters. Acta Agriculture Scandinavica 55, 49–56.
5. Gomez MD, Varona L, Molina A and Valera M 2011. Genetic evaluation of racing performance in trotter horses by competitive models. Livestock Science 140, 155-160.
6. Harvey RW 1990. Mixed Model Least Squares and Maximum Likelihood Computer Program. User, s Guide for LSMLMW and MIXMDL.
7. Hintz RL 1980. Genetics of performance in the horse. J. Anim. Sci. 51, 582–594.
8. Katona O and Distl O 1989. Sire evalution in the German trotter (Standardbred) population. EAAP Publication 42, 55-61.
9. Klemetsdal G 1994. Application of standardized, accumulated transformed earnings in breeding of Norwegian trotters. Livestock Production Science 38, 245–253.
10. Langlois B andVrijenhoek T 2004. Qualification status and estimation of breeding value in French trotters. Livestock Production Science 89, 187-194.
11. Langlois B and Blouin C 2007. Annual, career or single race records for breeding value estimation in race horses. Livestock Science 107, 132-141.
12. Ojala M and Van Vleck LD 1981. Measures of racetrack performance with regard to breeding evaluation of trotters. Journal of Animal Science 53, 611-619
13. Ojala M 1987. Heritabilities of annually summarized race records in trotters. Journal of Animal Science 64, 117–125.

Invited paper

REVIEW OF NUTRITION MODEL FOR GESTATION SOWS

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Abstract

Optimal feeding of sows is a complex problem due to their very different needs and use of nutrients during certain phases of the reproductive cycle. In pregnancy the sows need for nutrients is relatively small and slightly higher than maintenance requirements. In the last few years, the recommendations for feeding pregnant sows have been revised in such a way that greater protein (amino acid) and energy intake have been proposed for late gestation, compared to early gestation. The U.S. National Research Council (NRC, 2012) published the Nutrition models for swine, combined into a Microsoft Excel workbook and stored in the file NRC swine 2012. The program also includes a database with nutrient profiles of ingredients and a means to formulate diets and generate feeding programs. In the current paper, we will show agreements and differences between models and empirical data and discuss the implications and relevance for pregnant sow nutrition.

Key words*: needs, nutrition model, sows, recommendation*

Introduction

In pig production, sow nutrition is one of the most dynamic and most pre-stressed, so it is a challenge to meet the needs of nutrients and achieve satisfactory results. During exploitation, sow should produce about 70-75 piglets as is generally accepted. The key factors are: stage of gestation and feeding regimen, parity and anticipated litter size; and effects of housing and environmental conditions. These factors are main influence of nutrient requirements, and in the 2012 NRC model during evaluation different feeding programs they are indispensable. Pig farming is a process within which it is difficult to say "This stage is the beginning of the process". The question that arises is whether it is a second insemination or farrowing, or heat phase? But truly speaking it can be said that gestation is a phase of reproductive cycle which lasts more than 70% of the sow life. Also undisputable fact is that during pregnancy sows eat 2/3 of the total amount of feed consumed during the year. Therefore, as one of the measures for better efficiency Stančić et al. (2011) recommended early diagnosis of pregnancy, thereby reducing the number of unproductive feeding days per sow, significantly increasing the efficiency of utilization of accommodation space, and it is possible and appropriate timely intervention in each animal examined. With the ultrasound method, gestation can be diagnosed 17 days after insemination (Stančić et al., 2011). Over 95% of correct diagnosis is made by examination performed between the 25th and the 35th day of insemination. But significant deficiency, which significantly limits the large-scale use of this method in practical production is a high price of ultrasound equipment (Stančić et al., 2011). Reproductive problems, which

may result in the reduction of sows productivity or early culling, are often related to extreme variations in body reserves (Dourmad et al., 1994; Kovčin et al. 2009), although body reserves should be considered more as an indicator of the risk rather than as the real cause of problems. During pregnancy, sufficient body reserves must be built to compensate for the eventual nutritional deficit that may occur in the following lactation. However, these reserves should not be excessive in order to avoid the occurrence of farrowing problems that are typical for fat sows, or to impair feed intake after farrowing (Dourmad et al., 2008). The sow nutrients requirements in the beginning of gestation are small, slightly higher than maintenance requirements, which are the largest in the structure, compared with growth (maternal protein and fat deposition), fetus, mammary tissue, uterus, and placenta and fluids. Therefore, regardless of the small requirements for protein and energy during gestation, and due to the aforementioned facts, the impact on reproductive efficiency and cost of production of this stage is huge and very important.

Protein and energy requirements

The values for complete feed for pregnant sows and gilts according to the feed quality regulative of the Republic of Serbia are shown in (Table 1).

Table 1. *Values for complete feed for pregnant sows and gilts according to the feed quality regulative of the Republic of Serbia (the Official Gazette of the Republic of Serbia, no. 4/2010)*

Protein, % min.	13
ME, MJ/kg, min.	12.0
Lysine, %, min.	0.55
Meth+cist, %, min.	0.3
DM, %, min	86.5
Cellulose, %, max	9
Ash, %, max.	8
Ca, %	0.75 – 1.00
P, % min.	0.55

The use of diets with 13% CP and 12 MJ ME, during the whole period of pregnancy without a detailed analysis of the individual requirements of each sow is believed to show a retrograde attitude. Kleisiary (2007) showed increased piglet birth weight when the dietary protein content was increased from 11 to 13% for the last 30 day of pregnancy. According to the NRC (1998, 2012) models of the requirements, they should be coordinated with body weight of sows during mating, growth during pregnancy, as well as the expected number of piglets per litter. Although the gestation as a sow life period has been characterized like period with low need in proteins and other nutrients, the quantity and quality of protein in this stage according to many researchers may have a significant impact on the reproductive efficiency and profitability of production. This is particularly pronounced in the last stage of pregnancy. Therefore it is important to define nutrition program of sows that will ensure maximum production of piglets with a minimum amount of protein and minimal cost of feed. INRA and NRC have some differences that largely define their specificity, for example NRC (2012) includes AA balances for calculating sow requirements, but the model does not include manure composition and mass calculations.

On the other hand the InraPorc model (Dourmad et al., 2008) estimates the requirements of gestating and lactating sows, but does not estimate nutrient excretion and emission.

Table 1 shows that the same diet could be used for gestation sows and gilts. This can cause a potential problem if gilts are kept in groups without automatic feeding. In such circumstances, the efficiency of the entire herd of sows may be reduced. Wholesale exclusion of young sows is the consequence of inadequate nutrition of growing gilts and gilts just before the mating. Many studies indicate that gilts extremely meaty breed body weight of 100 kg should be reached between 150-160 days and inseminated in the second or third estrus at the age of 220-230 days, the body weight of 130 kg and the back fat of 20 mm (Foxcroft 2002; Foxcroft et al., 2005; Jonson et al., 2009; Patterson et al., 2010). Gilts should continue to develop and to deposit the reserves necessary to meet the needs of the nutrients contained in milk (Kustina et al., 1999; Kovčin et al., 2006). Soto et al. (2011) observed increased piglet and litter weights when gilts were given an extra 1.82 kg/d during the last 2 weeks of pregnancy. Anyway increased exclusion of gilts after farrowing or sows after first and second farrowing is a huge problem, because it reduces the reproductive efficiency of the sow herd (Beuković and Kovčin, 1995; Beuković, 1999) .

Protein and amino acid balance

In the gestating sow model (NRC 2012) Pd (predicted total protein gain) predicted in the various protein pools is dependent on either time or energy intake and in products of conception is varied with anticipated litter size and mean piglet birth weight. Based on changes in Pd with stage of gestation and across parities, the gestating sow model clearly shows the need to increase feeding levels and daily AA (amino acid) intake towards the end of gestation in order to satisfy increased energy and AA requirements for products of conception and to avoid negative maternal energy and body protein balances. It also supports reductions in daily amino acid requirements with increasing parity (NRC 2012).

The weight of uterus increases during gestation (Walker and Young, 1992). Growth of the mammary gland is limited until 80 day of gestation (Figure 1), but the growth accelerates afterwards (Kim et al., 2009). Besides the growth associated with pregnancy, the sow will continue to grow toward mature weight. Fetal weight, fetal protein content and mammary protein content increase 5-, 18- and 27-fold, respectively, in the last 45 d of gestation (McPherson et al., 2004; Ji et al., 2006; Moehn S. and Ball R. O. 2013).

Goodband et al. (2013) in their analysis made compare estimates for AA, the new model first subdivides protein deposition and retention during gestation into six tissue pools: fetus, placenta plus fluids, uterus, mammary tissue, time-dependent protein deposition, and energy-dependent protein deposition (Figure 1). The same authors have pointed out that models changes in the protein accretion of these tissues over time are based mainly on serial slaughter studies with gestating sows according to (McPherson et al., 2004; Ji et al., 2006).

Goodband et al. (2013) pointed that the greatest AA requirement throughout gestation is that of time-dependent protein deposition and energy-dependent protein deposition for weight gain (represented by the blue and yellow band, Figure 1) of the gilt or sow. The greatest fetal growth and mammary development are late gestation what can be clearly seen at (Figure 1). Because of the protein and AA demands the best opportunity for replenishing body protein reserves is in early gestation.

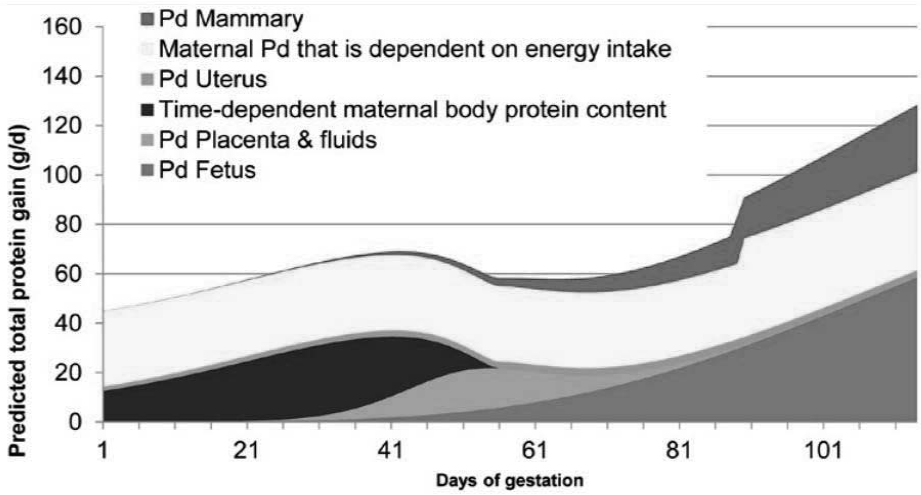

Figure 1. *Predicted total protein gain (Pd; g/d) in second-parity sows during gestation (Goodband et al. 2013)*

Analyzing the experimental data from Moehn S. and Ball R. (2013) research, group include the requirements in early and late gestation for lysine (Samuel et al., 2013), threonine (Levesque et al., 2011), isoleucine and tryptophan (Moehn et al., 2012a,b). In that analysis, each sow received each of six test diets in both early and late gestation. Feed allowance was kept constant throughout gestation. Amino acid requirements were determined using the indicator amino acid oxidation technique simultaneously with indirect calorimetry to measure energy expenditure. Key results of these experiments are compiled in Table 2.

Table 2. *Total lysine 1, threonine 2, tryptophan 3 and isoleucine 3 requirements of gestating sows (Moehn S. and Ball R., 2013)*

Amino acid	Phase	First parity (Srichana 2006)	Second parity (Samuel et al., 2010)	Third and forth parity (Levesque et al., 2011)
Lysine	EG	15.0	13.1	8.1
	LG	18.0	18.4	13.0
Threonine	EG	n/a	7.0	5.0
	LG	n/a	13.6	12.3
Tryptophan	EG	n/a	1.7	n/a
	LG	n/a	2.6	n/a
Isoleucine	EG	n/a	n/a	3.6
	LG	n/a	n/a	9.7

n/a – not available

Table 3. *Changes in sow performance during amino acid requirement studies in early (EG) and late gestation (LG) over 3 parities (Moehn S. and Ball R., 2013)*

Parity		BW, kg	Maternal gain, kg	ME intake, MJ/d	Protein retention, g/d	Energy retention, MJ/d	Litter size	Litter weight, kg
2	EG	177	44	34.2	32	3.0	13.8	19.5
2	LG	215		34.5	126	-0.7		
3	EG	205	40	36.1	38	1.2	13.6	20.1
3	LG	244		36.0	119	-0.9		
4	EG	240	25	35.6	4	1.5	15.8	22.1
4	LG	266		35.5	64	-1.3		

Table 3 shows body weight of sows increased from early to late gestation, regardless of parity, and increased from parity 2 to 4. Litter size and weight increased marginally over 3 parities. Protein deposition was greater in late than early gestation, across all parities, which is in accord with the fetal growth that occurs predominantly in late gestation. Thus, fetal growth drives amino acid requirements in late gestation, whereas maintenance and maternal growth are the principal factors affecting amino acid requirements in early gestation (Moehn S. and Ball R., 2013).

Changing amino acid requirements during gestation (Table 2) have important consequences for feeding sows. Since amino acid requirements increase to a much greater degree in late gestation than energy requirements it is nearly impossible to satisfy the requirements by simply feeding more of the same diet in late gestation. If the feed allowance of the same diet is increased sufficiently to cover amino acid intake, the sows will consume excessive amounts of energy. So what is more important: amino acid or energy intake? According to Moehn S. and Ball R. (2013) by (Shelton et al. 2009; Kleisiary, 2007; Kusina et al., 1999) meeting the protein (amino acid) requirement is more important than meeting the energy needs. Parity segregated phase feeding is the ideal tool to meet both the amino acid and energy requirements of pregnant sows of all ages.

Energy balance

When talking about need in energy for maintenance**,** metabolisable energy of maintenance (MEm) does not change throughout gestation. It was set to 440 kJ/kg BW0.75 for a sow with 240 min of standing activity per day (Hansen et al., 2014; Dourmad et al., 2008).

Fetal growth changes throughout gestation. In the first third of gestation, the daily weight accumulation (g/d) is relatively slow compared with fetal growth during the last part of gestation (Hansen et al., 2014). Similar to amino acid requirements, in the 2012 NRC model energy requirements have been divided into six tissue pools for maintenance, growth (maternal protein and fat deposition), foetus, mammary tissue, uterus, and placenta and fluids (Goodband et al., 2013; NRC, 2012). Huge energy requirement is for maintenance and maternal growth (Figure 2). The maintenance requirement can be expressed as 100 × BW0.75 (NRC 2012).

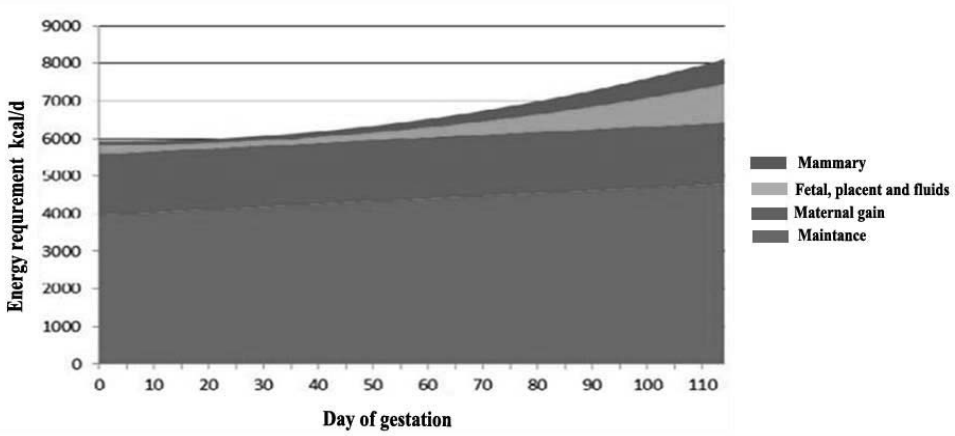

Figure 2. *Energy needs of gilts (kcal/d) during gestation based on different body tissues (Goodband et al., 2013)*

Thus, as BW increases, maintenance energy requirements increase as well. Insufficient amount of energy in the diet leads to slower growth and serious disturbances in reproduction. Excessive increases in fat can also cause negative effects on reproduction (Beuković, 1999; Maletić 2012).

Some studies have even shown that increasing the amount of protein and energy in pregnancy aims to provide the best possible preparation for the next lactation of sows and possibly increase the body weight in newborn piglets (Beuković, 1999; Young and Aherne, 2005; Smits et al., 2008; Maletić, 2012; Goodband et al., 2013). From further reproduction are usually excluded best sows, which had a huge and good litter, because of the insufficient preparation BW for lactation. Weng et al. (2009) observed that sows housed in individual stalls or grouped in pens during gestation on average do not spend more than four hours standing. If individually penned sows are housed at less than 20°C or group housed sows are housed at less than 16°C, additional adjustments must be made for the increased requirement for thermal regulation. Young gilts and second-parity sows will require more energy for weight gain than older sows where BW is simply maintained. In reality, weight gain is dictated by the level of feeding above the requirement for maintenance and requirement for foetal tissue, mammary tissue, placenta, and fluids, so the greatest opportunity for maternal gain is in early gestation, when requirements for foetal tissues and mammary gland growth are relatively low.

Conclusion

NRC model allows users to properly adjust and enter parameters related to production conditions depending on the situation. The model will estimate protein deposition, lipid deposition and bodyweight changes. In gestating sows, these changes will be estimated for several pools, including the sow, the fetuses and the reproductive tissue.

Requirement for SID amino acids is one of the benefits that this model provides. Besides that the sows should have individual amounts applied depending on average body weight and condition, and it should be modified for each sow or group, particularly if they are too lean or too fat. In any case heavier or lighter than average, and more or less efficient in their nutrient utilization. Single diet is not adequate to provide sufficient amino acids in

late pregnancy for young sows. Conversely, a single diet provides excess amino acids throughout pregnancy for older sows. The consequence is that the single diet may impair performance of young sows and will waste money because it is over-formulated for older sows.

References

1. Bauman DE and Currie WB 1980. Partitioning the nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63, 1514–1529.
2. Beuković M and Kovčin S 1995. Nivo proteina u obrocima krmača u laktaciji i njihova reproduktivna efikasnost. Zbornik naučnih radova,25-26, 99-106 Institut za stočarstvo Novi Sad.
3. Beuković M 1999.– Efekat nivoa proteina u ishrani mladih krmača tokom laktacije. PhD thesis. University of Novi Sad Faculty of Agriculture.
4. De Lange CFM 2013. New NRC 2012 Nutrient Requirements of Swine. Advances in Pork Production (2013) Proceedings, Volume 24, 17-48.
5. Dourmad JY, Etienne M, Prunier and Noblet J 1994. The effect of energy and protein intake of sows on their longevity: a review. Livest. Prod. Sci. 40, 87–97.
6. Dourmad JY, Etienne M and Valancogne A 2008. InraPorc: A model and decision support tool for the nutrition of sows. Animal Feed Science and Technology , Volume 143(1), 372 – 386.
7. Foxcroft G 2002. Fine tuning the breeding program. Saskatchewan pork industry Symposium 2002, November 12-14 2002. Proceedings 49-61 .
8. Foxcroft G, Beltranena E and Patte J 2005. Physiological limits to maximizing sow productivity. London Swine Conference – Production at the Leading Edge 6-7 April 2005. Proceedings P 29-46.
9. Goodband RD, Tokach MD, Goncalves MAD, Woodworth JC, Dritz SS and DeRouchey JM 2013. Nutritional enhancement during pregnancy and its effects on reproduction in swine. Animal Frontiers, 3 (4)68-75.
10. Hansen AV 2014. Energy and nutrient deposition and excretion in the reproducing sow: Model development and evaluation." Journal of animal science 92(6), 2458-2472.
11. Johnson R, Miller PS, Moreno R, Anderson MW, Perkins JM, Rhynalds K, Glidden TJ, McClure D and McGargill T 2009. Nutrition during gilt development and genetic line affect reproductive rate through Parity 1. Nebraska Swine Reports. Paper 247.
12. Ji F, HurleyWL, and Kim SW 2006. Characterization of mammary gland development in pregnant gilts. J. Anim. Sci. 84, 579–587.
13. Kim SW, Hurley LW, Wu G and Ji F 2009. Ideal amino acid balance for sows during gestation and lactation. J. Anim. Sci. 87, 123–132.
14. Kleisiari M 2007. Food protein level during sows pregnancy. XIII International Congress In Animal Hygiene, June 17–21, 2007, Tartu, Estonia. pp. 778-784.
15. Kovčin S, Pejin B, Stanaćev V, Stančić B, Beuković M, Korovljev and Stanaćev V 2009. Uticaj ishrane krmača u prvoj fazi suprasnosti na veličinu legla. Savremena poljoprivreda, 58(1-2), 27-32.
16. Kusina J, Pettigrew JE, Sower AF, White MA, Crooker BA and Hathaway MR 1999. Effect of protein intake during gestation and lactation on the lactational performance of primiparous sows. J. Anim. Sci. 77. 931-941.

17. Levesque CL, Moehn S, Pencharz PB and Ball RO 2011. The threonine requirement of sows increases in late gestation. J. Anim. Sci. 89, 93-102.

18. Maletić Z 2012. Reproductive efficiency of sows in dependency by the nutrition models at gestation and lactation. PhD thesis.

19. Moehn S and Ball RO 2013. Nutrition of Pregnant Sows. London Swine Conference – Managing For Production March 27 -28, 2013. Proceedings, p 55- 63.

20. Moehn S, Franco D, Josephson JK, Pencharz PB and Ball. RO 2012a. Tryptophan requirement of gestating sows. Mid-West ASAS Conf., Des Moines, IA.

21. Moehn S, Franco D, Josephson JK, Pencharz PB and Ball RO 2012b. Isoleucine requirement of pregnant sows. Mid-West ASAS Conf., Des Moines, IA.

22. McPherson RL, Ji F, Wu G, Blanton JR and Kim SW 2004. Growth and compositional changes in fetal tissues in pigs. J. Anim. Sci. 82:2534–2540.

23. Oldham JD 1991. AFRC technical committee on responses to nutrients, Theory of response to nutrients by farm animals. Pregnancy Lactation. Nutrition abstract and review 61, 683–722.

24. Patterson JL, Beltranena and Foxcroft GR 2010. The effect of gilt age at first estrus and breeding on third estrus on sow body weight changes and long-term reproductive performance. J Anim Sci vol. 88 no. 7 2500-2513.

25. Samuel RS, Moehn S, Pencharz PB and Ball RO 2013. The dietary lysine requirement of sows increases in late gestation. J. Anim. Sci. vol. 90 no. 13 4896-4904.

26. Shelton NW, DeRouchey JM, Neill CR, Tokach MD, Dritz SS, Goodband RD and Nelssen JL 2009. Effects of increasing feeding level during late gestation on sow and litter performance. Swine Day, Manhattan, KS, November 19, 2009.

27. Smits RJ, Henman DJ, King RH 2008. Increasing the dietary energy of diets fed to first litter sows on lactation performance and subsequent reproduction. Report prepared for Co-operative Research Centre for an internationally Competitive pork industry, November 2008. Web: http://apri.com.au/2G-102_FinalResearch_Report.pdf.

28. Soto J, Greiner L, Connor J and Allee G 2011. Effect of increasing feeding levels in sows during late gestation on piglet birth weights. J. Anim. Sci. 89, E-Suppl. 2: 124.

29. Srichana P 2006. Amino acid nutrition in gestating and lactating sows. PhD Diss. 479 University of Missouri, Columbia.

30. Stančić IB, Beuković M, Radović I, Stančić B, Dragin S and Erdeljan M 2011. Ultrazvučna dijagnostika rane gravidnosti kod svinja. Savremena poljoprivreda, 60(3-4), 413-425.

31. Walker B and Young BA 1992. Modelling the development of uterine components and sow body composition in response to nutrient intake during pregnancy. Livest. Sci. 30:251–264.

32. Young M and Aherne F 2005. Monitoring and Maintaining Sow Condition. Advances in Pork Production. Volume 16, pg. 299.

Original paper

THE EFFECT OF ADDITION OF LUCERNE BIOMASS AND NPN SUBSTANCES ON QUALITY OF GRAPE POMACE SILAGE

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Abstract

Grape pomace (GP) of white varieties without stalks was ensiled by the method of single factor trial in the two treatments: in the first treatment, with the addition of lucerne biomass (L) of the last cut, which was harvested at the stage of forming pods with equal shares in the weight ratio and in the second with the addition of Benural S (B) in the amount of 1%. Chemical analyses were conducted on GP and L to determine the suitability of biomass for silage and to determine the chemical composition and nutritional value and the process of lactic acid fermentation. It has been found that the biomass of GP had 2 times higher concentration of water-soluble carbohydrates (WSC 140.5:69.4 gkg^{-1}DM) relative to L, and a lower buffer capacity (BC) what makes it 10 times more favourable for ensiling (ratio WSC/BC 13.1 : 1.3). GP, as compared to L, had lower contents of CP, CF, and a lower nutritional value expressed in NE_L and NE_M units and a higher content of crude fat. Silage with the equal share of GP + L compared to silage with GP + B had a slightly lower CF and significantly less crude fat and ash, especially Ca. More favourable ratio Ca : P (2.93 : 1) was established in silage GP + L compared to 10.1:1 silage GP + B. Silages GP + L in the fermentation process were scored/rated one class higher according to the DLG and Zelter assessment methods, compared to the silages GP + B.

The aim of the study was to investigate the possibility of ensiling grape pomace with equal proportion of biomass of lucerne and added NPN substances and to determine the detailed chemical composition, nutritive value and silage quality on the basis of the process of lactic acid fermentation.

Key words: *Benural S, ensilability, grape pomace, lucerne, NE_L and NE_M, silage quality*

Introduction

Providing feed for ruminants in Serbia has been a growing problem in recent years, both because of rising prices of production inputs (oil, fertilizers, pesticides) and climate changes and droughts in the years 2012 and 2013. Decreased production of animal feed can cause a reduction in the number of ruminant animals (cattle and sheep) and therefore reduction in the production of milk and meat.

This problem of lack of fodder can be partly alleviated by using additional products of food industry (sugar, beer, alcohol, juice, etc.). By-products are usually characterized by a high water content, rapid fermentation and perishability and present real environmental problems because they pollute water and soil (Nikolić and Jovanović, 1986). Dehydration

of these products requires a large amount of energy and it is economically unprofitable. Some by-products can be used as raw material for making the compost, but the most cost effective and sustainable solution is processing and preservation/conservation so that they can be used as part of the animal diet.

GP, as a by-product of wine and fruit juice industry, is a major problem for storage and for the environment. Large quantities of obtained pomace, hundreds of tons annually, must be stored in a safe, environmentally friendly, sustainable and cost-effective manner. One way that meets the aforementioned criteria is its preservation and the use as silage for animal nutrition.

GP is the residue produced by pressing the grapes and juice extraction and consists mainly of fruit skin, seeds, insoluble fruit parts and of the juice remains. Approximately 22-25 % of fresh pomace is obtained from the total amount of grapes, and it contains 40-50 % dry matter (Pavličević et al., 1988). The content of nutrients in the GP is variable and depends on the grape variety, processing technology, climate and soil conditions of production. In nutrition of ruminants, fresh, dried and ensiled grape pomace can be used. It is not possible to use fresh pomace as animal feed in the long run due to rapid fermentation and spoilage. In previous years, dried GP was used in the nutrition of ruminants, primarily for cattle fattening (Nikolić et al., 1980; Zeremski, 1982, Stojanović et al., 1989), but today this kind of feed is not cost effective because of the high cost of energy required for dehydration. For this reason ensiling GP becomes the most economical solution for its long-term storage and use.

Considering that the GP is a typical carbohydrate feed, the goal of this study was to determine the possibility of increasing the CP content in order to optimize the nutrition of ruminants. The best solution is to add biomass of perennial legumes, but the problem occurs at the time when grape is processed (September and October) when there is very little lucerne. Another possibility, extremely simple and cheap, is to add a non-protein source of nitrogen to the silage or diet. Symbiotic bacteria in rumen use this nitrogen for the synthesis of their own proteins of high biological value, which will be digested and utilized in the small intestine of ruminants together with protein in the diet (Đorđević and Dinić, 2011). Potential problem for the using value of GP silage could be insufficient aerobic stability due to the very broad WSC and BC ratio and improper unload and low daily consumption.

Material and methods

GP of white varieties (Rkatsiteli and Chardonnay) without stalks was taken from the production facility "Rubin" Kruševac immediately after pressing and was ensiled according to the method of single factor trial in the two treatments a) with the addition of lucerne biomass (L) of the last cut, which was harvested at the stage of forming pods with the weight ratio of 1:1, and b) with the addition of Benural S (B) in the amount of 1%. Ensiling was carried out on September 15th 2012, on a private farm in the village of Pepeljevac in experimental containers holding 130 litres.

Benural S containing 42 % of urea, 56 % of bentonite and 2 % of sulphur was used as the NPN substance. Bentonite allows slower release of ammonia in the rumen, and thereby a more efficient utilization by rumen micro-organisms. It binds gases and toxic substance and contains some important elements of the alkali (K, Na, Mg etc.). Sulphur from Benural S enables more efficient synthesis of amino acids methionine and cystine.

The parameters relevant to the suitability of biomass for silage were determined in the initial material. WSC were determined colorimetrically by the method of DuBois et al. (1956), while BC was determined according to the method of Weissbach (1967). The following parameters were determined in GP and silage: content of dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), NDF, ADF, ash and content of macro elements (Ca and P).

The samples of silage were taken 90 days after ensiling, and at the same time the chemical analysis of the process of lactic acid fermentation was performed. The degree of acidity (pH) and the content of soluble nitrogen and ammonia, acetic, butyric and lactic acids were determined in the silage. Chemical analyses were conducted in the laboratory of the Institute of Forage Crops, Kruševac, according to the standard methods (AOAC, 2002). In the assessment of the quality of silage, three methods were applied: DLG, Zelter and Weissbach (Đorđević and Dinić, 2003). The results of chemical analysis were processed by the analysis of variance while the statistical significance of differences was tested using the LSD test.

Results and discussion

The suitability of the initial material for ensiling

In order to ensure successful lactic acid fermentation and stable silage without the presence of butyric acid, the WSC/BC ratio should be equal to or greater than 3.0 (Weissbach, 1967). The tested silages showed favourable share of DM (380 gkg^{-1}), low BC (0.7 meq LA - lactic acid$100g^{-1}$DM), high concentration of monosaccharides (117 gkg^{-1}DM) and WSC (140.5 gkg^{-1}DM) and a very favourable WSC/BC ratio (13.12) (Table 1). Such favourable WSC/BC ratio in GP suggests that it may be successfully ensiled and can serve as a supplement to biomasses that are difficult for ensiling, such as perennial legumes. However, silages made from easy ensiled biomasses can often subsequently ferment due to residual WSC in the silage and insufficient generated fungicidal acids (acetic, propionic, etc.). In contrast to GP, withered lucerne biomass is extremely poor in monosaccharides and WSC, and has an unfavourable SC/BC ratio (1.32), indicating that it cannot be successfully ensiled on its own. Wilted lucerne biomass has a high content of DM as a result of wilting and mowing at the stage of forming the pods. The suitability of the lucerne biomass for ensiling in this study is slightly greater than in the research by Dinić et al. (2012). The results of these studies show WSC content in the GP decreased by 28.5 gkg^{-1}DM (169 gkg^{-1}DM) compared to the results of Alipour and Rouzbehan (2007) and more than 2 times lower than in the studies of Zheng et al. (2012), which could be interpreted as a result of favourable climatic conditions and varietal characteristics.

Table 1. *Parameters of suitability of grape pomace and lucerne biomass for ensiling*

Biomass	DM, gkg^{-1}	BC, meq LA$100g^{-1}$ DM	Monosah. gkg^{-1}DM	WSC gkg^{-1}DM	Ratio WSC/BC
Alfalfa wilted	410	52.4	42	69.4	1.32
Grape pomace, fresh	380	10.7	117	140.5	13.1

Legend: DM – dry matter; BC – buffer capacity; LA – lactic acid., WSC – water-soluble carbohydrates

Chemical composition and nutritive value of the initial material and silage

Chemical composition and nutritional value of organic matter of the initial material of GP and lucerne biomass harvested in the last stage of pod formation were different in all tested parameters particularly in the content of ether extract (92.1 : 18.2 gkg^{-1}DM) what can be interpreted by the high concentration of oil in the grape seeds released after milling with a sieve (Table 2).

It is considered that fat present in ruminant diet containing GP or GP silage is not available to animals because it is imprisoned in seeds. A significant difference in the content of CP in favour of lucerne (29 gkg^{-1}DM) was determined, as well as nutritional value expressed in NE_L and NE_M units, also in favour of lucerne. Statistically significant differences were determined between silages for all tested parameters, except for the content of CP and CF. Similar values for the concentration of CP and CF were the result of late cutting of lucerne. Had the cutting of lucerne been done in a timely manner (at budding stage) the concentration of CP, minerals and nutritional value would have been higher and the content of structural carbohydrates significantly lower (Dinić et al., 1997).

Higher NFE, NDF, ADF values and nutritional value expressed by NE_L and NE_M were determined in silage GP + L (Table 2). Lower nutritive value of silages GP + B was the result of lower digestibility coefficients, primarily high share of lignin in biomass and silage from white grape pomace (Zalikarenab et al., 2007).

Table 2. *Chemical composition and nutritive value of the initial material and silages, gkg^{-1} DM*

Treatments	CP	CF	EE	NFE	NDF	ADF	NE_L, MJ kg^{-1}SM	NE_M, MJ kg^{-1}SM
	The starting material							
GP	128.4	330.6	92.1	395.9	625.6	590.4	2.87	2.17
L	157.4	360.1	18.2	371.2	560.4	470.7	4.57	4.29
	Silage							
GP+L	141.9a	339.2a	60.76b	403.2a	621.7a	586.0a	3.69a	3.13a
GP+B	145.a	331.4a	94.9a	332.2b	573.3b	503.6b	2.74b	2.06b
LSD 0.05	3.74	8.97	6.72	13.09	23.59	9.04	0.09	0.08

GP– grape pomace; L–alfalfa biomass; B–benural S; CP– crude protein; CF–crude fiber; EE – ether extract

The concentration of mineral substances in the silage with the equal shares of GP + L (54.9 gkg^{-1}DM) is almost two times lower than in silage GP + B (96.1 gkg^{-1} DM) and this difference was statistically significant (Table 3). This difference was the result of high concentrations of bentonite, urea and sulphur in Benural S. In addition, a higher concentration of Ca in silage GP + B was determined, as well as less favourable (broader) Ca : P ratio.

Table 3. *Concentration of mineral substances in the initial material and silages, gkg^{-1} DM*

Treatments	Ash	Ca	P	Ratio Ca:P
The initial material				
Grape pomace	53.0	5.0	2.6	1.92
Alfalfa	93.1	14.1	2.5	5.64
Silage				
Grape pomace + Alfalfa	54.9b	7.33b	2.5a	2.93b
Grape pomace + Benural S	96.1	24.5a	2.43a	10.11a
LSD 0.05	10.46	2.33	0.19	1.71

Concentration of calcium of 7.33 gkg^{-1} DM in the silage GP + L can meet cows` nutritional needs according to NRC recommendations (1989 and 2001) (0.43-0.66% and 0.57-0.67%), and Đorđević et al. (2009), while the concentration of P of 2.5 gkg^{-1}DM did not meet the requirements. The concentration of P in the dry period of cows should be 0.3-0.4% and during lactation from 0.38 to 0.55% in the dry matter of the ration. The concentration of Ca in the silage GP + B was three times higher compared to the cows' requirements.

Biochemical changes occurring during ensiling process

It was established that very good silages were obtained on the basis of sensory perception expected on the basis of the advantages of GP for ensiling (WSC/BC ratio 13.1), which contributed to the successful ensiling of lucerne. High DM content, favourable H values, favourable share of ammonia and soluble nitrogen were established particularly in silage GP + L, the absence or traces of butyric acid and a favourable ratio of lactic and acetic acids (Table 4). It is a well-known fact that DM levels above 300 gkg^{-1} can prevent the activity of butyric acid and other undesirable bacteria that cause decay and spoilage of silage and to reduce the losses in the form of effluent - silage juice (Ensilage, 1978, Đorđević and Dinić, 2003), which was confirmed by the results of the present study.

Table 4. *Parameters of biochemical changes in silages*

Treatments	DM	pH	% NH_4-N /ΣN	% H_2O-N /ΣN	Acids in silage					
					Acetic		Butyric		Lactic	
					gkg^{-1}DM	% u TA	gkg^{-1}DM	% u TA	gkg^{-1}DM	% u TA
GP+L	413.3a	3.80b	2.89b	23.77b	24.6b	37.19	0.0b	0.00	41.3b	62.81
GP+B	353.3b	4.98a	10.01a	60.71a	37.5	42.48	0.77a	0.87	50.2a	56.65
LSD 0,05	52.35	0.255	1.69	9.37	6.84	-	0.092	-	8.55	-

The degree of acidity (pH 3.80) of silage GP + L was at the level of acidity which can be realized in whole maize silage or silage with easy ensiled plant material and was evaluated as the maximum score for this parameter, as well as the share of ammonia nitrogen in relation to total nitrogen (%NH_4-N/NΣ) and water-soluble in relation to total nitrogen (%H_2O-N/ΣN). The silage GP + B was found to have higher values of pH (lower acidity) as well as the share of NH_4-N/ΣN and % H_2O-N/ΣN which had negative impact on its quality. Most of the methods for assessing the quality of silage allocate maximal number of points for the pH in the range of 3.5 to 4.8 (depending on the level of dry matter), for the NH_4-N/NΣ up to 10%, for the H_2O-N/ΣN up to 50%, butyric acid up to 4kg^{-1}DM of silage, and acetic acid up to 40% of relative share in relation to the total acid (Ensilage 1978).

Table 5. *Evaluation of the silage quality by various methods*

Treatments	DLG		ZELTER		WEISSBACH	
	Points	Class	Points	Class	Points	Class
GP + L	47	I	16	II	70	II
GP + B	39	II	14	III	65	II

In order to assess the quality of silage three methods were used: DLG, which is based on the degree of acidity and the relative proportion of lactic, acetic and butyric acids, Zelter's method which assesses the percentage of NH_4-N/NΣ and the content of acetic and butyric acids in fresh silage, and Weissbach method, which scores the content of butyric acid gkg^{-1}DM, % NH_4-N/ΣN, pH in relation to the level of DM, and points are deducted, if the total content of acetic and butyric acids is in the excess of 40 gkg^{-1}DM and based on share of moulds in silage. The results of quality evaluation of silage are shown in Table 5. The silage GP + L in relation to the silage GP + B was scored one class higher based on the DLG and Zelter methods, while according to Weissbach method, it was classified into the same class, but with a greater number of points (70:65).

Conclusion

On the basis of the conducted research on ensiling GP with biomass L from final cut in a weight ratio of 1:1 and ensiling GP with 1% Benural S the following can be concluded:

GP in relation to L has 5 times lower buffer capacity (10.7: 52.4 g LA/100gDM), 2 times higher content of WSC (140.5:69.4gkg^{-1}DM), i.e. 10 times more suitable for ensiling compared to lucerne (WSC/BC ratio = 13.1:1.32).

Biomass L compared to the GP had a higher content of CP, CF, nutritional value expressed in NE_L and NE_M units, higher mineral content and unfavourable (broader) ratio of Ca : P.

Silages GP + L compared to silages GP + B had higher values for NDF, ADF, and higher nutritional value and lower value for the content of EE, ash and Ca.

Silage GP + L compared to silages GP + B had more favourable values for the acidity (pH), for the lower values of ammonia and soluble nitrogen in relation to total nitrogen, and more favourable relative share of acids, i.e. were scored one class higher based on DLG and Zelter's methods.

The general conclusion is that the GP can be successfully ensiled together with lucerne biomass to provide somewhat better silage compared to silage supplemented with NPN substances. The diet in ruminant nutrition will comprise parts of both silages.

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References

1. Alipour D and Rouzbehan Y 2007. Effects of ensiling grape pomace and addition of polyethylene glycol on *in vitro* gas production and microbial biomass yield. Animal Feed Science and Technology 137, 138-149.
2. Association of Official Analytical Chemistry (A.O.A.C.) 2002. Official Methods of Analysis. 17th ed., Washington, DC.
3. Dinić B, Stošić M, Negovanović D, Ignjatović S, Tomić Z and Jevtić G 1997. Influence of plant growth phase, dry mass level and chemical conservators on lucerne ensilage. Proceedings of the 8th International symposium Forage Conservation 29th Semptember - 1st October, Brno, Czech Republic, 122-123.

4. Dinić B, Đorđević N, Blagojević M, Terzić D and Đokić D 2012. Quality of alfalfa haylage depending on the aplication of inokulant and ground corn. Proceedings of The First International Syposium on Animal Science, November Belgrade, Serbia, Book I 488-495.
5. DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F 1956. Colorimetric method for determination of sugars and related supstances. Anal. Chem. 28, 350-356.
6. Đorđević N and Dinić B 2003. Siliranje leguminoza. Monografija. Institut za istraživanje u poljoprivredi SRBIJA – Beograd.
7. Đorđević N, Makević M, Grubić G and Jokić Ž. 2009. Ishrana domaćih i gajenih životinja. Univerzitet u Beogradu, Poljoprivredni Fakultet, Beograd.
8. Đorđević N and Dinić B 2011. Proizvodnja smeše koncentrata za životinje. Institut za krmno bilje Kruševac, Kruševac.
9. Ensilage 1978. MAI № 15. Bases theoriques de 1' ensilage. Paris.
10. NRC - National Research Council 1989. Nutritient Requirements of Dairy Cattle. 6th rev. ed., National Academy Press, Washington, D.C.
11. Nikolić A, Negovanović D and Stoičević Lj. 1980. Mogućnosti povećanja hranljive vrednosti grožđane komine. Krmiva, 11.
12. Nikolić JA and Jovanović M. 1986. Some properties of apple pomace ensiled with and without additives. Animal Feed Science and Technology, 15, 57-67.
13. NRC - National Research Council 2001. Nutritient Requirements of Dairy Cattle. 7th rev. ed., National Academy Press, Washington, D.C.
14. Pavličević A, Zeremski D, Grubić G and Jokić Ž. 1988. Mogućnost siliranja grožđane i jabučne komine u kombinaciji sa zelenom lucerkom, pivskim tropom i svežim rezancima šećerne repe. Zbornik radova: proizvodnja i korišćenje krmnih kultura (u funkciji jevtinije produkcije mesa i mlijeka). VI Jugoslovenski simpozij o krmnom bilju, 22-24.06., Osijek, 396-404.
15. Stojanović S, Stojsavljević T, Vučurević N, Vukić-Vranješ M i Mandić A. 1989. Hemijski sastav, hranljiva i upotrebna vrednost suve grožđane komine u ishrani tovne junadi. Stočarstvo, 43: (7-8) 313-319.
16. Weissbach F. 1967. Die Bestimung der Pufferkapazitat der Futterpflanzen und ihre Bedeutung der Vergarbarkeit, Aus: Tagungberichte Nr. 92 der Deutschen Akademie der Landwirtschafts wissen schaften zu Berlin 211-219.
17. Zalikarenab L, Pirmohammadi R and Teimuriyansari A 2007. Chemical Composition and Digesttibility of Dried White and Red Grape Pomace for Ruminants. J. of Anim. Vet. Adv. 6, 1107-1111.
18. Zeremski D 1982. Nusproizvodi prehrambene industrije kao hrana za tov goveda. Krmiva, 24**,** 7, 145-150.
19. Zheng Yi, Lee K, Yu S, Cheng YS, Simmons WC, Zhang R, Jenkis BM, Vander Gheynst JS. 2012. Ensilage and Bioconversion of Grape Pomace into Fuel Etanol. J. Agric. Food Chemistry, 60 (44), 11128-11134.

Original paper

EFFECT OF RAPESEED MEAL AS PROTEIN SUPPLEMENT IN FEEDLOT LAMB DIETS ON EXCRETION OF URINARY PURINE DERIVATES AND MICROBIAL PROTEIN SYNTHESIS

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Abstract

The objectives of our work were to establish the feeding effects of rapeseed meal (*RSM*) on urinary purine derivatives (*PD*) excretion as an indicator of ruminal synthesized and yielded microbial crude protein (*MCP*), and nitrogen (*N*) balance. Experimental animals (n=5, Tsigai breed wethers, 4-y aged, 65±2kg *BW*) averaging 1.1±0.1kg digestible organic matter intake (*DOMI*) randomly assigned to dietary treatments (n=2) in the two-stage 21–d periods–preliminary (14-d) and collection (7-d). Diets were consisted of 36% meadow hay, 34% cereal (corn, tritikle), 3% vitamin–mineral premix, 27% protein supplement. Diets were either control (with sunflower meal=*SFD*) or experimental (with rapeseed meal=*RSD*). They're iso-nitrogenous (crude protein, *CP*=16%), iso-caloric (net energy as feed units for gain, *FUG*=1.0/kg *DM*), equal in protein digestible in small intestines (*PDI*=86g/kg dry matter) and *Ca:P* ratio (2:1). The results have revealed that *RSD* gained (p=0.2) in higher *DOMI* (1.1 vs. 1.0kg) and tended to improve *N* balance (p=0.4) compared with *SFD*. *PD*-s value (24.0 vs. 22.3Mm), as allantoin (14.8 vs. 11.7Mm), xanthine+hypoxanthine (7.2 vs. 8.6Mm) and uric acid (1.9 vs. 2.0), respectively weren't affected (p=1.0, 0.9, 0.5 and 0.7, respectively) among the dietary treatments (*SFD* vs. *RSD*). Similarly, established ratios, as allantoin (2.1 vs. 1.9) and *PD* to creatinine (2.4 vs. 2.1, respectively), were not differ (p=0.3, respectively) between diets (*SFD* vs. *RSD*). Evaluated microbial *N* yield (20.7 vs. 19.3g, respectively) wasn't affected (p=0.9), and gained 19.3 vs. 17.1 microbial protein synthesis efficiency, respectively (p=0.8). Observed relationship between *DOMI* level and allantoin value (R^2=0.62), and between *PD* value and *MCP* level (R^2=0.99) suggested good parity among the treatments. In conclusion, the usage of high level *RSM* (27% of total ration), as a protein source in feedloot lamb diets tended to improve *DOMI* and dietary *N* utilization, but didn't affect *PD* excretion and microbial *N* yield.

Key words: *dietary protein source, ruminants, microbial N, purine derivatives, rapeseed meal*

Abbreviations: *Ca*-calcium; *CP*-crude protein; *DM*-dry matter; *DOMI*-digestible organic matter intake; *FUG*-feed units for gain; *GE*-gross energy; *MCP*-microbial crude protein; *N*-nitrogen; *P*-phosphorus; *PD*-purine derivatives; *PDI*-protein digestible in small intestines; *RSD*-rapeseed meal–based diet; *RSM*-rapeseed meal; *SFD*-sunflower meal–based diet; *TR*-total ration.

Introduction

In regards to sustainable ecosystems policy and eco–agro–socio–economic efficiency (Yossifov, 2014) of diet balancing, new feeding strategies with alternative feedstuffs and optimization of productive systems are required.

So, the ruminant nutrition must meet the N requirements of host organism and ruminal microorganisms in regards to improve N utilization for animal performance and to limit N excretion (Tamminga, 1992). The microbial protein synthesis (*MCP*) must cover protein requirements in ruminants, especially high-productive animals (Tas and Susenbeth, 2007). But, the conversion of dietary protein into microbial protein and the microbial protein yield is related to some factors, such as feeding level and passage rate and available energy and amino acids (*AA*) in synchronous rate (Firkins et al., 2007). For higher and strong microbial efficiency, substrate energy must be transformed at short chain fatty acids (*FA*) and adenosine triphosphates molecules (*ATP*). So, diet nutrients must ensure energy and N supply to rumen microbes.

The MPC could be predicted by the concentration of renal excreted purine derivates (*PD*) in regards to relationship to MCP (Chen et al., 1992; Shingfield, 2000). The duodenal purine bases (*PB*) are used as a microbial marker as an efficiently absorbed and the majority of their derivatives excreted via the kidney. So, the ratio of PDs in urine closely reflects, and therefore may be used to predict, microbial protein flow.

The objectives of current work were to 1/ establish the effects of feeding rapeseed meal–based diets (RSD) on urinary purine derivatives (PD) excretion as an indicator of ruminal synthesized and yielded MCP, 2/ estimate nitrogen (N) balance, and 3/ draw relationship model between established parameters and among the treatments. We hypothesized that the usage of high levels rapeseed meal (27 % in total ration), as a protein source in sheep diets, would affect DOMI, N balance, PD excretion and microbial N yield.

Material and methods

Experimental design. Experimental units were conducted at Experimental Base of Institute of Animal Science, Kostinbrod, BG. Animals averaging 1.1 ± 0.1 kg digestible organic matter intake (DOMI = (Coefficient of OM digestibility x OM content) x Feed intake) randomly assigned to dietary treatments (n= 2) in the two-stage 21–d periods – preliminary (14-d) and collection (7-d) (adopted from Yossifov and Kozelov (2014). Wethers were housed in individual metabolic cages with free access of fresh water and were fed twice a day (8.00 and 16.00 h).

Animals and treatments. Five Tsigai breed wethers (4-y aged, 65 ± 2 kg BW) were used in an experiment. Diets were consisted of 36 % meadow hay, 34 % cereal (corn and tritikle), 3 % vitamin–mineral premix and 27 % protein supplement (Table 1). Dietary treatments were either control (with sunflower meal – SFD) or experimental – with rapeseed meal (RSD). Offered diets were iso-nitrogenous (crude protein, CP= 16 %), iso-caloric (net energy as feed units for gain, FUG= 1.0/kg DM), equal in protein digestible in small intestines (PDI= 86 g/kg DM) and Ca:P ratio (2:1).

Sampling and procedures. Feed, faeces and urine samples were quantitatively collected per animal during the collection period. Feeds were dried at 65 ^{o}C, ground and then analyzed

(AOAC, 2002) for chemical composition (DM, CP, ether extract (EE), crude fiber (CF), Ash). Consumption (feed and water) was calculated for each animal as a difference between offered and refused (if orts were presented). Total digestible nutrients (TDN), energy and protein values were investigated and reported elsewhere (Yossifov and Kozelov, 2014). The faeces samples were analyzed for total N. Urine samples were analyzed for total N and purine derivatives (allantoin, xanthine + hypoxanthine, uric acid) as described in IAEA (1997). The amount of microbial purine derivatives was calculated as discribed by Chen and Gomes (1995).

Calculations and biostatistics. Analyses of variance and simple linear regression were performed. All observed data were presented as mean with SEM. The treatment means were compared by Student's T-test and were accepted as representing statistically significant differences at $p< 0.05$ and trends were discussed at $0.05 < P < 0.20$. The deduced relationships between DOMI level (kg/d) and allantoin excretion value (Mm/d) and between PDs excretion value (Mm/d) and MCP level (g N/d) were prepared by MS Office Statistic Package.

Results and discussion

The composition of the experimental diets were similar for SFD and RSD (Table 1). The difference was observed at diet digestibility (Yossifov and Kozelov, 2014), affecting TDN (11 %) and ME (14 %) concentration among the treatments.

The higher digestibility of OM significantly improved the values of the digestible OM (*DOM*) for RSD than SFD ($p<0.05$). So, thus reflected on the intake of DOM (*DOMI*) among the diets trending ($p= 0.18$) with the positive effect for RSD (Table 2). This considers the effective digestibility of ruminant diets in depends on the amount of soluble, potentially digestible and indigestible fractions, as well as the rate of passage and their ruminal digestion (Van Soest, 1982). Increased *DOM* might be related to increased OM outflow from the rumen with a high proportion of bacterial cells being associated with the particulate matter. So, it is apparent greater flow of particles from the rumen at an early stage of digestion with more attached microbes (Faichney, 1980; Van Soest, 1982; Merchen et al., 1986).

The investigation on value of metabolizable energy (ME) tended to higher levels for RSD than SFD (14 %). Such higher levels must to reflect on the amount of microbial protein synthesized in rumen as an energy source.

The effect of dietary protein source on nitrogen balance (*N*) is presented below (Table 2). The between-animal variation in N balancing trial was markedly higher in the present study, so the differences were not significant and shown only trends. As shown, N intake (N_i) is similar between diets (N_i= 38.2 g/d). The N balance in terms of N available (N_a, $p= 0.7$) and urinary N (N_u, $p= 0.6$) also did not be affected by the treatments. Although, N retention (N_r) and fecal N (N_f) tended to be increased (14 %) by rapeseed meal supplementation ($p= 0.4$). Such trendline was observed among calculated N balancing ratios in RSD fed animals ($p> 0.05$) than SFD fed animals (Table 2). The strongest trend (15 %) was estimated for the values of utilized N per 1 g N_i ($p= 0.19$). In regards to N utilization some authors noted that N excretion and N retention should reflect the differences in N metabolism, but the latter index was the most important of the protein nutrition status (Zinn, 1988). Also, this data confirmed the opinion that N_u decreased in order of energy supply (Balch, 1967).

Table 1. -*Experimental design (%) and diet composition (%)*[1]*

		Treatments	
		SFD	RSD
Ingredient			
Meadow hay		36.6	36.3
Cereal			
Corn		17.2	17.0
Tritikale		17.2	17.0
Protein supplement			
Sunflower meal		26.3	-
Rapeseed meal		-	27.2
Premix[2]		2.7	2.5
Nutrient			
Dry matter		1584.1	1606.28
Organic matter		95.3	94.8
Digestible OM		73.3[a]	78.9[a]
Total digestible nutrients		61.5	68.0
Energy	Gross	17.9	18.0
	Net, as FUG	1.0	1.0
	ME[3]	2.2	2.5
Crude protein		16.4	16.5
PDI		8.6	8.6

[*]Adopted from Yossifov and Kozelov (2014); [1]as DM basis, except DM (g) and energy (GE (kcal), ME (Mcal) and FUG (units); [2]Vitamin-mineral premix + limestone + dicalcium phosphate; [3]Metabolizable energy (ME, Mcal/kg DM)= (TDN×0.04409×0.82); [aa]$p<0.05$; RSD-rapeseed meal- based diet; SFD-sunflower meal-based diet; PDI-Protein digestible in small intestine; FUG-feed units for gain.

The mean values for daily excretion of the PDs are shown in the table above (Table 2). The between-animal variation in PD excretion also was markedly higher in the present study, so the differences were not significant and shown only trends. The allantoin and PD excretion values decreased with 21 and 7 % feeding RSD. The RSD increased with 19 and 7 % for xanthine + hypoxantine and uric acid values, respectively. Also, insignificant reduction in PD excretion was observed when ME intake was higher in other reports (Giesecke et al., 1984).

As also shown for N and PD, the between-animal variation in efficiency of microbial protein synthesis (*EMPS*) was higher in the present study, so the differences were not significant and shown only trends. The *EMPS*, presented as different indices, in SFD and RSD fed animals was not altered ($p> 0.8$) with the exception of the N_i/DOMI values ($p= 0.3$). These values were similar to those obtained in sheep by other authors (White et al., 2002; Yu et al., 2002).

Table 2. *Effect of RSM vs. SFM supplementation on productive parameters (per day)*

Item	Treatments		SEM	P-value	r^2
	SFD	RSD			
DOMI, g/d	1.05	1.13	0.04	0.18	0.54
N balance, g/d					
N intake (N_i)	38.16	38.29	1.73	0.98	0.92
Fecal N (N_f)	9.21	8.16	0.59	0.46	0.98
Available N (N_a)	28.94	30.13	1.32	0.72	0.89
Urinary N (N_u)	13.17	12.21	0.75	0.60	0.99
Retention N (N_r)	15.77	17.93	1.09	0.42	0.88
N_r / N_i	37.03	42.57	2.61	0.38	0.88
N_r / N_a	54.48	59.15	1.98	0.33	0.97
Utilized N per g N_i	0.41	0.47	0.02	0.19	0.97
N_r/N_i per 100g CP/кg DMI	5.93	6.81	0.42	0.38	0.88
Renal Excretion, Mm/d					
Allantoin	14.83	11.72	4.92	0.54	0.96
Xanthine + Hypoxanthine	7.24	8.60	2.66	0.63	0.90
Uric acid	1.89	2.02	0.74	0.86	0.53
PD/ $BW^{0.75}$	1.05	0.98	0.18	0.85	0.93
Efficiency					
MCP, g N	20.65	19.28	4.48	0.90	0.94
N_i/DOMI (g/kg)	36.36	33.67	1.09	0.31	0.93
MCP N/DOMI (g/kg)	19.28	17.11	3.92	0.83	0.87
MCP N/ME intake (g/Mcal)	0.0064	0.0055	0.001	0.78	0.89
MCP N/N_a	0.68	0.67	0.12	0.95	0.87
Ratios					
Allantoin:Creatinine	2.13	1.88	0.44	0.25	0.95
PD:Creatinine	2.44	2.14	0.51	0.33	0.93

SFD-sunflower meal-based diet; RSD-rapeseed meal-based diet. DOMI-digestible organic matter intake; CP-crude protein; PD-purine derivatives; BW-body weight; MCP-microbial protein; ME-metabolizable energy.

The deduced relationship models between established parameters and among the treatments are presented below (Fig. 1 and 2). The graph of relationship between DOMI level ($kg.d^{-1}$) and allantoin excretion value ($Mm.d^{-1}$) is shown in Fig. 1.

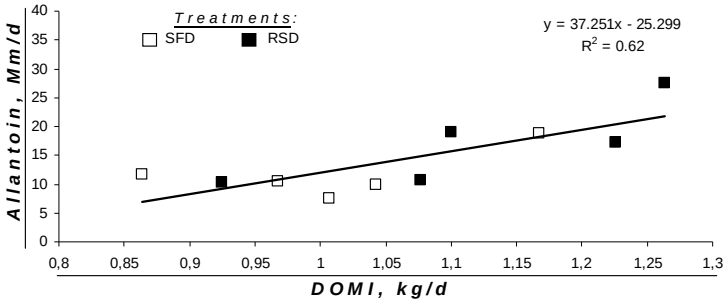

Figure 1. *The relationship between DOMI level (kg/d) and allantoin excretion value (Mm/d).*

Observed correlation suggested good parity among treatments (R^2= 0.62). Regression analysis indicated that the values of allantoin excreted (y) were closely related to estimated level of

DOMI (x). The slope of 0.1807 and small intercept of 0.1807 indicate that both parameters gave comparable results (Fig. 1). Such strong relationship confirmed the results of others (Perez et al., 1996).

The graph of relationship between PD excretion value (Mm/d) and MCP level (g/d) is presented in Fig. 2.

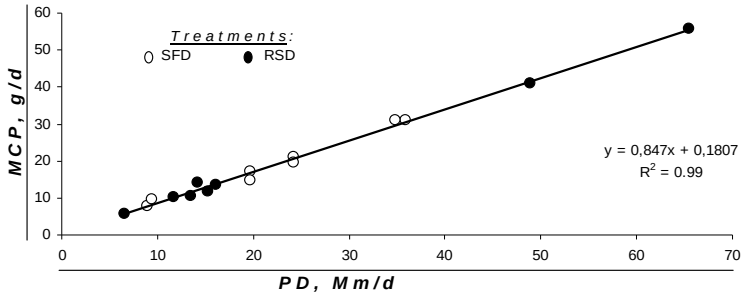

Figure 2. *The relationship between PD excretion value (Mm/d) and MCP level (g N/d).*

Observed correlation suggested good parity among treatments (R^2= 0.99). Regression analysis indicated that the values of microbial protein synthesized (y) were closely related to estimated level of PDs excreted (x). The slope of 0.1807 and intercept of 0.847 indicate that both parameters gave comparable results (Fig. 1).

Conclusion

Results from this study indicate that rapeseed meal (RSM) was superior to sunflower meal (SFM) in terms to having higher dietary digestible organic matter (DOM), total digestible nutrients (TDN) and metabolizable energy (ME), and to optimizing N utilization. Based on this research unit, it can be concluded that substitution of dietary protein source and diet supplementation with RSM vs. SFM improves diet composition as DOM, TDN and ME with 8, 11 and 14 %, respectively. The higher (8 %) digestible organic matter intake (DOMI) at rapeseed meal-based diet (RSD) compared to sunflower meal-based diet (SFD) optimizing N utilization by lower N excretion (9 %) and higher N retention (14 %) at equal N intake. The level of the purine derivatives (PD) excretion and efficiency of microbial protein synthesis has been not affected. Close relationships were found between DOMI level and allantoin excretion value, and between PD excretion value and MCP level. This study recommended RSM to be used as local feedstuff and alternative protein and energy supplement to improve animal performance at intensive feeding systems.

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References

1. AOAC International, 2002. Official methods of analysis, 17th ed., Rev. 1. AOAC Int., Gaithersburg, MD.
2. Balch C 1967. Problems in predicting the value of non-protein nitrogen as a substitute for protein in rations for farm animal ruminants, World Review of Anim. Production 3, 8491.
3. Chen X, Chen Y, Franklin M, Orskov E, Shand W 1992. The effect of feed intake and body weight on purine derivate excretion and microbial protein supply in sheep, J. Anim. Sci. 70, 1534-1542.
4. Chen X and Gomes M 1995. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives - an overview of the technical details.Publication 1992, Int. Feed Resources Unit, Rowett. Research Institute, Aberdeen, UK.http://macaulay.ac.uk.pdf.
5. Faichney G 1980. Measurement in sheep of the quantity and composition of rumen digesta and of the fractional outflow rate of digesta constituents, Aust. J. Agric. Res. 31:1129.
6. Firkins J, Yu Z, Morrison M 2007. Ruminal nitrogen metabolism: Perspectives for integration of microbiology and nutrition for dairy, J. Dairy Sci. 90 (E. Suppl.):E1-E16.
7. Giesecke D, Stangassinger M, Tiemeyer W 1984. Nucleic acid digestion and urinary purine metabolites in sheep nourished by intragastric infusions, Can. J. Anim. Sci. 64, 144-145.
8. IAEA-TECDOC-945, 1997. Estimation of rumen microbial protein production from purine derivatives in urine, ISSN 1011-4289.
9. Merchen N, Firkins J, Berger L 1986. Effect of intake and forage level on ruminal turnover rate, bacterial protein synthesis and duodenal flows in sheep, J. Anim. Sci., 62:216.
10. Perez J, Balcells J, Guada J, Castrillo C 1996. Determination of rumen microbial-nitrogen production in sheep: a comparison of urinary purine excretion with methods using I5N and purine bases as markers of microbial-nitrogen entering the duodenum, British J. Nutr. 75, 699-709.
11. Shingfield K 2000. Estimation of microbial protein supply in ruminant animals based on renal and mammary purine metabolite excretion. A review, J. Anim. Feed Sci., 9:169.
12. Tamminga S 1992. Nutrition management of dairy cows as a contribution to pollution control, J. Dairy Sci., 75:345–357.
13. Tas B and Susenbeth A 2007. Urinary purine derivates excretion as an indicator of in vivo microbial N flow in cattle:A review, Livestock Sci. 111, 181-192.
14. Van Soest P 1982. Nutritional Ecology of the Ruminants, Comstock Publishing Assoc., Cornell Univ., Ithaca, USA, 152-345.
15. White C, Hanbury C, Young P, Phillips N, Wiese S, Milton J, Davidson R, Siddique M, Harris D 2002. The nutritional value of Lathyrus cicera and Lupinus angustiflolius grain for sheep, Anim. Feed. Sci. Technol. 99, 45-64.
16. Yossifov M 2014. Sustainable Food Production Chain for sustainable Ecosystems. Livestock, Climate Change and Food Security Conference - Madrid 2014, p.120. Available in: http//:*animalchange.wordpress.com/abstract-book.pdf.*

Original paper

EFFECT OF CARBOHYDRATE SOURCE AND ADDITION CANOLA SEED ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF LAMBS

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Abstract

The objective of this study was to investigate effects of partial replacement of starch with neutral detergent soluble fiber with or without roasted canola seed as a fat source, and possible interactions on apparent digestibility and growth performance of growing lambs. For this purpose, 24 male lambs were used in a completely randomized design with a 2×2 factorial arrangement of treatments. Dietary treatments were 1- starch from barley, 2- starch from barley with roasted canola seed, 3- soluble fiber from beet pulp, and 4- soluble fiber from beet pulp with roasted canola seed. The experimental period was 84 days and forage:concentrate ratio was 10:90 for all diets. Treatments had no significant effect on the dry matter intake, feed conversion ratio, apparent digestibility of protein and organic matter ($p>0.05$). High starch diets compared with high soluble fiber diets caused a significant decrease in the digestibility of dry matter, organic matter, ADF and NDF ($p<0.01$). Canola seed addition to the diets significantly decreased ADF and NDF digestibility ($p<0.01$). The carbohydrate source by canola seed interaction was significant for weight gain and rumen pH ($p<0.01$) indicating that addition of canola seed to high starch diet increased weight gain and rumen pH more dramatically in comparison with soluble fiber diet ($p<0.01$). It is concluded that addition of canola seed to high starch diet had favorable effects on growth performance and rumen pH but because of its negative effect on fiber digestibility and its different response depend on carbohydrate source, more research is needed for conclusion.

Key words: *Dietary energy source, Digestibility, Fattening lamb*

Introduction

Neutral detergent-soluble carbohydrates (NDSC) vary in their fermentation and digestion characteristics, including the profile of metabolizable nutrients that they provide. Ruminal fermentation of starch tend to produce relatively more propionate than acetate, and may produce lactic acid. In contrast, fermentations of neutral detergent-soluble fiber (NDSF) carbohydrates, such as pectic substances, tend to produce more acetate than propionate and do not generate appreciable amounts of lactic acid (Strobel and Russell, 1986). Starch has the potential to be digested in the small intestine, and the resulting monosaccharides are absorbed. The nonstarch polysaccharides of NDSF are digested only by microbial enzymes. It may be possible to influence both the nutrient digestibility and animal growth

by modifying the supply of metabolizable nutrients to the fattening lamb through altering the proportions of NDSC types in the diet.

Barley is starch rich and beet sugar pulp is one of the pectin rich feedstuffs commonly fed to fattening lamb. In the most common lamb-finishing system in Iran, feedlot lambs are fed high-concentrate diets. However, this feeding program, is usually associated with digestive disorders, such as ruminal acidosis, causing reduced feed intake, impaired nutrient absorption and depressed animal performance (Bodas et al., 2007). Acidosis also prejudices the reticulo-rumen epithelial tissues producing parakeratosis (Wang et al., 2009). In the study by Strobel and Russell (1986), pectin utilization decreased by 53 % when the pH decreased from 6.7 to 6.0. These researchers concluded that pectin-utilizing bacteria are very sensitive to low pH.

Inclusion of whole oilseeds in the diets of ruminants may provide a high quality protein and increase the energy density of the diet. Whole canola seed contains high levels of lipid (approximately 43%) and large amount of protein (approximately 20.5% CP), with a similar to slightly lower amino acid availability than that of soybean meal (Bell, 1984).

Little information is found in the literature with respect to the effects of partial substitution of cereal grains with pectin-rich feedstuffs, such as sugar beet pulp in the high concentrate diets. Also, few data are available concerning the interaction between NDSC type and fat supplementation. Therefore, the objective of this study was to investigate partial substitution of barley grain with beet pulp with or without roasted canola seed supplementation on growth performance and nutrient digestibility of lambs fed by high concentrate diets.

Material and methods

This study was undertaken at the Experimental Farm of Agriculture College, Ramin Agriculture and Natural Resources University of Ahvaz, Iran. Twenty-four male lambs with an average age of 118 ± 10 days and average weighing 23.7± 2.5 kg, were used in a completely randomized design with a 2 * 2 factorial arrangement of treatments. Dietary treatments consisted of 1) barley starch, 2) barley starch with roasted canola seed, 3) beet pulp soluble fiber, 4) beet pulp soluble fiber with roasted canola seed. Ingredients and chemical composition of the experimental diets are reported in Table 1. Before doing the experiment, all lambs were maintained under the same feeding management and environmental condition. The lambs were weighed and randomly allocated to one of four dietary treatments according to live weight. The lambs were individually penned (1*1.5M) and fed for 84 days with two week of adaptation to the diets. Before starting the experiment, the lambs were dewormed by dosing with Dieverm and vaccinated against enterotoxaemia (Razi Institute, Hesarak, Karaj, Iran). The experimental diets were formulated based on NRC (2007) requirements of a finishing lamb. The roughage:concentrate ratio was 10:90. The animals were fed ad libitum a TMR twice daily, morning (0600 h) and afternoon (1800 h). During the experimental period, to calculate the daily gain, the lambs were weighed every 7 days from weaning until the end of the experiment. The weights were taken after about 14 h fasting to minimize error. In order to estimate apparent digestibility of dry matter and nutrients, feed intake and refusals were collected each day, weighed to accurately determine DMI, and discarded also, fecal output was collected thrice (At days 20, 40, and 80 of age), weighed, and recorded. Samples were stored frozen (-20°C) until analysis. Feed, orts and faeces were analysed for DM by drying at 55 ◦C for 48 h. The OM was determined by ashing at 550 ◦C for 6 h (968.08; AOAC, 2006) and CP by a Kjeldahl technique (988.05; AOAC, 2006). Neutral detergent fiber was

determined by a procedure of Van Soest et al. (1991) without sodium sulphite or α-amylase and acid detergent fiber was determined according to the method described by Van Soest (1981).

Data analysis was performed using a statistical model based on a completely randomized design with repeated measurements using the model:

$$Y_{ijk} = \mu + T_i + W_l + A_j + D_k + (T*D)_{ik} + \alpha_{iljk}$$

Y_{ijk} = Dependent variable; μ = The overall mean; T_i = Treatments (NDSC source, Addition of roasted canola seed and interaction among these factors); W_l = The effect of initial weight, as covareat; A_j = Random residual effects in the treated lambs; D_k = Effect of Time; $(T*D)_{ik}$ = Interaction of treatment and time, α_{iljk} = The residual error.

Results and discussion

Table 1. *Ingredient and chemical composition (g/kg DM) of the basal experimental diet*

Ingredients (% DM)	Experimental diets®			
	Starch		Beet pulp	
	-RCS	+RCS	-RCS	+RCS
Alfalfa hay	10	10	10	10
Milled barley	64	62	28	28
Dried beet pulp	0	0	36	36
Roasted Canola seed	0	7	0	7
Wheat bran	4	4	0	0
Soybean meal	10	12	14.2	16.2
Canola meal	10	3	10	3
Limestone	1.2	1.2	1.0	1.0
Salt	0.2	0.2	0.2	0.2
Vitamins and minerals supplement*	0.6	0.6	0.6	0.6
Chemical composition(%)				
Dry matter[a]	92.5	93.6	93.7	94.3
Organic matter[a]	90.8	90.2	89.9	90.0
Crude protein[a]	18.3	17.2	18.5	18
Neutral-detergent fiber[a]	24	23	32	32
Acid-detergent fiber[a]	11.6	11.1	17.1	16.6
Non-fiber carbohydrates1[b]	55.3	50.5	46.3	41.0
Ether extract[a]	2.5	5.1	2.0	4.6
Metabolisable energy (MJ/kg DM)[c]	2.83	2.97	2.75	2.82

*–RCS=Without roasted Canola seed; +RCS = With roasted Canola seed

*Vitamin-trace mineral pre-mix provides per kg of mixed ration: 18,750 IU Vitamin A; 3750 IU Vitamin D3; 7.5 IU Vitamin E; 5mg Co; 1.25 mg Cu; 75 mg Fe; 1250 mg Mg; 150 mg Mn; 0.375 mg Se; 100 mg Zn.

[a]Obtained from measurements in the laboratory

[b]Non-fiber carbohydrates (NFC) were calculated using the formula: %NFC = 100 - (%CP + %EE + %NDF + %ASH). [c] Metabolisable energy was calculated according to NRC (2007).

Table 2. *Growth performance of fattening lambs fed by experimental diets (kg)*

Parameters	Experimental diets*				S.E	P values of effects**					
	Starch		Beet pulp								
	-RCS	+RCS	-RCS	+RCS		NDSC	RCS	NDSC * RCS	TRT	Time	TRT×Time
Initial body weight,	23.4	23.8	24.1	23.6	0.5	0.89	0.88	0.78	---	---	---
Final body weight	44.9	47.8	46.7	47.9	1.33	0.87	0.92	0.30	0.11	<0.01	<0.01
Average daily intake	1.41	1.51	1.48	1.48	0.05	0.52	0.75	0.66	0.50	<0.01	0.07
week 4	1.24	1.28	1.29	1.30	0.05	0.70	0.74	0.67	---	----	----
week 8	1.47	1.58	1.52	1.50	0.07	0.76	0.74	0.65	---	----	----
week 12	1.53	1.68	1.63	1.65	0.05	0.74	0.76	0.64	---	----	----
Feed conversion	5.5	5.3	5.4	5.2	0.72	0.46	0.28	0.51	0.57	<0.01	0.004
week 4	4.1	4.4	4.3	4.1	0. 64	0.43	0.29	0.51	---	----	----
week 8	5.4	5.3	5.7	4.6	0.68	0.49	0.25	0.46	---	----	----
week 12	7.1	6.6	6.5	7.1	0.85	0.46	0.33	0.52	---	----	----
Average daily gain	0.256	0.286	0.269	0.289	0.04	0.04	0.35	0.03	0.31	<0.01	<0.01
week 4	0.306	0.299	0.301	0.311	0.05	0.51	0.59	0.71	---	----	----
week 8	0.248	0.305	0.269	0.325	0.03	0.007	0.03	0.006	---	----	----
week 12	0.215	0.255	0.239	0.232	0.03	0.56	0.44	0.51	---	----	----

** -RCS= Without roasted Canola seed; +RCS = With roasted Canola seed

**NDSC =Starch vs. Neutral detergent-soluble; RCS= Diets with Roasted canola seed vs. Diets without roasted canola seed and diets interaction

Table 3. *Growth performance of fattening lambs fed by experimental diets (%)*

Nutrient	Experimental diets*				S.E	P values of effects					
	Starch		Beet pulp								
	-RCS	+RCS	-RCS	+RCS		NDSC	RCS	NDSC * RCS	TRT	Time	TRT×Time
DM	77.6	81.5	83.3	85.6	2.97	0.007	0.65	0.65	0.001	0.006	0.39
week 4	77.7	77.6	80.5	83.0	2.75	0.012	0.62	0.68	---	----	----
week 8	79.4	82.1	86.3	86.2	3.01	0.008	0.65	0.63	---	----	----
week 12	79.6	84.2	87.2	87.4	3.27	0.001	0.66	0.67	---	----	----
OM	58.8	68.8	71.7	69.8	3.62	0.002	0.67	0.63	0.71	0.80	0.65
week 4	59.4	67.5	70.3	70.1	3.76	0.003	0.65	0.64	---	----	----
week 8	58.6	69.7	72.7	69.3	3.43	0.002	0.68	0.66	---	----	----
week 12	58.4	69.2	72.1	69.9	3.62	0.002	0.66	0.61	---	----	----
CP	72.6	70.4	70.1	68.7	1.72	0.65	0.61	0.77	0.63	0.44	0.45
week 4	73.2	70.2	69.8	68.6	1.33	0.67	0.67	0.79	---	----	----
week 8	71.2	69.2	69.8	67.6	1.56	0.67	0.63	0.77	---	----	----
week 12	73.2	71.7	70.7	69.7	1.93	0.66	0.65	0.79	---	----	----
NDF	30.1	27.7	50.8	46.1	2.21	0.01	0.003	0.48	<0.001	0.53	0.36
week 4	30.5	26.7	48.9	43.1	1.77	0.003	0.004	0.51	---	----	----
week 8	30.1	27.2	49.9	43.9	1.68	0.031	0.003	0.44	---	----	----
week 12	31.7	29.5	53.6	51.3	2.02	0.011	0.005	0.47	---	----	----
ADF	22.5	21.4	45.9	38.1	1.68	0.021	0.003	0.48	<0.001	0.72	0.02
week 4	19.6	18.8	39.5	35.9	1.62	0.003	0.038	0.5	---	----	----
week 8	21.6	20.7	46.0	39.3	1.52	0.013	0.063	0.46	---	----	----
week 12	26.4	24.7	52.3	39.4	1.73	0.042	0.043	0.49	---	----	----

** -RCS= Without roasted Canola seed; +RCS = With roasted Canola seed

**NDSC =Starch vs. Neutral detergent-soluble; RCS= Diets with Roasted canola seed vs. Diets without roasted canola seed and diets interaction

Mean values of the growth performance trial containing initial body weight, final body weight, average feed intake, feed conversion and average daily gain are report in Table 2. Initial body weight showed no significant difference due to stratification within dietary treatments ($P > 0.05$). High starch diets compared with high soluble fiber diets had no significant effect on final body weight, average daily DM intake and feed conversion ratio. Roasted canola seed addition to the diets also had no significant on these parameters ($P > 0.05$). A similar trend was observed by a comparison diets interaction for traits ($P > 0.05$). In an experiment by Mandebu and Galraith (1999) examined the effects of diets containing different proportions of barley and beet pulp on growth performance of male lambs and no significant differences observed for feed intake and feed conversion ratio. Similar results have been reported by Bodas et al. (2007). In contrast, Rouzebhan et al. (1999) reported the decrease in dry matter intake with increasing level of beet pulp in the diet. Different factors could affect the dry matter intake including stretch of gastrointestinal tract. Amount of stretch that could limit DMI is different in the animals with different levels of production and among diets with various concentrations of energy, nutrients, dry matter and physical properties (Allen, 1996). Effectiveness of stretch of rumen wall in reduction of DMI is likely attributable, in part, to the weight and bulk of consumed feed. Beet pulp could increase filling effect of consumed feed with its higher water holding capacity and therefore reduce feed intake (Robinson et al., 1990). It look like that the level of beet pulp in the rations used in current study was not high enough that could limit feed intake.

In agreement with the current study, Several researchers found no adverse effects of supplemental fat on DM intake and feed conversion of sheep (Goulas et al., 2003; Zhang et al., 2007). Similar findings have also been reported for dairy cows fed canola seed (Khorasani et al., 1991), sunflower seed (Markus et al., 1996) or flaxseed (Mustafa et al., 2003). Feeding supplemental fat in the form of oilseeds is expected to have less detrimental effects on DM intake than if a similar amount was added as free oil (Kennely, 1996).

An interaction between NDSC and RCS was observed for daily gain ($P < 0.05$) because the level of increase in weight gain with addition of RCS was different among carbohydrate sources. Limited research has been conducted investigating the interaction between carbohydrate source and fat supplementation.

Bhattacharya *et al*. (1975) reported that growing fattening sheep fed on a diet contained 45% beet pulp + 45% corn gained faster and required less feed per unit of gain than those fed on either 90% corn or 90% beet pulp. Similar conclusions were also reported by Bouaque *et al*. (1976) on young bulls and Mandebvu and Galbraith (1999) on lamb. The significant difference in average daily gain observed in lambs fed with high soluble fiber could be the result of high water holding capacity of dry beet pulp due to the existence of pectic substances, methyl and carboxyl groups in its molecular structure that might be the reason of its better digestion. Inclusion of beet pulp in feeding of ruminants delays rate of passage outside the rumen (Vollker and Allen, 2003) and increased methanogenic bacterial count, Lactobacilli and Streptcocci and enzymatic yield of polygalacturonase and pectinesterase (Vollker and Allen, 2003).

Addition of canola seed to the high starch diet caused dramatic increase in weight gain in comparison with high soluble fiber diet. Data is lacking using full fat oilseeds in high starch diets. This difference in response might be attribute, in part, to the correlated change in rumen pH (results are not reported). An increase in rumen pH observed when RCS included in the high starch diet. Wallace and Cotta (1989) indicated that at low pH, bacteria spend part of the available energy in maintaining the proton-motive force across

the cell membrane, increasing maintenance requirements at the expenses of growth (Nagaraja and Titgemeyer., 2007). The result is the reduction of microbial nitrogen flow to the small intestine. Hoover and Miller (1992) summarized several studies in which pH was modified in in vitro conditions and observed that the efficiency of microbial protein synthesis was affected when pH decreased below 5.5 (high starch diet without RCS supplementation).

Effects of experimental diets on digestibility of dry matter, organic matter, crude protein, NDF and ADF are shown in Table 2. Neutral detergent-soluble carbohydrates significantly affect dry matter and organic matter digestibility ($p<0.05$). High-starch diets compared with diets containing soluble fiber reduced the digestibility of dry matter and organic matter. Addition of roasted canola seed had no significant effect on the digestibility of these two parameters ($p<0.05$).

NDSC source had a significant effect on NDF and ADF digestibility and replacement of starch with soluble fiber resulted in increase in NDF and ADF digestibility ($P<0.001$). Increase in dietary fat with RCS incorporation in the ration significantly decreased NDF and ADF digestion. No significant interaction observed between NDSC source and RCS.

In agreement with the current study, Voelker and Allen (2003) reported greater ruminal and total tract NDF digestion when dried, pelleted beet pulp replaced corn in lactating dairy cow diets. Also, Mojtahedi and Danesh Mesgaran (2011) have reported increased digestibility of dry matter, organic matter by partial substituting of barley with beet pulp in the diet of steers. Beet pulp NDF has a shorter lag time and more rapid digestion rate than most other sources of fiber (Bhatti and Firkins, 1995) partly because it has been previously soaked in hot water (Bichsel,1988). Therefore, increasing the contribution of beet pulp NDF to total NDF can increase the overall rate of NDF digestion, independent of any associative effects of beet pulp NDF on the digestion of NDF from other sources. Substituting the readily degraded pectin and NDF of beet pulp for barely may also increase the rate of digestion of other dietary fiber through associative effects of both fiber and starch. Adding beet pulp might have increased the population of fibrolytic bacteria and fibrolytic enzyme activity by providing excess available substrate for fiber degraders. Dilution of the concentration of dietary starch would also reduce the negative effects of starch fermentation on cellulolytic bacteria (Voelker and Allen, 2003). It has been suggested that the decrease in pH caused by rapid fermentation of starch and lactate production can also affect the reduction in fiber digestion (Voelker and Allen, 2003). However, even if the pH is kept constant, the lower NDF digestibility can occur with the addition of starch (Mandebu and Galraith, 1999).

Stanford et al (2000) reported the decrease in digestibility of NDF and ADF with increase in dietary fat from canola seed source. These researchers conclude that the result is due to the toxic effects of fat on cellulose-degrading bacteria and protozoa. In vitro studies (Henderson, 1973; Maczulak et al., 1981) demonstrated that the unsaturated fatty acids, particularly C18:1, inhibit ruminal cellulolytic microbes. MacLeod and Buchanan-Smith (1972) postulated that the depressing effects of fat on fiber digestion might also be partially due to a physical coating of fiber particles, forming a lipid barrier that impedes enzyme penetration.

Conclusions

Partial replacement of starch with neutral detergent soluble fiber in high concentrate diets had positive effects on weight gain and nutrient digestibility. Also, incorporation of canola

seed in high concentrate diets, specially high starch diets, improved growth performance but because of its adverse effect on the digestibility of fiber fraction more reseach is needed for conclusion.

References

1. Allen MS 1996. Physical constraints on voluntary intake of forages by ruminants. Journal of Animal Science 74,3063-3075.
2. AOAC 2006. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists (AOAC), Arlington, VA, USA.8.
3. Bell J M 1984. Nutrients and toxicants in rapeseed meal: A review. Journal of Animal Science 58, 996-1010.
4. Bhattacharya AN, Khan MT and Uwayjan M 1975. Dried beet pulp as a sole source of energy in beef and sheep rations. J. Anim. Sci. 41, 616-621.
5. Bhatti SA and Firkins JL 1995. Kinetics of hydration and functional specific gravity of fibrous feed products. Journal of Animal Science 73, 1449–1458.
6. Bodas R, Giraldez FG, Lopez SA, Rodriguez A and Mantecon AR 2007. Inclusion of sugar beet pulp in cereal asked diets for fattening labms. Small Ruminant Research 71, 250-254.
7. Goulas C, Zervas G and Papadopoulos G 2003. Effect of dietary animal fat and methionine on dairy ewes milk yield and milk composition. Animal Feed Science and Technology 105, 43–54.
8. Henderson C 1973. The effects of fatty acids on pure culture in rumen bacteria. Journal of Agricultural Science (Camb.) 81, 107–112.
9. Hoover WH and Miller KT 1992. Rumen digestive physiology and microbial ecology. Bull. 708T, Agric. Forestry Exp. Sta., West Virginia University, Morgantown, WV.
10. Kennely JJ 1996. The fatty acid composition of milk fat as influenced by feed in oilseeds. Animal Feed Science and Technology 60, 137–152.
11. Khorasani RG, Robinson PH, De Boer G and Kennelly JJ 1991. Influence of canola fat on yield, fat percentage, fatty acid profile, and nitrogen fractions in Holstein milk. Dairy Scince 74, 1904–1911.
12. MacLeod GK and Buchanan-Smith GJ 1972. Digestibility of hydrogenated tallow, saturated fatty acids and soybean oil-supplemented diets by sheeps. Journal of Animal Science 35, 890–895.
13. Maczulak AE, Dehority AB and Palmquist LD 1981. Effect of long chain fatty acids on growth of rumen bacteria. Applied and Environmental Microbiology 42, 856–862.
14. Mandebu P and Galraith H 1999. Effect of sodium bicarbonate supplementation and variation in the proportion of barley and sugar beet pulp on growth performance and rumen, blood and carcass characteristics of young entire male lambs. Animal Feed Science and Technology 82, 37–49.
15. Mandebu P and Galraith H 1999. Effect of sodium bicarbonate supplementation and variation in the proportion of barley and sugar beet pulp on growth performance and rumen, blood and carcass characteristics of young entire male lambs. Animal Feed Science and Technology 82, 37–49.
16. Markus SB, Wittenberg KM, Ingalls R and Undi M 1996. Production responses by early lactation cows to sunflower seed or tallow supplementation of a diet based on barley. Journal of Dairy Science 79, 1817–1825.

17. Mojtahedi M and Danesh Mesgaran M 2011. Effects of the inclusion of dried molasses sugar beet pulp in a low-forage diet on the digestive process and blood biochemical parameters of Holstein steers. Livestock Science 141, 95–103.

18. Mustafa AF, Chouinard PY and Christensen DA 2003. Effects of feeding micronized flaxseed on yield and composition of milk from Holstein cows. Animal Feed Science and Technology 83, 920–926.

19. Nagaraja TG and Titgemeyer CE 2007. Ruminal Acidosis in Beef Cattle: The Current Microbiological and Nutritional Outlook. Journal of Dairy Science E17-E38.

20. Ramsey PB, Mathison GW and Goonewardene LA 2002. Effect of rates and extent of ruminal barley grain dry matter and starch disappearance on bloat, liver abscesses, and performance of feedlot steers. Animal Feed Science and Technology 97, 145-157.

21. Robinson PH, Burgess PL and McQueen RE 1990. Influence of moisture content of mixed rations on feed intake and milk production of dairy cows. Journal of Dairy Science 73, 2916-2921.

22. Stanford K, Wallins1 GL, Smart WG and McAllister TA 2000. Effects of feeding canola screenings on apparent digestibility, growth performance and carcass characteristics of feedlot lambs. Journal of Animal Science 80, 355–362.

23. Strobel HJ and Russell BJ 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. Journal of Dairy Science 69, 2941-2947.

24. Voelker JA and Allen MS 2003. Pelleted beet pulp substituted for high-moisture corn: 2. Effects on digestion and rumen digestion kinetics in lactating dairy cows. Journal of Dairy Science 86, 3553-3561

25. Wallace RJ and Cotta AM 1989. Metabolism of nitrogen-containing compounds. Pages 217–250 in The Rumen Microbial Ecosystem. P. N. Hobson, ed. Elsevier Applied Science, New York.

26. Wang YH, Xu M, Wang FN, Yu ZP, Yao JH, Zan LS and Yang FX 2009. Effect of dietary starch on rumen and small intestine morphology and digesta pH in goats. Livestock Science 122, 48–52.

27. Zhang RH, Mustafa AF and Zhao X 2007. Effects of feeding oilseeds on nutrient utilization by lactating ewes. Small Ruminant Research 67, 307–311.

Review paper

NUTRITION SPECIFICITIES OF GOAT KIDS IN SUCKLING PERIOD

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Abstract

In this paper the up to date methods and regulation of goat kids feeding as the new normatives and nutrient requirements are presented. In kids feeding remarkable attention has to be made on optimal needs concerning the accurate growth and progress. In order to make the best solution the updated normatives with large number of parameters have to be used.

Growing goat kids require energy, protein, vitamins and minerals for optimum growth. Determining quantities of these nutrients needed will make possible to determine how much of grain mix and hay should be used to support maintenance and growth. The nutrient requirements of growing goats show a different trend than those of mature animals.

The latest investigation results on protein level recommendation in kids feeding (NRC, 2007) have grown over 45%, while energy needs only infinitesimally more in regard to goat nutrient requirement needs considering the NRC recommendation from 1981. However, it must be remembered that energy requirements are based on minimal activity needed to secure feed and the actual requirement may very. Thus, the needs of energy for growing kids with a body mass of 20 kg is 5.69 MJ ME and 75.6 g in proteins. The first three days after birth are the most critical days in the life of a newborn kid. If the mother rejects the kids, colostrum of kids should be fed three times a day, a total of 2 to 3 pint per day. After three days, the kid diet is based on milk or milk replacer, which is given twice a day and does not exceed three liters per day. Feeding with milk or milk replacer may continue until 8-12 weeks or until the time the kids are weaned and able to consume 0.2 kg grain milk per day.

At weaning, for feeding the 30 days old kids we used a mixture concentrate for early suckling kids. By own nutrient compounds this grain mixtures must have better quality than concentrate mixtures used in goat kids feeding.

Provide a grain mix (kid starter) containing high levels of protein (16%) and high levels of fiber (11%) as well as good quality hay to encourage rumen development. After weaning up to 6 months, continue feeding with kid starter 0.2 to 0.4 kg/day and plenty of good quality forage and pasture. When the growing kids start to use large amounts of hay or green forage in feeding, they should be given a mixture of concentrate with something simpler composition. Do not feed silage and non-protein nitrogen at this age.

Key words: *goats, kids, nutrient requirements, nutrition, weaning*

Introduction

Adequate nutrition and rearing of kids is the basis for future high production in goats. The production values of each head is greatly dependent on feeding in early age (Memiši and Bauman, 2003; Žujović et al., 2002). Future goat production will therefore depend largely on the quantity and quality of these nutrients. In doing so, the quality of roughages is of great importance (Poore and Luginbühl, 2002). In such cases, depending on quality of roughage a part of it involves a certain amount of concentrate feed, because the forage crops are not sufficient to meet all the needs of animals in nutrients. Concentrate mixtures are given to the younger categories with the aim to increase the share of energy and protein in the diet and also to enrich meal in certain mineral elements (Memiši et al., 2007a and 2008a). Special attention should be given to feeding goats in gestation period. In regard to this, a good diet in the last gestation period resulted in coarser and more vital kids, which later enabled their intense and successful development (Memiši et al., 2008b).

Intensive production of kid meat in countries with developed goat production is becoming more and more prominent (Memiši, 2009). In addition, of particular interest are systems that contribute to solving the problem of efficient kids weaning, in other words, a faster transition to feeding with dry food (Memiši and Bauman, 2007b). Taking into account that in our country there is a great interest in breeding goats, and the problem of rearing the kids is still greatly unknown, we wanted, in this paper, to give a contribution to clarifying the issues related to the effects of proper weaning of the kids. One more reason is that there are real possibilities to adequately rear offspring in our conditions with great success, which will allow breeders to produce larger quantities of milk for the production of various products for the market and it will also lead to the intensification of goat production, which currently is not on a high level.

Nutritional requirements of goat kids

It is most important for kids to receive colostrum (the doe's first milk) during the first 24 hours of their life. A healthy newborn kid rarely needs help to suck from its dam. Colostrum contains essential antibodies (immunoglobulins) that protect kids from disease and represents a concentrated source of nutrients. The newborn kid can absorb the essential antibodies in colostrum only during its first 24 hours or so of life. Kids eat solid food from about 1 week of age and are often seen ruminating at about 2 weeks, so it is important to give them good-quality hay and suitable concentrate from 1–2 weeks of age (Ensminger, 2002).

When the young goats begin to eat solid food (forages and grains), these feeds may stay in the rumen what can lead to development of the microbial population. The rumen-reticulum and the large intestine begin to increase more rapidly at the expense of the abomasum and small intestine. The change from preruminant to ruminant is a gradual process. Fibrous feedstuffs (forages) encourage rumen development and appear to speed up the development of the muscles of the rumen wall (Ezeasor and Nwaogu, 2008), which are important in rumen digestion and mixing of rumen contents.

These changes in the digestive system have a large impact on the feeding methods used in raising young goats and should be kept in mind during all feeding management decisions. Growing goat kids require energy, protein, vitamins and minerals for optimum growth and profitability. Determining needed quantities of these nutrients will

enable you to determine how much of grain mix and hay should be used to support maintenance and growth **(**Memiši and Bauman, 2007b).

Growth is a period of high energy requirement and the NRC (1981) recommends an allowance of 1732.8 kJ ME/kg of weight gain be made in the diet. The difficulty in estimating energy requirements for growth are due to the immense differences in the energy costs of protein and fat deposition and the change in rates of deposition with age, weight and diet (AFRC 1997). Growth has high protein requirements due to the fast lay down of protein rich tissue. The NRC (1981) recommendation of protein allowance for growth was 195g DP/w kg 0.75. For replacement females in a diary system the female weaners need to gain 50-100g/day. In order to achieve this, the diet should contain 12 MJ/kg DM and 140g CP/ kg DM (AFRC 1993). To achieve growth rates of 200g/day, the diet needs to be based on cereal grains and in an intensive lotfeeding type situation (AFRC 1993). The metabolisable protein requirements for growth as recommended by the AFRC (1997) are detailed in Table 1.

Table 1. *Requirements in metabolisable protein for the growth of kids (AFRC 1997).*

Expected growth	Requirements in metabolisable protein (g/day)		
	The body weight of kids		
	15 -20 kg	20-25 kg	25-30 kg
100 g/day	24.6	24.0	23.4
200 g/day	49.2	48.0	46.8
300 g/day	73.8	72.0	70.2

The nutrient requirements of growing goats show a different trend than those of mature animals (Table 2). In the research of group of authors (Luo et al., 2004 and 2004a; Sahl et al., 2004), recommendations for the level of protein in the diet of goats, according to these studies, increased by over 45% while the needs of the energy were only slightly higher compared to those of 1981 (NRC, 1981). However, it must be remembered that energy requirements are based on minimal activity needed to secure feed and the actual requirement may very (Memiši et al., 2009).

Table 2. *Energy and protein requirements of a 20 kg growing kid (Luo et al., 2004)*

Parameters	NRC 1981	2004
ME mJ	5.53	5.69b
Crude Protein, g	51.76	76.5c

a 50 g gain
b 2004 requirements for energy based on doelings and wethers
c metabolizable protein converted to crude protein utilizing NRC 1996

On the basis of these studies, in 2004, the amount of information available about the actual needs of the offspring of goats in nutrients increased, which is essential for making decisions concerning the expected performance of the wide variety of nutritional status. Based on that research NRC in 2007 published new regulations in nutrient requirements of goats which represent a step forward compared to the former regulations from 1981.

NRC (2007) provides a detailed summary of the nutrient requirements for kids and differentiates between dairy, Boer, indigenous local (assumed to be equivalent to

Australian rangeland, but may not be) and Angora. Daily requirements vary according to current or target liveweight, rate of daily gain, gender and are too extensive to list within this review; however Table 3 provides an extract to highlight the differences.

Table 3. *Daily dry matter intake (DMI), dietary NDF tolerance, crude protein, protein (CP) and energy (ME) of four goat genotypes at 20 and 30kg LW growing at 200g/d (adapted from NRC 2007).*

Genotype	Live-weight	Growth rate	Fiber growth rate	DMI	DMI	NDF limit	CP	ME
	kg	g/day	g/d	kg/day	% LW	%	g/d	MJ/d
Dairy	20	200		0.73	3.7	33	130	9.66
Boer	20	200		0.66	3.3	36	163	8.91
Indigenous local	20	200		0.62	3.1	39	130	8.24
Angora	20	40	8	0.74	3.7	32	84	7.45
Dairy	30	200		1.14	3.8	32	145	11.47
Boer	30	200		1.04	3.5	35	179	10.42
Indigenous local	30	200		0.97	3.2	37	145	9.75
Angora	30	40	8	0.91	3.0	40	100	9.12

Supplemental feeding

The first three days after birth are the most critical days in the life of a newborn kid. If mother rejects the kids, colostrum of kids should be fed three times a day, a total of 2 to 3 pint per day. After three days, kid diet is based on goat milk or milk replacer, which is given twice a day and does not exceed three liters per day. In some cases, the female goats cannot produce enough milk for feeding their kids, especially when it comes to their greater numbers (twins or triplets). For providing adequate daily increment of kids a sufficient amount of the initial starter mixture and high-quality hay must be available from the first days after birth.

Table 4. *Nutrition technique for kids under 3 months of age (INRA, 1989)*

Nutrition technique	Age of kids	Quantity of milk (kg)	
Goat milk in 2 meals	By the third day	Colostrum (max. 1.5 kg)	From the 7th day after kidding feeding hay and concentrates ad libidum
	31	1.5 kg goat milk	
	32	1.0 kg goat milk	
	33 – 42	0.5 kg goat's milk in the 1st meal	
	85 – 90	Weaning	

Table 4 contains examples of the composition of concentrate mixtures for goats feeding. If the kid feeding is used as milk replacer, it should contain not less than 5% fat, and at least 20% crude protein, which should originate from by-products of milk (Solaiman, 2014). In any case, substitute for milk from cow's milk is less expensive compared to the replacement of goat milk and can be a very good quality food for kids (Memiši and

Bauman, 2003a). Feeding milk or milk replacer could continue up to 8-12 weeks or up to the time the kids are weaned or able to consume 0.2 kg of grain mix daily. Provide a grain mix (kid starter, Table 5) containing high levels of protein (16%) and high levels of fiber (11%) as well as good quality hay to encourage rumen development.

Table 5. *Kid starter grain mix (Solaiman, 2014)*

Ingredient	% of ration
Cracked corn	29.0
Crushed oats	29.0
Wheat bran	29.0
Soybean meal	11.5
Trace mineral salt	0.5
Dicalcium phosphate	0.5
Vitamins ADE	0.5
Protein	15-16%
Fiber (minimum)	10%

For additional weight gain, animals also need nutrients for growth such as 0.55 Mega calorie (Mcal) for every 100 g gain/d, 57 g protein (CP) for every 100 g gain/d., 2.8 g calcium (Ca), 1.3 g phosphorus (P).

Table 6. *Examples of complete meal for starter and mixtures for the growth in nutrition of kids (Sahlu et al., 1992)*

Feedstuff	Kid Starter %	Growing Ration %	Feedstuff	Kid Starter (% dry matter basis)
Corn	27.6	12.9	Corn	70.17
Oats	37.9	10	Oats	2
Soybean meal (44%)	10	8.6	Soybean meal (44%)	24
Alfalfa leaf meal	18	10	Alfalfa meal	2
Cane molasses	5	5	Calcium carbonate	0.81
Cottonseed hulls	0	51.9	Dicalcium phosphate	0.37
Trace mineral salt	1	1	Trace mineral salt*	0.45
Limestone	0.3	0.4	Vitamins A, D and E (premix)**	0.2
Vitamins A, D and E (premix)	0.2	0.2		
Crude protein = 20% Calcium = 0.6% Metabolizable energy, Mcal/kg = 3.06 Phosphorus = 0.5% * Composition: 98% salt (NaCI), 0.35% zinc, 0.28 manganese, 0.175% iron, 0.035% copper, 0.007% iodine and 0.008% cobalt. ** Contents (for 2.2 lbs): 6600 IJ vitamin A, 2200 IJ vitamin D and 33 IJ vitamin E.				

The kids that are in the flock for overhaul and further reproduction purposes, which are selected and graded on the basis of production and morphological characteristics of their parents should be weaned at the age of three months. Feeding base of weaned kids should be a good quality pasture with the addition of small amounts of concentrate. The amount of concentrate depends on the condition and the mass of kids, where certain recommendations suggest providing around 100-150 g of concentrate a day. However,

if the pasture is of a lower quality, it is necessary to provide sufficient quantities of good quality lucerne hay. Otherwise, during the weaning, the kids gain decreases, and thereby the consumption of feed per kilogram of gain increases.

Conclusion

Adequate nutrition and rearing of kids is the basis for future high production goats. The production value of each head is greatly dependent on feeding in early age. Poor and inadequate nutrition in this age can have adverse consequences of a permanent character. Kids are mostly fed milk suckling their mother, but in some cases this can also be supplemented. If we use the supplementation in kids nutrition, the number of servings for kids should be the same as the suckling.

Nutrition of weaned kids very much depends on whether the animals are intended for rearing or to be used for breeding. Feeding base of weaned kids should be a good quality pasture with the addition of small amounts of concentrate. The amount of concentrate depends on the condition and the mass of kids. In feeding kids intended for breeding we should strive for those in the age 7-8 months to attain body weight 30-35 kg. In this way they will be in a good shape and well prepared for mating.

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References

1. Agricultural and Food Research Council 1993. Energy and protein requirements of ruminants. An advisory manual prepared by the AFRC technical committee on responses to nutrients. Commonwealth Agricultural Bureau, Farnham Royal, UK.
2. Agricultural and Food Research Council 1997. Technical committee on responses to nutrients report No. 10. The nutrition of goats. Nutrition abstracts and reviews (series B) 61, 573-612.
3. Brien A. 1998. Nutrition of the Young Goat: Birth to BreedinG. Retreived April 10, 1998, from http://www omafra.gov.on.ca/.../goat/.../goatnutrition.
4. Ensminger ME 2002. Sheep and goat science (6th ed.). Danville, IL: Interstate Publishers, Inc.
5. Ezeasor IC and Nwaogu DN. 2008. Morphological studies on rumen development in West African Dwarf goats (Capra hircus). Sokoto Journal of Veterinary Sciences 7 (2), 1-6.
6. National Institute for Agricultural Research 1989. Ruminant nutrition: Recommended allowances and feed tables. John Libbey & Co. Ltd, Paris, France.
7. Luo J, Goetsch AL,, Nsahlai IV, Johnson ZB, Sahlu T, Moore JE, Ferrell CL Galyean ML and Owens FN 2004. Maintenance energy requirements of goats: predictions based observations of heat and recovered energy. Small Rum. Res. 53, 221-230.

8. Luo J, Goetsch AL, Nsahlai IV, Sahlu T, Ferrell CL, Owens FN, Galyean ML, Moore JE and Johnson ZB 2004a. Metabolizable protein requirements for maintenance and gain of growing goats. Small Rum. Res. 53, 309-326.
9. Matthew H. Poore and Jean-Marie Luginbuhl 2002. Improving Meat Goat Nutrition with Forages and Supplementation. Kentucky Ruminant Nutrition, pp. 11-20.
10. Memiši N 2009. Proizvodne i klanične karakteristike jarića domaće balkanske koze. Tehnologija mesa 50 (3-4), 205-210.
11. Memiši N, Frida Bauman 2003. Sistemi odgoja i ishrane jaradi u dojnom periodu. Poljoprivredne aktuelnosti 3-4, 87-96.
12. Memiši N, Frida Bauman 2003a. The feeding of early weanned kids. Poljoprivredne aktuelnosti 3-4, 97-102.
13. Memiši N, Frida Bauman, and Biserka Pavlov 2007a. Significance microelement in nutrition of goats on their production traits. In Proceedings of XXInd Conference of Agronomist, Veterinarians and Technologiest, 26-28 February2007, Padinska Skela, Serbia, pp. 143-150.
14. Memiši N, Frida Bauman 2007b. Zamene za mleko u ishrani jaradi. I Međunarodni kongres „Tehnologija, kvalitet i bezbednost hrane“, XII međunarodni simpozijum Tehnologija hrane za životinje. 13-15. November 2007, Novi Sad, Serbia, pp. 191-198.
15. Memiši N, Frida Bauman and Biserka Pavlov 2008a. Normative of energy for goat in intensive production. In Proceedings of XXIInd Conference of Agronomist, Veterinarians and Technologiest, 26-27 Februar 2008, Padinska Skela, Serbia, pp. 119-128.
16. Memiši N, Žujović M, Bogdanović V and Petrović MP 2008b. The level concentrate in meal of goat on production milk in lactation period. Biotehnology in Animal Husbandry 24, 481-490.
17. Memiši N, Jovanka Lević and Đorđević N 2009. New recommendations and contemporary achievements in preparing feeds for goats feeding. 13th International Feed Technology Symposium, 29-30 September 2009, Novi Sad, Serbia, pp. 153-163.
18. National Research Council 2007. Nutrient Requirements of Sheep, Goats, Cervids and Camelids. National Academy Press. Washington D.C.
19. National Research Council 1981. Nutrient Requirements of Goats: Angora, Dairy and Meat Goats in Temperate and Tropical Countries. National Academy Press, Washington D.C.
20. Poore MH and Luginbuhl JM 2002. Improving Meat Goat Nutrition with Forages and Supplementation, Kentucky Ruminant Nutrition, 11-20.
21. Sahlu T, Carneiro H, El Shaer HM and Fernandez JM, 1992. Production performance and physiological responses of Angora goat kids fed acidi®ed milk replacer. J. Dairy Sci. 75, 1643-1650.
22. Sahlu T, Goetsch AL, Luo J, Nsahlai IV, Moore JE, Galyean ML, Owens FN, Ferrell CL and Johnson ZB 2004. Nutrient requirements of goats: developed equations, other considerations, and future research to improve them. Small Rum. Res. 53, 191-219.
23. Solaiman S 2014. Feeding management of a meat goat herd. Technical Paper No. 06-11, Tuskegee University Retreived April 10, 2014, from http://www.agmrc.org/media/cms/feedingmanagement_.
24. Žujović M, Petrović PM, Josipović S, Zorica Tomić, Cmiljanić R, Dušica Tomašević, Stela Stresoglavec and Memiši N 2002. Uticaj ranog odlučivanja jaradi blizanaca na njihov razvoj i proizvodnju mleka i mesa. 15th Symposium on innovation in animal science and production. Biotechnology in animal husbandry 18, 5-6, 81-86.

Original paper

THE EFFECT OF FEEDING PROGRAMMES ON BROILER PERFORMANCE AND PRODUCTION EFFICIENCY

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Abstract

The goal of the paper was to investigate the effect of feeding programmes with different protein levels on broiler performance and production efficiency. The research was conducted on 600 chicks of Cobb-500 and Ross-308 provenience, previously divided into four groups (by feeding treatments).

The groups (treatments) differed in the type of mixture given in the starter period. The feeding programmes were, as follows: T1 (control group) – 23% protein mixture (Day 1 to Day 21); T2 - 23% protein mixture (Day 1 to Day 14), and 20% protein mixture (Day 14 to Day 21); T3 – 23% protein mixture (Day 1 to Day 7), and 20% protein mixture (Day 7 to Day 21); T4 - 20% protein mixture (Day 1 to Day 21). The chicks were given 20% protein mixture from Day 21 to Day 35, and 18% protein mixture from Day 35 to Day 42. A standard technology for chicken fattening was carried out. Production efficiency was determined by observing the ratio of the total cost and total revenue, i.e. profit per chick. From the value of the European Production Efficiency Factor (EPEF), a summary indicator of production results, the authors came to a conclusion that T1 (control) group of the Cobb-500 and Ross-308 chicks had achieved higher EPEF than the experimental groups, whereby the values of T2 group were similar to the values of T1. The highest profit per chick was found for T1 group of Cobb-500 chicks and for T2 group of Ross-308 chicks. The research showed that a drastic protein reduction in the starter period had resulted in negative tendencies in the chicks of the both genotypes. From the aspect of development of more profitable feeding programmes, further research should therefore focus on T2 feeding programme.

Key words: *broiler, genotype, production efficiency, production performance*

Introduction

In broiler production, a proper diet is one of the most important preconditions for expressing the genetic potential of modern hybrids. Broiler diets comprise feeding programmes that follow recommendations given in NRC Standards (National Research Council, 1994),

programmes of breeding centres – producers of line hybrids, and programmes aimed to satisfy some specific market requirements (e.g. the production of meat of special quality) as well as environmental and other requirements for modern broiler production.
A diet is also one of the key factors affecting broiler production efficiency, since feed costs make about 70% of the total cost of production (Marcu et al., 2013). The cost of the protein component in broiler mixtures generates the largest share of feed costs, together with energy sources (Moosavi et al., 2011). Over the last decade, one can see the tendency of protein reduction in broiler mixtures, due to economic and environmental requirements. Proteins have an important role not only in enabling the carrying out of the basic life functions but also in providing conditions for expressing the genetic potential, in terms of broiler productivity. Therefore, nutritionists try to develop such feeding programs that will optimally meet broiler requirements and requirements for production efficiency. There are also some aggravating circumstances – protein requirements can fluctuate a lot, depending on the genotype, sex, age, production programme, etc., and one should have in mind that the efficiency of protein utilisation can also differ depending on the protein source and levels of other nutrients in a diet (Jokić et al., 2004).
To increase production efficiency, some feeding programmes are designed to maximise profits instead of maximising production performance. The research conducted by Eits (2004) was aimed to develop a model to make choosing of optimal feeding strategies much easier, in terms of achieving preferable performances with minimal costs.

In order to determine an optimal amount of time for giving basic mixtures, and considering broiler performance and production efficiency, the subject-matter of the research of numerous authors was phase feeding programmes, i.e. programmes based on different frequencies of change of feeding mixtures – starters, growers and finishers (Watkins et al., 1993; Saleh et al., 1996; Pope and Emmert, 2001; Roush et al., 2004). The aforementioned authors mostly indicated a necessity for questioning traditional feeding programmes and duration of each feeding phase. They also pointed out some genetic improvements in broilers, a shorter time for gaining body weight, as well as a growing need for the economic optimisation of broiler production. The economic effect of changing starter mixtures with growers is based on the difference in costs of starters and growers. According to Warren and Emmert (2000), the duration of a feeding phase significantly affects fattening efficiency, i.e. phase feeding programmes can reduce feed costs, without affecting weight gain and body yield.

The goal of the paper was to investigate the effect of feeding programmes comprising different protein levels, i.e. different frequencies of change of starter and grower mixtures on performances of different genotype broilers and their production efficiency.

Materials and methods

The experiment comprised 1.200 chicks, i.e. 600 chicks of Cobb-500 and 600 chicks of Ross-308 provenience. Within each genotype, four groups (treatments) were formed, with two repetitions per treatment. The object was divided in 16 pens (75 chicks each). The treatments and repetitions were assigned by a randomised block design. Each treatment comprised 150 chicks, which makes 600 chicks per provenience investigated. Feeding differed only in the starter period (from Day 1 to Day 21), according to the following programme:

- T1 (control group) - 23% protein mixture (starter) from Day 1 to Day 21;

- T2 - 23% protein mixture (starter) from Day 1 to Day 14, and 20% protein mixture (grower) from Day 14 to Day 21;
- T3 - 23% protein mixture (starter) from Day 1 to Day 7, and 20% protein mixture (grower) from Day 7 to Day 21;
- T4 - 20% protein mixture (grower) from Day 1 to Day 21.

From Day 21 to Day 42, all the chicks were fed in the same way. They were given 20% protein mixture (grower) from Day 21 to Day 35, and then 18% protein mixture (finisher) to Day 42. Feeding was *ad libitum*. A standard fattening technology was carried out, following all technological norms of intensive fattening.

All the chicks were marked, after which control weighing on a precision balance was conducted on the Day 1 and then on a weekly basis. Daily gain was calculated on the basis of the data on body weight. Data on the used feed and achieved gain were used to calculate feed intake and conversion.

The obtained production parameters were then used to calculate the European Production Efficiency Factor (EPEF = liveability (%) * body weight (kg) / number of days of fattening * feed conversion) * 100) and the European Broiler Index (EBI = liveability (%) * average daily gain (g) / feed conversion *10).

Production efficiency was determined after taking into account the value of production and the total cost, i.e. profit per chick. A profit index per chick was calculated for each feeding programme, whereas the obtained profit per chicken for the control group was used as a basis for calculating the profit index per chicken for experimental feeding programmes.

The data were statistically processed using the computer programme Statistica (version 5) (Stat.Soft.Inc, 2006). Individual differences in means were compared with the LSD test.

Results and discussion

Having analysed the data given in Table 1, the authors determined that at the end of the starter and the fattening period (after 42 days) the Cobb-500 chicks from the group T1 had achieved the largest body weight. Statistically, the value of their body weight was significantly higher than the value of the body weight in the groups in which feed restriction had started in the first (T4) and second week (T3). No statistical significance was detected between T1 and the group in which protein reduction had started in the third week (T2). As for the chicks of Ross-308 provenience, after comparing T1 with the experimental groups and comparing the experimental groups among themselves, the results showed T4 group (largest protein reduction) was the only one with significantly smaller body weight in the aforementioned periods. This result showed certain differences among hybrids, i.e. that the Ross-308 chicks were more tolerant to protein reduction and to a shorter period of feeding with the starter mixture. Different reactions of hybrids were also detected by Madrigal et al. (1994) when investigating the effect of feeding programmes. Smith and Pesti (1998) also investigated the effect of different feeding programmes on two proveniences and determined that the hybrid and the level of protein in mixtures had affected production performances - body weight and feed intake, which is in line with the results of this research. Saki et al. (2010), however, studied three feeding programmes applied on two genotypes in their starter period and found no statistically significant interactions between the hybrids and the feeding programmes.

Table 1. *Broiler production performances*

Traits	Measures of variation	Broiler production performances							
		Cobb-500				Ross-308			
		T1	T2	T3	T4	T1	T2	T3	T4
Day 1 to Day 21									
Body weight	$\bar{x}$	642[a]	621[a]	614[b]	530[c]	642[a]	638[a]	623[a]	554[b]
	Sd	116.54	87.12	85.87	92.08	93.28	104.57	100.71	94.54
Average daily gain (g)	$\bar{x}$	28.81[a]	27.80[a]	27.48[a]	23.48[b]	28.78[a]	28.55[a]	27.90[a]	24.63[b]
	Sd	5.51	4.12	4.05	4.38	4.41	7.26	4.79	4.47
Average feed intake (g)		50.229	49.029	55.465	56.357	50.382	50.402	56.820	51.232
Day 1 to Day 42									
Body weight (g)	$\bar{x}$	2279[a]	2216[a]	2203[b]	2048[c]	2177[a]	2166[a]	2136[a]	1963[b]
	Sd	261.96	244.05	210.31	234.56	266.24	264.00	287.00	247.72
Feed conversion		1.871	1.918	1.994	2.058	1.914	1.927	2.022	2.126
Mortality, (%)		2.67	2.33	2.50	2.17	2.50	2.17	2.33	2.50
EPEF		282.27	268.68	256.47	231.79	264.04	261.82	245.66	214.35
EBI		277.63	264.04	252.11	227.65	257.55	257.24	241.32	202.2

a-b - values with different superscript letters in the same line for hybrids are statistically significantly different ($P < 0.05$)

In the chicks of Cobb-500 and Ross-308 provenience, the smallest, insignificant difference in finished body weight was found by comparing T1 with T2 group, which consumed the starter mixture for two weeks. This result raises a question whether the chicks in the starter period need starter mixtures for longer than 14 days. The obtained results are in line with the research of Saleh et al. (1997), who estimated the periods of giving starter, grower and finisher mixtures to fattening chicks in the fattening period of 42 days. These authors pointed out the importance of targeting finished body weight when creating feeding programmes, and they gave recommendations for duration of certain feeding phases, considering the finished body weight of 1 kg, 2 kg or 3 kg (Saleh et al., 1996, 1997a and 1997b). For the both investigated genotypes, after comparing T1 with the experimental groups, significant and also the largest difference in finished body weight was found between T1 and T4 group, observed by periods and in the whole experiment. A lag in weight gain, found in T4 group during the starter period but also in the second fattening phase, was a result of a drastic protein reduction, since these chicks were fed with the grower mixture from the first day. The obtained results are in line with the other researches, where chicks subjected to drastic protein reductions in their early period lagged behind in weight gain, not being able to compensate it later (Watkins et al., 1993).

The analysis showed best feed conversion in T1 group and poorest in the experimental groups with the highest protein reduction (T3 and T4). The obtained results can be somewhat compared with the one of Jianlin et al. (2004), who investigated eight feeding programmes in the starter period of broilers, where the control group was given feed with 22.48% crude protein, and each following experimental group was given feed with reduced protein levels,

the lowest of which was 16.61%. Body weight and feed conversion was smaller than in the control group, and the more reduced protein levels were, the smaller was the body mass and feed conversion. Dozier et al. (2006) also investigated the effect of phase programmes with different protein levels, and found that chicks fed with mixtures with higher protein levels and nutrients had achieved larger body weight and better feed conversion.

The broilers of Cobb-500 and Ross-308 provenience were of satisfying liveability, which implies that the feeding programmes did not affect this trait. The obtained results are in line with the results of Watkins et al. (1993), in which different time distribution of mixtures did not affect broiler mortality. However, Salmon et al. (1983), when investigated the effect of different feeding programmes on liveability, found high protein levels in starter mixtures that resulted in increased total mortality.

From the obtained value of EPEF, a summary indicator of production results, the authors came to a conclusion that T1 (control) group of the Cobb-500 and Ross-308 chicks had achieved higher EPEF than the experimental groups, whereby the values of T2 group were similar to the values of T1. The results of EBI showed that the highest profit per chick of Cobb-500 provenience had been identified for T1 group, with the smallest difference between T1 and T2 programmes. EBI for T1 and T2 group of the chicks of Ross-308 provenience were completely uniform. The obtained results for EPEF and EBI for the chicks of the both provenience implies that further research should focus on T2 feeding programme. Differences in EPEF and EBI were also found by Marcu et al. (2013), when investigated feeding programmes with different protein and energy levels.

The production efficiency of the feeding programmes, determined after comparing the value of production and the total cost, i.e. profit index per chick, is shown in Table 2.

Table 2. *Broiler production efficiency, depending on the feeding programme*

	Profit index per chick, depending on feeding programmes (%)							
	Cobb-500				Ross-308			
Parameter			Feeding programmes					
	T1	T2	T3	T4	T1	T2	T3	T4
Index	100	93.64	80.98	38.73	100	116.31	75.62	15.58

The highest profit index per chick was found in T1 group of the Cobb-500 chicks and T2 group of the Ross-308 chicks. The feeding programmes with drastic protein reduction (T3 and T4) achieved the lowest profit indices, which can be related to the smallest finished body weight and poorest feed conversion that resulted in the lowest profits for these groups. The results are in line with the research of Moosavi et al. (2011), in which feed costs per kg were different. They were lower for mixtures with lower protein levels, yet production costs were then higher due to poorer feed conversion, considering the chicks were fed with mixtures with lower protein and energy levels. Differences in profit indices of different feeding programmes were also detected by Petričević et al. (2012).

Conclusion

Based on the obtained results, it was determined that the applied feeding programmes with different protein levels (different frequencies of change of starter, grower and finisher mixtures) for Cobb-500 and Ross-308 chicks had affected the production performance and efficiency. When observing the production results for the whole experiment, in the both provenience, T1 (control) group achieved the highest European Production Efficiency Factor (EPEF) compared to the experimental groups (T2, T3 and T4). The applied feeding programmes with a drastic protein reduction in the starter period (T3 and T4) resulted in negative tendencies from the aspect of production results and efficiency. In the both provenience, the experimental group T2 achieved similar values of EPEF to T1. Moreover, when it comes to the Ross-308 chicks, T2 group also achieved the highest profit index per chick. From the aspect of the development of more profitable feeding programmes, the focus of further research on optimising the periods of giving starter, grower and finisher mixtures should be put on T2 programme.

References

1. Dozier WA, Gordon RW, Anderson J, Kidd MT, Corzo A and Branton SL 2006. Growth, meat yield and economic responses of broilers provided three and four - phase schedules formulated to moderate and high nutrient density during a fifty –six day production period. Journal of Applied Poultry Research 15, 312-325.
2. Eits R 2004. Modeling responses of broiler chickens to dietary balanced protein. PhD, Wageningen Univ.
3. Jianlin S, Fritts CA, Burnha DJ and Waldroup PW 2004. Extent to Which Crude Protein May Be Reduced in Corn-soybean Meal Broiler Diets through Amino Acid Supplementation. International Journal of Poultry Science 3 (1), 46-50.
4. Jokić Ž, Kovčin S and Joksimović - Todorović M 2004. Ishrana živine, Poljoprivredni fakultet, Beograd, Srbija
5. Madrigal SA, Watkins SE and Waldroup PW 1994. Feeding Programs Designed to Modify Early Growth Rates in Male Broilers Grown to 56 Days of Age. Journal of Applied Poultry Research 3, 319-326.
6. Marcu A, Vacaru - Opriş I, Marcu A, Dănăilă L and Kelciov B 2013. Effect of feed protein and energy levels on economic efficiency of broiler chickens growth. 23rd International symposium „New Technologies in Contemporary Animal Production", Novi Sad, Serbia
7. Moosavi M, Moosa E, Morteza C and Boujarpour M 2011. Economic value of diets with different levels of energy and protein with constant ratio on broiler chickens. Journal of Animal and Veterinary Advances 10 (6), 709-711.
8. National Research Council 1994. Nutrient Requirements of Poultry, 9th rev.ed. National Academy Press, Washington, DC
9. Petričević V, Lukić M, Škrbić Z, Pavlovski Z, Stojanović Lj 2012. The effect on nutrition using commercial mixtures of different nutritive value on production performance and economical efficiency of broiler fattening. Proceedings of the First International Symposium on Animal Science, Belgrade, Serbia, 645-653.

10. Pope T and Emmert JL 2001. Phase – feeding supports maximum growth performance of broiler chicks from forty – three to seventy - one days of age. Poultry Science 80 (3), 345-352.

11. Roush WB, Boykin D and Branton SL 2004. Optimization of phase feeding of starter, grower and finisher diets for male broilers by mixture experimental design: forty-eight day production period. Poultry Science 83(8), 1264-1275.

12. Saki AA, Momeni M, Tabatabaei MM, Ahmadi A, Rahmati MMH, Hemati Matin HR and Janjan A. 2010. Effect of Feeding Programs on Broilers Cobb and Arbor Acres plus Performance. International Journal of Poultry Science 9 (8), 795-800.

13. Saleh EA, Watkins SE, Waldroup AL and Waldroup PW 2004. Effect of Dietary Nutrient Density on Performance and Carcass Quality of Male Broilers Grown for Further Processing. International Journal of Poultry Science 3 (1), 1-10.

14. Saleh EA, Watkins SE and Waldroup PW 1997a. Changing Time of Feeding Starter, Grower and Finisher Diets for Broilers. 2. Birds grown to 2.2 kg. Journal of Applied Poultry Research 6, 64-73.

15. Saleh EA, Watkins SE and Waldroup PW 1997b. Changing Time of Feeding Starter, Grower and Finisher Diets for Broilers. 3. Birds grown to 3.3 kg. Journal of Applied Poultry Research 6, 290-297.

16. Saleh EA, Watkins SE and Waldroup PW 1996. Changing Time of Feeding Starter, Grower and Finisher Diets for Broilers. 1. Birds grown to 1 kg. Journal of Applied Poultry Research 5 (3), 269-275.

17. Salmon R.E., Classen H.L. and McMilan R.K. (1983): Effect of starter and finisher protein on performance, carcass grade and meat yield of broilers. Poultry Science, 62, 837-845.

18. Smith ER and Pesti GM 1998. Influence of broiler strain cross and dietary protein on the performance of broilers. Poultry Science 77, 276-281.

19. Stat.Soft. Inc, 2006. STATISTICA, version 5

20. Warren WA and Emmert JL 2000. Efficacy of phase – feeding in supporting growth performance of broiler chicks during the starter and finisher phases. Poultry Science 79 (5), 764-770.

21. Watkins SE, Waldroup AL and Waldroup PM 1993. Effect of dietary amino acid level diets for broiler chickens on change from starter to grower. Poultry Science 72 (2), 137.

Original paper

PRESENCE OF AFLATOXIN IN COMPLETE FEEDING MIXTURES FOR DIFFERENT CATEGORIES OF PIGS IN SERBIA

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Abstract

A total of 106 samples of complete feeding mixtures for different categories of pigs were analyzed for presence of aflatoxin. Feeding mixtures were composed of feed materials harvested during 2012 harvest season in Serbia. These samples included complete feeding mixtures for fattening pigs (49), lactating sows (19), pregnant sows (10) and piglets (28). Analysis was performed using ELISA test kits. The presence of aflatoxin was the highest in complete feeding mixtures for fattening pigs (43%), somewhat lower in complete feeding mixtures for lactating sows (37%) while in complete feeding mixtures for piglets was 25% of positive samples. Incidences ranged from 4 µg/kg to 66 µg/kg, both in complete feeding mixtures for fattening pigs. Out of 10 samples of complete feeding mixtures for pregnant sows, aflatoxin was detected in only one sample in the concentration of 14 µg/kg. Contamination levels were above the maximum limit established by the European Union and Serbian regulation in 7 (14%) samples of complete feeding mixtures for fattening pigs, 4 (21%) samples of complete feeding mixtures for lactating sows and in 7 (25%) samples of complete feeding mixtures for piglets. None of the samples of complete feeding mixtures for pregnant sows contained aflatoxin above the maximum permitted level.

Key words*: aflatoxin, ELISA, feedstuffs, pigs, Serbia*

Introduction

Mycotoxins, as natural food and feed contaminants, became a public health problem of considerable importance. Fungal invasion and subsequent mycotoxins production can occur during plant growth, maturity, harvesting, storage, processing of grains and is influenced by various factors (temperature, relative humidity, oxygen availability, damaged or broken grain kernels) (Bernardo, 2004; Lanyasunya et al., 2005). The Food and Agriculture Organization (FAO) estimates that 25% of the world's food crops are affected by mycotoxins, of which the most notorious are aflatoxins (WHO, 1999). Aflatoxins (AFB1, AFB2, AFG1, AFG2, AFM1, AFM2), a group of potent carcinogenic and teratogenic compounds, are secondary metabolic products of some *Aspergillus spp.: Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius* (Kurtzman et al., 1987; Martins, 1989). Among all aflatoxins, the aflatoxin B1 (AFB1) is the most potent hepatocarcinogenic substance known; recently, after a thorough risk evaluation, it has been proven to be also genotoxic (Van Egmond and Jonker, 2004; Zain, 2011).

Aflatoxins may cause various performance and health problems in pigs, including significant effects on growth performance, decreased average daily gain and feed gain ratio, increased relative weights of liver, kidney, spleen and pancreas, impaired liver function, the increase of serum enzyme activities and the decrease of liver enzyme activities (Shi et al., 2007). Aflatoxins have a severe influence on reproduction in sows which may include endometritis, mammary glands edema and reduced rate of piglets' survival (Liu et al., 2009).

All mammals that ingest AFB1 during lactation excrete aflatoxin M1 in their milk (Wood, 1991), even though its amount depends on many factors. In case of dairy cows, the excreted amount of AFM1 is up to 6.2% of ingested AFB1 (Veldman et al., 1992). It was reported that in case of pigs, an AFB1 conversion index from feed to liver is 800:1 and that the carry-over of AFB1 from sow feed to milk was not known (Stoloff, 1977). Crenshaw (2008) reported the presence of AFM1 in the milk of sows fed diets containing aflatoxins. It was found that pigs nursing sows consuming feed with 500 to 750 ppb of aflatoxin had higher death rates and slower growth. Also, even though pigs were not exposed to aflatoxins after weaning, they were permanently stunted and performance was reduced throughout the growing/finishing period. Bertuzzi et al. (2003) investigated the AFB1 carry-over in lactating sows. Starting from parturition, lactating sows were fed 5 kg of contaminated feed for 7 days. Colostrum and milk samples were collected daily for 10 days after parturition, from each sow. The feed was prepared by adding 5% of a peanut meal naturally contaminated by aflatoxins and contained a mean level of 6.4±0.5 and 0.67±0.05 ppb of AFB1 and AFB2, respectively. The authors concluded that the AFB1 carry-over as AFM1 in the milk was much lower in sows compared to cows (1 - 3%).

To reduce the risk of exposure, many countries have regulated the levels of aflatoxin in feed. Currently, the legal limits of aflatoxin in feedstuffs are highly variable from the European Union (EU) countries to other countries. The EU has a limit of 20 μg/kg for complete feedingstuffs for pigs and poultry (except young animals) and 10 μg/kg for other complete feedingstuffs (European Commission, 2003). In Serbia, proposed maximum permissible levels of aflatoxins in complete and complementary feedingstuffs for pigs and poultry (except young animals) is 20 μg/kg and in complete and complementary feedingstuffs for calves, lambs, kids, piglets, chicks, turkeys, ducklings is 5 μg/kg (the Official Gazette of the Republic of Serbia, 27/2014).

Aflatoxin has long been monitored by the United States Food and Drug Administration, and a level of 20 μg/kg has been set as the limit of aflatoxin content for corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals (United States Food and Drug Administration, 2000). The mentioned regulation also establishes maximum level of aflatoxin in cottonseed meal intended for beef, cattle, swine, or poultry (300 μg/kg), corn and peanut products intended for finishing swine of 100 pounds or greater (200 μg/kg) and corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry (100 μg/kg) (United States Food and Drug Administration, 2000).

The purpose of this study is to give the information on the occurrence of aflatoxin in complete feeding mixtures for different categories of pigs.

Materials and methods

Samples- One hundred and six samples of complete feeding mixtures for different categories of pigs were collected randomly from different farms in Serbia during 2013

year. Immediately after sampling, 1000 g of each sample were prepared by grinding in a laboratory mill in such a way that >93% passed through a sieve with pore diameter of 1.0 mm. After that, the sample was homogenized by mixing. Samples thus prepared were packed in plastic bags and stored in a freezer at -20 ºC until analysis. Prior to each analysis, the samples were allowed to reach room temperature.

Extraction- Exactly 20 g of samples were weighed in a 150 ml beaker. Aflatoxin was extracted with 100 ml of 70% methanol on an Ultra Turrax T18 homogenizer for 3 min at 11,000 rpm. Crude extract was then filtered through 6 Advantec filter paper.

Analysis- The immunochemical analysis was performed using the Veratox, Aflatoxin (Total), Quantitative Test Kit (Neogen, Lansing, MI, USA) with four calibration standard solutions (0, 5, 15 and 50 µg/kg). Analytical procedure was carried out according to manufacturer's procedure. Optical densities on the basis of which aflatoxin content was calculated, were read using the reader of microtitration plates with a 630 nm filter (BioTec Instruments, USA).

Results and discussion

Validation parameters of method for aflatoxin determination were estimated according to the European Commission (2006) and Reason (2003). Limit of detection (LOD) for aflatoxin in feed samples was 0.45 µg/kg, while limit of quantification (LOQ) was 1.37 µg/kg. Average recovery value, based on analysis of certified reference material, was 106.2% which is within acceptable limits according to the European Commission (2006). Precision was estimated in terms of repeatability and reproductivity. Both parameters can be described as "acceptable". Measure uncertainty was calculated in accordance with Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories (Magnusson et al., 2012) and it was 34.55%. The obtained results showed that the proposed analytical method fits well for control purposes of aflatoxin in feed samples.

Table 1. *Presence of aflatoxin in samples of complete feeding mixtures for different categories of pigs*

Feeding mixture	No of samples	No of positive samples (%)	No of samples above maximum permitted level (%)
Fattening pigs	49	21 (43)	7 (14)
Lactating sows	19	7 (37)	4 (21)
Pregnant sows	10	1 (10)	-
Piglets	28	7 (25)	7 (25)
Total	106	36 (34)	14 (13)

The occurrence of aflatoxin was investigated in 106 samples of complete feeding mixtures for different categories of pigs and the results are presented in Table 1 and Table 2. Out of 106 samples, aflatoxin was present in 36 (34%) samples with the average content of 20 µg/kg, ranged from 4 to 66 µg/kg. The highest rate of positive samples (43%) and the greatest concentration of aflatoxin (66 µg/kg) was established in samples of feeding mixtures for fattening pigs. It needs to be pointed out that this was the largest group of samples (nearly half of samples). Despite the smaller number of samples in case of feeding mixtures for lactating sows, the presence of aflatoxin was quite high (37%) with the highest mean value of 27 µg/kg. Somewhat lower incidence was established in samples of

feeding mixtures for piglets (25%) with the average aflatoxin content of 16 μg/kg, while in samples of feeding mixtures for pregnant sows, aflatoxin was found in only one sample.

Regarding legislative in our country and the European Union, contamination levels were above the maximum limit in 7 (14%) samples of complete feeding mixtures for fattening pigs, in 4 (21%) samples of complete feeding mixtures for lactating sows and in 7 (25%) samples of complete feeding mixtures for piglets.

Table 2. *Aflatoxin concentration in samples of complete feeding mixtures for different categories of pigs*

Statistical parameters	Feeding mixture				
	Fattening pigs	Lactating sows	Pregnant sows	Piglets	Total
No of samples	49	19	10	28	106
Average (μg/kg)	19	27	14	16	20
Std. dev. (μg/kg)	16	17	-	19	17
Minimum (μg/kg)	4	9	-	6	4
Maximum (μg/kg)	66	59	-	58	66

During 2012 the presence of aflatoxin was found in most grains in the Republic of Serbia, particularly in maize (Kos et al., 2013a; Jajić et al., 2013b) which may explain the obtained results. Jajić et al. (2013b) reported aflatoxin contamination in 63.6% of the analyzed maize samples with concentration range of 5-367 μg/kg. Somewhat lower contamination (56.4%) was reported by Kos et al. (2013a) with a concentration range of 1-80 μg aflatoxin/kg. All this has contributed to the aflatoxin contamination of compound feed (Jajić et al., 2013a). Extreme aflatoxin contamination of crops and feed seems to be a consequence of favorable weather conditions for Aspergillus mold growth and aflatoxin production. Kos et al. (2013b) compared climatic conditions in period 2009 – 2012 and concluded that very high average temperatures and greater number of days with temperatures higher than 30 °C and 35 °C characterized summer of 2012. According to Republic Hydrometeorological Service of Serbia (2012) drought period (July-August) was characterized with extremely high temperatures and lower amount of precipitation for up to 95% than multiannual average. Kos et al. (2013b) noted that different weather conditions during 2012 in comparison to previous years resulted in presence of aflatoxins in maize.

As for our neighboring countries, aflatoxin contamination was monitored in Romania (Braicu et al., 2008; Tabuc et al., 2010) and Croatia (Šegvić Klarić et al., 2009). Tabuc et al. (2010) analyzed 56 cereal samples (corn, wheat, barley and oats). They determinated the level of fungal contamination and aflatoxin content where Aspergillus species were present in over 80% of the samples and aflatoxin B1 has been identified in almost 30% of the samples, mainly corn with the low concentrations <10 μg/kg. Braicu et al. (2008) investigated forty-three samples of different cereals (wheat, maize, rye and Triticale). It was found that 58.1% of samples were contaminated with different mycotoxins in various concentrations: aflatoxin B1 (1.6–5.7 μg/kg), aflatoxin B2 (0.89–4 μg/kg), aflatoxin G1 (1.2–5.76 μg/kg), aflatoxin G2 (0.96–3.4 μg/kg) and/or 4.3–30 μg/kg ochratoxin A. The concentration of total aflatoxin contamination ranged from 11.2 to 10.8 μg/kg. Among the different cereals, the highest number of contaminated samples was found to be in the wheat samples (62.5%). Šegvić Klarić et al. (2009) analyzed 37 samples of cereals and feed randomly collected in 2007 from households of an endemic nephropathy area in Croatia. The incidence of aflatoxins was 24.3 % with the average value of 4.6 μg/kg.

Conclusion

Based on everything stated above, it can be concluded that the presence of aflatoxin was highest in complete feeding mixtures for fattening pigs (43%), while in complete feeding mixtures for pregnant sows, aflatoxin was detected in only one sample with concentration of 14 µg/kg. This may be explained by smaller amount of maize used for preparation of complete feeding mixtures for pregnant sows. However, there was no information about their actual composition, so this is just a theoretical assumption. Contamination levels were above the maximum limit established by the European Union and Serbian regulations in 14 (13%) samples which can be characterized as quite high contamination. Since Serbian regulations for control of mycotoxins in feed was harmonized with EU regulations in 2014 it can be assumed that in the future there will be more frequent controls of aflatoxins and more data about its occurrence in Serbia.

Acknowledgments

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References

1. Bernardo F 2004. Micotoxicoses: Gestão de Risco. Prova de agregação. Faculdade de Medicina Veterinária, Lisboa, 172 pp.
2. Bertuzzi T, Pietri A, Barbieri G and Piva G 2003. Aflatoxin residues in milk of sows fed a naturally contaminated diet. Italian Journal of Animal Science 2, 234-236.
3. Braicu C, Puia C, Bodoki E and Socaciu C 2008. Screening and quantification of aflatoxins and ochratoxin A in different cereals cultivated in Romania using thin-layer chromatography-densitometry. Journal of Food Quality 31, 108–120.
4. Commission Directive 100/2003 of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. Official Journal of the European Union, L 285, 33-37.
5. Commission Regulation 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of the European Union, L 70, 12-34.
6. Crenshaw M 2008. Mycotoxin in Swine Diets. Retrieved December 17, 2013, from: http://en.engormix.com/MA-pig-industry/health/forums/article-mycotoxin-swine-diets-t4093/165-p0.htm.
7. Jajić I, Krstović S, Perišić B and Glamočić D 2013a. Presence of aflatoxin in complete feeding mixtures for milking cows in Serbia. 23rd International Symposium „New technologies in contemporary animal production”, Novi Sad, Serbia, 28 pp.
8. Jajić I, Perišić B, Krstović S and Kos J 2013b. Aflatoxins – maize safety economy challenge in 2012. Savremena poljoprivreda 62 (3-4), 133-138.
9. Kos J, Janić Hajnal E, Mastilović J, Milovanović I and Kokić B 2013a. The influence of drought on the occurrence of aflatoxins in maize. Proceedings for Natural Sciences Matica Srpska 124, 59-65.

10. Kos J, Mastilović J, Janić Hajnal E and Šarić B 2013b. Natural occurrence of aflatoxins in maize harvested in Serbia during 2009-2012. Food Control 34, 31-34.
11. Kurtzman CB, Horn BWE and Hesseltine CW. 1987. Aspergillus nomius, a new aflatoxin-producing species related to Aspergillus flavus and Aspergillus parasiticus. Antonie van Leeuwenhoek 53, 147-158.
12. Lanyasunya T, Wamae LW, Musa HH, Olowofeso O and Lokwaleput IK 2005. The Risk of Mycotoxins Contamination of Dairy Feed and Milk on Smallholder Dairy Farms in Kenya. Pakistan Journal of Nutrition 4, 162-169.
13. Liu CL, Wang P, Yang YH and Wu WG 2009. Effects of Aflatoxins on Reproduction in Sows. Journal of Hebei North University (Natural Science Edition) 3, 19.
14. Magnusson B, Näykki T, Hovind H and Krysell M 2012. Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories. Nordic Innovation, Oslo, Norway.
15. Martins LM 1989. Capacidade de produção de aflatoxinas por Aspergillus flavus em substratos naturais. Repositório de trabalhos do LNIV 21, 123-132.
16. Pravilnik o kvalitetu hrane za životinje. Službeni glasnik Republike Srbije, 27/2014. Retrieved May 5, 2014, from: http://www.e-glasnik.rs/SlGlasnikPortal/pages/home.xhtml.
17. Reason AJ 2003. Validation of Amino Acid Analysis Methods. In Methods in Molecular Biology: Protein Sequencing Protocols, (eds BJ Smith), pp. 181-194. Humana Press Inc., Totowa, NJ, USA.
18. Republic Hydrometeorological Service of Serbia 2012. Agrometeorološki uslovi u proizvodnoj 2011/2012 godini na teritoriji Republike Srbije.
19. Šegvić Klarić M, Cvetnić Z, Pepeljnjak S and Kosalec I 2009. Co-occurrence of aflatoxins, ochratoxin A, fumonisins, and zearalenone in cereals and feed, determined by competitive direct enzyme-linked immunosorbent assay and thin-layer chromatography. Arhiv za higijenu rada i toksikologiju 60(4), 427-433.
20. Shi YH, Fang LY, Sun Y, Xu ZR and Wang CZ 2007. Effects of aflatoxin on growth performance and liver function of pigs. Journal of Northwest A & F University (Natural Science Edition) 6, 11.
21. Stoloff L 1977. Aflatoxins – an overview. In Mycotoxins in Human and Animal Health (eds JV Rodncks, CW Hesseltine and MA Mehlman), pp. 7-28. Pathotox, Park Forest South, Illinois, USA.
22. Tabuc C, Stroia C and Neacşu A 2010. Incidence of Aspergillus strains and of aflatoxin B1 in cereals in south-western Romania. Agricultural Science Research Journal 42(2), 322-327.
23. United States Food and Drug Administration (FDA). 2000. Retrieved March 12, 2014, from: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm077969.htm.
24. Van Egmond HP and Jonker MA 2004. Worldwide regulations for mycotoxins in food and feed in 2003. The Food and Agriculture Organization of the United Nations (FAO). Food and nutrition paper no. 81.
25. Veldman A, Meijs JAC, Borggreve GJ and Heeres-van der Tol JJ 1992. Carry-over of aflatoxin from cows' food to milk. Journal of Animal Production 55, 163-168.
26. Wood GE 1991. Aflatoxin M1. In Mycotoxins and Phytoalexins (eds RP Sharma and DK Salunkhe), pp. 145-164. CRC Press, Inc., Boca Raton, Florida, USA.
27. World Health Organization 1999. Basic Food Safety for Health Workers. 17-28.
28. Zain ME 2011. Impact of mycotoxins on humans and animals. Journal of Saudi Chemical Society 15, 129–144.

Original paper

RELATIONSHIP BETWEEN ENDOCRINE PROFILE, ENERGY BALANCE AND MILK YIELD IN DAIRY COWS DURING LACTATION

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Abstract

The objective of the present study was to investigate relationship between endocrine profile with energy balance (EB) and milk yield (MY) in Simmental dairy cows during lactation. Fifteen late pregnant cows, 15 early lactation cows and 15 mid lactation cows were chosen for the analysis. Blood samples were collected to measure growth hormone (GH), insulin, triiodothyronine (T3) and thyroxine (T4). Early lactation cows were found to have higher blood serum concentrations of GH ($p<0.05$) and lower blood serum concentrations of insulin ($p>0.05$), T3 ($p<0.05$) and T4 ($p>0.05$) compared to dry and mid lactation cows. Insulin and thyroid hormones were in positive correlation with EB ($p<0.05$), and in negative correlation with MY (nonsignificant). GH was in positive correlation with MY ($p<0.05$), but in negative correlation with EB (nonsignificant). Relationship between hormones showed significant positive correlation between insulin and thyroid hormones. These relations are not principaly determinated by EB or MY. Negative correlations between insulin or thyroid hormones with GH were observed. These relations are principaly determinated by EB or MY.

Key words: *cows, correlation, energy balance, hormones*

Introduction

Negative energy balance in dairy cows during transition period (21 days before and 21 days after calving) leads to many metabolic changes. These metabolic changes occur as a result of the entry of the mammary gland in the metabolic processes. Negative energy balance is the result of decreased food intake, higher consumption of glucose in the udder and decreased insulin sensitivity. These changes are in relation with many endocrine and metabolic adaptations. Characteristic endocrine adaptation is increased concentration of growth hormone (GH), decreased concentration of thyroid hormone (T3 and T4), decreased insulin and IGF-I concentration (Ingvarsten and Andersen, 2000).

Interdependent changes occur in the GH - insulin - IGF-I - glucose signalling pathway in early lactation (Lucy et al., 2001). GH concentration increases at this time; this increase is accompanied by an increase in IGF and IGF binding proteins in mammary secretions, suggesting a role for these factors in mammogenesis and lactogenesis (Tucker, 1994). When cows are in a negative EB, GH stimulates lipolysis; it alters the sensitivity of

adipose tissue to b -adrenergic agents (Bauman and Vernon, 1993). Similarly, plasma concentrations of insulin, another homeorhetic hormone, would be changed by prepartum nutrition and this would affect nutrient supply to the udder. Insulin plays a role in the adaptation of organic matter metabolism in dairy cows during the transitional period and lactation, particularly in terms of nutrient redistribution and partitioning towards the mammary gland (insulin resistance) (Butler et al., 2003; Balogh et al., 2008). Thyroid hormones, primarily triiodothyronine (T3), are important in regulating the energy metabolism. Blood levels of thyroid hormones in peripartal cows decrease, particularly in early lactation, when body reserves are mobilized for high milk production (Bonczek et al., 1988; Tiirats, 1997; Huszenicza et al., 2002). Circulating thyroid hormone concentrations correlate positively with EB and negatively with daily MY in cattle (Blum et al., 1983; Tiirats, 1997; Eppinga et al., 1999; Capuco et al., 2001; Reist et al., 2002; Cassar-Malek et al., 2001).

The objective of the present study was to investigate the nutritional, metabolic and endocrine status in Simmental cows during transition period and mid lactation based on the relationships between blood metabolic hormones, EB and MY**.**

Material and methods

Fifteen late pregnant cows, 15 early lactation cows and 15 mid lactation cows were chosen for the analysis. Blood was sampled from 25 to 1 (13 ± 9) days before partus, in the first month of lactation (16 ± 9 days), and in mid lactation cows between 3 to 5 months of lactation (115 ± 29 days). Blood samples were collected at 10 a.m. by puncture of the jugular vein into sterile disposable test tubes. Blood samples were collected to measure growth hormone (GH), insulin, triiodothyronine (T3) and thyroxine (T4). Serum concentrations of GH, insulin, T3 and T4 were determined by ELISA methods (Endocrine Technologies Inc. CA, USA) using Humareader Single plus (Human, Germany). Diet was suited to the energy requirements of late pregnancy, early and mid lactation cows. Weende methodology was used for the chemical analysis of the feed. Energy balance was calculated by NRC recommendation (2001). Actual energy balance was calculated as a difference between DMI and NEL of the ration offered minus DMI and NEL of the rest of the ration after feeding. Feeding space was provided to each individual cow in order to prevent mixing of their rations. MY was recorded every day by farm software.

Model and statistics: Difference between hormone concentration, EB and MY between three periods of lactation were calculated by ANOVA analysis and posthock LSD test. Relationship between hormones, EB and MY was calculated by Pearson correlation coefficient. Finally, correlation and partial correlation between metabolic parameters were evaluated by Pearson correlation analysis. Partial correlation analysis is used to examine the correlation between endocrine and metabolic parameters with the effects of EB removed. Software used: Statgraphic Centurion, Statpoint Technologies Inc.Warrenton, Va, Virginia, USA.

Results and discussion

Early lactation cows were found to have higher blood serum concentrations of GH ($p<0.05$) and lower blood serum concentrations of insulin ($p>0.05$), T3 ($p<0.05$) and T4 ($p>0.05$) compared to dry and mid lactation cows.

Table 1. *Blood hormones, energy balance and milk yield in late pregnant, early and mid lactation dairy cows (n=15 in each group). Results are expressed as mean ±SD.*

Parameter	Late pregnant cows	Early lactation cows	Mid lactation cows
GH (ng/ml)	11.4 ±8.67 [a]	17.13 ±3.87 [b]	11.45±4.42 [a]
Insulin (ng/ml)	0.55±0.44 [a]	0.39±0.21 [b]	0.65±0.47 [c]
T3 (ng/ml)	0.77± 0.36 [a]	0.73 ±0.41 [b]	1.29± 1.01 [bc]
T4 (ng/ml)	32.70±13.67 [a]	31.93±18.30 [a]	33.06± 17.04 [a]
EB	10.15±9.5	3.56±10.19 [b]	12.15±8.4
MY	/	19.6±5.5	24.8±3.2

Values marked by letters (a, b, c) in one row describe significant differences at level $p< 0.05$ or higher

Insulin and thyroid hormones were in positive correlation with EB ($p<0.05$), and in negative correlation with MY (nonsignificant). GH was in positive correlation with MY ($p<0.05$), but in negative correlation with EB (nonsignificant).

Table 2. *Relationship between hormones, EB and MY.*

Parameter	GH	Insulin	T3	T4
EB	-0.26	0.32*	0.3*	0.31*
MY	0.51**	-0.29	-0.19	-0.17

*p < 0.05; **p < 0.01

Relationship between hormones showed significant positive correlation between insulin and thyroid hormones. These relations are not principaly determinated by EB or MY. Negative correlations between insulin or thyroid hormones with GH were observed. These relations are principaly determinated by EB or MY.

Table 3. *Relationship (correlation coefficients) between hormones in function of energy balance and milk yield.*

Parameter	Insulin	GH
T3	0.35* 0.32* 0.28	-0.21 -0.32* -0.34*
T4	0.37* 0.3* 0.29	-0.26 -0.33* -0.31*
Insulin	/	-0.29 -0.55** -0.33*

[a] correlation between parameters after exclusion of EB or MY; [b] correlation between parameters controlled by energy balance; [c] correlation between parameters controlled by milk yield; *p < 0.05; **p < 0.01

GH is a homeorhetic controller of metabolism, shifting the partitioning of nutrients between the various parts of the body during late pregnancy and lactation (Bonczek et al., 1988; Lucy et al., 2001). The transition and early lactation periods were considered as time periods that have the potential to enhance lactation performance. In the current study, early lactation cows had significantly higher GH levels than late pregnant and mid lactation

cows. GH dramatically increases lipid mobilization from the adipose tissue, and increases blood NEFA and BHB in early lactation cows (Tucker, 1994; Jindal and Ludri, 1994). In this study, GH was significantly positively correlated with MY and NEFA, but negatively with EB and DMI. These correlations have been reported by other authors (Jindal and Ludri, 1994; Balogh et al., 2008) and show that under NEB conditions, blood GH concentration increases what results in fat lipomobilisation, and stimulates MY in dairy cows during lactation. Therefore, lipolysis must be an important pathway to provide the precursors needed in the early postpartum period of cows especially to supply the energy required for milk production (Bonczek et al., 1988; Bauman and Vernon, 1993; Butler et al., 2003).

Insulin has an important homeostatic effect in regulating lipid metabolism. GH inhibits the ability of insulin to initiate lipogenesis in adipose tissue. Accordingly, GH reduces the action of insulin, restricts lipogenic enzyme activity, and reduces glucose utilization (Balogh et al., 2008). Blood insulin levels during the same period were non-significantly lower in early lactation cows than in late-pregnant and mid lactation cows. A significantly positive correlation coefficient between insulin and EB and DMI and a negative but non-significant coefficient with NEFA and BHB were obtained. These relationships were found in earlier studies (Jindal and Ludri, 1994; Xia et al., 2007) and are due to increased fat mobilization during insulin insufficiency. The decrease in blood insulin levels under NEB, reduced DMI and high blood GH values can cause an increase in blood NEFA and BHB levels, suggesting that the reduced anabolic effect of insulin on lipid metabolism leads to sudden uncontrolled mobilization of NEFA from body reserves, and ketogenesis in the liver. Similar results were reported elsewhere (Bonczek et al., 1988; Veenhuizen et al.,1991; Jindal and Ludri, 1994; Butler et al., 2003; Remppis et al., 2011).

Thyroid hormones, particularly T3 whose activity is 4 times greater than that of T4, are of importance in adapting the endocrine system during lactation, since their very low blood levels in transitional cows lead to a decrease in energy metabolism, mobilization of body fat reserves and their partitioning toward high milk production (Tiirats, 1997; Cassar-Malek et al., 2001; Huszenicza et al., 2002). This also involves disorders of metabolic balance and uncontrolled mobilization of lipids which, apart from being used for milk synthesis, very often remain within parenchymatous organs, liver, in particular. Blood levels of T3 and T4 in this experiment were lower in puerperal cows than in late pregnant and mid lactation cows, and exhibited a generally significantly positive correlation with EB and DMI, but a negative non-significant correlation with NEFA and BHB. These findings are consistent with those of other authors (Aceves et al., 1985; Jindal and Ludri, 1994; Tiirats, 1997; Eppinga et al., 1999; Capuco et al., 2001; Huszenicza et al., 2002; Đoković et al., 2007) suggesting that blood levels of thyroid hormones decrease in puerperal cows, particularly in those suffering from metabolic disorders, under marked NEB which involves increased mobilization of NEFA from body reserves.

Conclusion

Endocrine changes during lactation are in relation with energy balance and milk yield. Relationship between insulin and thyroid hormones was not controled by milk yield and energy balance. Contrary to that, negative correlations between insulin or thyroid hormones with growth hormone are principaly determinated by EB or MY.

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References

1. Aceves C, Ruiz A, Romero C and Valverde C 1985. Homeorhesis during early lactation. Euthyroid sick-like syndrome in lactating cows. Acta Endocrinol. (Copenh) 110, 505-509.
2. Balogh O, Szepes O, Kovacs K, Kulcsar M, Reiczigel J, Alcazar JA, Keresztes M, Febel H, Bartyik J, Gy. Fekete S, Fesus L and Huszenicza Gy 2008. Interrelationships of growth hormone Alu I polymorphism, insulin resistance, milk production and reproductive performance in Holstein-Friesian cows. Vet. Med. 53, 604-616.
3. Bauman DE and Vernon RG 1993. Effects of bovine somatotropin on lactation. Ann. Rev. Nutr. 13, 437-461.
4. Blum JW, Kunz P, Leuenberger H, Gautschi K and Keller M 1983. Thyroid hormones, blood plasma metabolites and hematological parameters in relation to milk yield in dairy cows. Anim. Prod. Sci. 36, 93-104.
5. Bonczek RR, Young SW, Wheaton JE and Miller KP 1988. Responses of somatotropin, insulin, prolactin and thyroxine to secretion for milk yield in Holsteins. J.DairySci. 71, 2470-2475.
6. Butler ST, Marr AL, Pelton SH, Radcliff RP, Lucy MC and Butler WR 2003. Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: Effects on expression of IGF-I and GH receptor 1A. J. Endocrinol. 176, 205-217.
7. Capuco AV, Wood DL, Elsasser TH, Kahl S, Erdman RA, Van Tassell CP, Lefcourt A and Piperova LS 2001. Effect of somatotropin on thyroid hormones and cytokines in lactating dairy cows during ad libitum and restricted feed intake. J.Dairy Sci. 84, 2430-2439.
8. Cassar-Malek I, Kahl S, Jurie C and Picard C 2001. Influence of feeding level during postweaning growth on circulating concentrations of thyroid hormones and extrathyroidal 5'-deiodination in steers. J. Anim. Sci. 79, 2679-2687.
9. Djoković R, Šamanc H, Jovanović M and Nikolić Z 2007. Blood concentrations of thyroid hormones and lipids in the liver in dairy cows in transitional period. Acta Vet. Brno 76, 525-532.
10. Eppinga M, Suriyasathaporn W, Kulcsar M, Huszenicza Gy, Wensing T and Dieleman SJ 1999. Tyroxin and triiodothyronine in association with milk yield, βOH-butyrate, and non-esterified fatty acid during the peak of lactation. J. Dairy Sci. 82, 50-56.
11. Huszenicza Gy, Kulcsar M and Rudas P 2002. Clinical endocrinology of thyroid gland function in ruminants: A review of literature. Vet. Med. 47, 191-202.
12. Ingvartsen KL and Andersen HR 2000. Integration of Metabolism and Intake Regulation: A Review Focusing on Periparturient Animals. J.Dairy Sci. 83, 1573-1597.
13. Jindal SK andLudri RS 1994. Relationship between some hormones, metabolites and milk yield in lactating crossbred cows and buffaloes. Asian-Aust J Anim Sci. 7, 239-248.
14. Lucy MC, Hauser SD, Eppard PJ, Krivi GG, Clark JH, Bauman DE and Collier RJ 2001. Variants of somatotropin in cattle: gene frequencies in major dairy breeds and associated milk production. Dom. Anim. Endocrinol. 10, 325-333.
15. Reist M, Erdin D, von Euw D, Tschuemperlin K, Leuenberger H, Delavaud C, Chilliard Y, Hammon HM, Morel C, Philipona C, Zbinden Y, Kuenzi N and Blum JW 2002. Estimation

of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. J. Dairy Sci. 85, 3314-3327.

16. Remppis S, Steingass H, Gruber L and Schenkel H 2011. Effects of energy intake on performance, mobilization and retention of body tissue, and metabolic parameters in dairy cows with special regard to effects of pre-partum nutrition on lactation. Asian-Aust J. Anim. Sci. 24, 540-572.
17. Tiirats T 1997. Thyroxine, triiodothyronine and reverse-triiodothyronine concentrations in blood plasma in relation to lactational stage, milk yield, energy and dietary protein intake in Estonian dairy cows. Acta Vet. Scand. 38, 339-348.
18. Tucker HA 1994. Lactation and its hormonal control. In The Physiology of Reproduction (ed. E. Knobil and J.D. Neill), pp. 2235–2263. Raven Press Ltd, New York.
19. Veenhuizen JJ, Drackley JK, Richard MJ, Sanderson TP, Miller LD and Young JW 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. J.Dairy Sci. 74, 4238-4253.
20. Xia C, Wang Z, Li YF, Niu S, Xu C, Zhang C and Zhang HY 2007. Effect of hypoglycemia on performances, metabolites, and hormones in periparturient dairy cows. Agri Sci China 6, 505-512.

Original paper

EFFECTS OF PHYTOGENIC FEED ADDITIVE AND ENZYME ON GROWTH PERFORMANCE OF BROILERS FED DIETS WITH REDUCED ENERGY CONCENTRATIONS

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Abstract

The effects of dietary supplementation with phytogenic feed additives (PFA) and enzyme (E) on performance parameters were investigated using Ross 308 as hatched broilers fed standard diets or diets with reduced energy concentrations. Birds were assigned to 5 treatments with 5 replications each and fed either a standard basal diet or a re-formulated basal diet with reduced energy concentrations. Reduction was made according to enzyme matrix (Ronozyme WX, DSM). Treatments were: (1) Standard diet; (2) Negative control (NC) – 4% reduction in ME (3) NC + E; (4) NC + PFA (5) NC + E + PFA. Body weight and feed consumption were recorded weekly. Mortality was recorded on daily basis. Foot pad lesions were scored at day 35 using scale from 0 (no lesion) to 2 (lesion extending through skin). The results showed that birds fed Negative control diets had a significantly lower body weights ($P<0.05$) compared to Positive control, Negative control + E and Negative control + E + PFA. Birds fed with Negative control + PFA had higher body weights compared to Negative control (+ 83 g on day 42), but the difference was not significant ($P>0.05$). Mortality and FCR did not differ significantly between treatments. Average foot pad lesion score was the highest in Negative control (1.05) and the lowest in NC+E (0.55).

In conclusion, re-formulation of diets for 4% energy reduction decreased broiler growth rate. Supplementation of diets with PFA improved live weight especially in combination with enzyme, hence confirming a growth-promoting effect of both phytogenics and enzymes in broilers.

Key words: *broilers, enzymes, performance, phytogenic feed additives*

Introduction

Use of antibiotics as growth promoters is no longer acceptable in EU and many other countries. Because of that it is necessary to offer other feed additives as an alternative such as enzymes or phytogenic feed additives which can have a beneficial effect on broiler performance even when added in feed with lower nutrient density. Studies reporting the positive effect of enzymes added to feed with lower energy content are very extensive and numerous authors have established that by application of enzymes production performances can be improved up to 10% (Acamovic, 2001; Cowieson and Ravindran

2008; Montahini et al., 2012). Also in recent years there has been growing interest in plant-derived substances as ingredients or supplements in broiler production. Essential oils contain a number of antimicrobial, antifungal and antioxidative compounds predominantly belonging to the groups of phenols, terpenes or aldehydes. An increasing number of scientific reports is available pertaining to the efficacy of essential oils in broiler production (Windisch et al., 2008; Perić et al., 2009; Wallace et al., 2010). It was hypothesized that, due to a nutrient-sparing effect, dietary supplementation with essential oils might compensate for a reduction in nutrient density in terms of growth performance in broilers.

The objective of this study was to determine the effects of phytogenic feed additives and enzymes on growth performance of broilers fed standard diets or diets with reduced energy concentration.

Materials and methods

The trial was carried out on 1050 Ross 308 broilers which were distributed in 6 groups with 5 replicates. Each replicate consisted of the floor pen with 35 birds per pen. Feed and water supply were *ad libitum* and birds were fed with Starter, Grower and Finisher diets (Table 1). The Positive Control diet was formulated to meet the actual breed standard. The Negative Control diets were formulated according to enzyme matrix. Used enzyme (Ronozyme WX, DSM) was added at level of 200 mg/kg and the phytogenic additive (Digestarom® Poultry) was added at level of 150 mg/kg. Feed was in the mash form for all groups.

Table 1. *Feed composition*

Parmeters	Positive control			Negative control		
Days	1-14	15-28	29-42	1-14	15-28	29-42
Ingredients,%	Starter	Grower	Finisher	Starter	Grower	Finisher
Corn	37.75	37.61	35.57	46.51	42.82	43.81
Wheat	15	20	25	15	20	25
Wheat middlings	6	2.42	5.17	0	0	0
Soybean meal (47% CP)	18.14	17.58	11.83	29.45	26.32	23.25
Full fat soya	18.26	16.91	16.72	4.05	5.29	2.1
Soybean oil	0	1.5	2	0	1.5	2
Threonine L – 98	0.13	0.05	0.04	0.17	0.09	0.08
Lysine	0.27	0.1	0.07	0.33	0.15	0.14
Methionine DL-99	0.12	0.02	0	0.12	0.02	0
Monocalcium phosphate	1.14	0.91	0.75	1.13	0.88	0.73
Limestone	1.61	1.33	1.29	1.64	1.36	1.32
Sodium bicarbonate	0.19	0.12	0.1	0.24	0.15	0.15
Salt	0.2	0.25	0.26	0.16	0.22	0.22
CAPTEX	0.2	0.2	0.2	0.2	0.2	0.2
PREMIX	1	1	1	1	1	1
TOTAL	100	100	100	100	100	100
The chemical composition of mixtures						
Crude protein, %	22.00	21.00	19.00	21.85	20.85	18.83
ME, MJ/kg	12.65	13.20	13.40	12.14	12.69	12.84

Ca, %	1.05	0.90	0.85	1.05	0.90	0.85
P (total), %	0.82	0.75	0.71	0.78	0.72	0.67
P (available), %	0.50	0.45	0.42	0.50	0.45	0.42
Lysine, %	1.43	1.24	1.09	1.42	1.24	1.09
Methionine, %	0.72	0.61	0.57	0.72	0.61	0.56
Methionine + cystine, %	1.07	0.95	0.89	1.06	0.95	0.88

Average body weights per pen were measured weekly. Average daily feed intake per pen was recorded for the feeding periods (1-14 days (starter), 15-28 days (grower) and 29-42 days (finisher)) and for the whole period (1-42 days).

Average feed intake and average body weight per pen are used to calculate the feed conversion ratio (FCR). Mortality was recorded daily during inspection. Birds that died were noted and their bodyweight was used to adjust the FCR accordingly.

Foot pad lesions were recorded at 35 days of age using 4 birds/pen with a 0–2 scoring as:

0 – No lesions

1 – Small lesions affecting skin, no ulcers

2 – Lesions extending through the skin

Data were analyzed by ANOVA followed by Duncan's post hoc test using StatSoft software (STATISTICA 12).The level of significance to indicate differences stated in the ANOVA model are $P<0.05$

Results and discussion

Results of the average body weights of birds per weeks and per treatments are shown in Table 2.

Table 2. *Average body weight of birds, g*

Days	Treatments				
	Positive control	Negative control			
		-	ENZYME	PFA	E + PFA
7	169.5^{a}	156.9^{b}	167.9^{ab}	164.3^{ab}	170.1^{a}
14	437.3^{a}	382.9^{b}	428.2^{a}	422.8^{ab}	418.3^{ab}
28	1158^{ab}	1105^{b}	1187^{ab}	1157^{ab}	1193^{a}
35	1642^{ab}	1543^{b}	1671^{a}	1597^{ab}	1645^{ab}
42	2143^{a}	2008^{b}	2135^{a}	2091^{ab}	2187^{a}

$^{a-c}$ Values between row with no common superscript are significantly different ($P<0.05$)

Average body weights of birds fed Negative control (NC) diets were lower compared to Positive control (PC) and other experimental groups from the beginning of the trial. At the end of the trial (42 days) the difference was statistically significant ($P<0.05$) compared to PC, NC+ E and NC+ E + PFA. Birds fed with NC+ PFA had higher body weights compared to NC (+ 83 g on day 42), but the difference was not significant ($P>0.05$). It is obvious that energy reduction in Negative control group resulted in decreased body weights but the addition of enzyme either individually or in combination with PFA significantly improved final body weight. Addition of PFA without enzyme tended to

improve body weight of birds fed diets with insufficient energy content, but in slightly lower extent compared to combination with enzymes.

Positive effect of enzymes added to feed with lower energy content is reported in many studies (Acamovic, 2001; Montahini et al. 2012; Zou et al., 2013). In the research reported by Cowieson and Ravindran (2008) it is stated that supplementation of both the standard and energy reduced diets with the enzyme improved weight gain and feed efficiency compared with the non-supplemented diets. However, some authors did not report a positive effect of enzymes on body weight of broilers when added into low feed with lower energy content (Iji et al., 2003, Zu et al., 2013). Studies examining the nutrient sparing effect of essential oils are also diverse. Perić et al. (2010) reported that supplementation of the standard diets with essential oils significantly increased ($P<0.05$) body weight of broilers at 42 days of age. Similar results were found by Windisch et al. (2008), Cross et al. (2008) and Bozkurt et al. *(*2012) who reported improved weight gain of broilers fed diets supplemented with essential oils. On the contrary, Buchanan et al. (2008) reported no improvement in final body weight by using a mixture of essential oils.

It has been suggested by many authors (Acamovic, 2001; Cross et al., 2007; Perić et al., 2009) that the results of the trials depend on the differences in the feed composition, level of energy or protein reduction, type and level of used enzyme or phytogenic additive, as well as environmental and management conditions.

Table 3. *Average daily feed consumption, g*

Period, days	Treatments				
	Positive control	Negative control			
		-	ENZYME	PFA	E + PFA
1-14	39.04	38.62	39.67	39.17	38.87
15-28	97.76	95.83	102.11	97.07	101.15
29-42	168.72^{a}	146.93^{c}	154.66^{bc}	155.98^{bc}	164.37^{ab}
Total, 1-42	100.85^{a}	93.89^{b}	98.82^{a}	97.24^{ab}	101.28^{a}

Feeding treatments significantly affected feed consumption. Birds from Negative control group consumed significantly ($P<0.05$) less feed compared to the others, except for the group NC+PFA. However, lower feed consumption had no significant effect on the feed conversion ratio (table 4). The same effect was established in the work of Perić et al. (2011) but Buchanan et al. (2008) reported a reduction in FCR (1.81 vs. 1.84) when a diet with standard nutrient concentration was supplemented with a mixture of essential oils.

Table 4. *Feed conversion ratio*

Period, days	Treatments				
	Positive control	Negative control			
		-	ENZYME	PFA	E + PFA
1-14	1.251^{a}	1.413^{c}	1.296^{b}	1.297^{b}	1.301^{b}
15-28	1.899	1.858	1.884	1.851	1.828
29-42	2.398	2.278	2.290	2.338	2.315
Total, 1-42	1.978	1.965	1.947	1.952	1.945

Mortality rate did not differ significantly between treatments and for all groups it was lower than 5%.

Table 5. *Mortality rate*

Mortality	Treatments				
	Positive control	Negative control			
		-	ENZYME	PFA	ENZ + PFA
No. of birds	6	4	8	5	7
%	3.43	2.29	4.57	2.86	4.00

Average foot pad lesion score was the highest in NC (1.05) and the lowest in NC+E (0.55). That could indicate that addition of enzymes has a positive effect on digesta viscosity and litter condition (Garcia et al., 2008). It is interesting that foot pad lesion score was lower in NC+PFA group compared to NC+E+PFA but the difference was not statistically significant.

Table 6. *Foot pad lesions*

Treatments	No. of birds	No. of birds with lesions			% of birds with lesions			Average score
		0	1	2	0	1	2	
Positive control	20	5	13	2	25	65	10	0.85
Negative control (NC)	20	3	13	4	15	65	20	1.05
NC+ Enzyme	20	9	11	0	45	55	0	0.55
NC +PFA	20	5	11	4	25	55	20	0.95
NC+ Enz+PFA	20	3	13	4	15	65	20	1.05

Conclusion

In conclusion, re-formulation of diets for 4% energy reduction decreased broiler growth rate. Supplementation of diets with PFA improved live weight especially in combination with enzyme, hence confirming a growth-promoting effect of both phytogenics and enzymes in broilers.

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References

1. Acamovic T 2001. Enzymes for poultry. World's Poultry Science Journal 57, 225-242.
2. Bozkurt M, Küçükyılmaz K, Çatlı AU, Çınar M, Çabuk M and Alçiçek A 2012. Effects of administering an essential oil mixture and an organic acid blend separately and combined to diets on broiler performance. Archiv fur Geflugelkunde 76, 81-87.
3. Buchanan NP, Hott JM, Cutlip SE, Rack AL, Asamer A and Moritz JS 2008. The effects of a natural antibiotic alternative and a natural growth promoter feed additive on broiler performance and carcass quality. Journal of Applied Poultry Research 17, 202–210.

4. Cowieson AJ and Ravindran V 2008. Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: growth performance and digestibility of energy, minerals and amino acids. British Poultry Science 49, 37-44.
5. Cross DE, McDevitt RM, Hillman K and Acamovic T 2008. The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. British Poultry Science 48, 496–506.
6. Garcia M, Lazaro R, Latorre MA, Gracia MI and Mateos GG 2008. Influence of enzyme supplementation and heat processing of barley on digestive traits and productive performance of broilers. Poultry Science 87, 940-948.
7. Hernández F, Madrid J, Garcia V, Orengo J and Megías MD 2004. Influence of two plant extracts on broilers performance, digestibility and digestive organ size. Poultry Science 83,169–174.
8. Iji PA, Khumalo S, Slippers RM and Gous R 2003. Intestinal function and body growth of broiler chickens on diets based on maize at different temperatures and supplemented with a microbial enzyme. Reproduction Nutrition Development 43, 77-90.
9. Jamroz D, Wiliczkiewicz A, Wertelecki T, Orda J and Sukorupinska J 2005. Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. British Poultry Science 46, 485-493.
10. Montanhini RN, Ceccantini ML and Fernandes JIM 2012. Productive performance, intestinal morphology and carcass yield of broilers fed conventional and alternative diets containing commercial enzymatic complex. International Journal of Poultry Science 11, 505-516.
11. Perić L, Žikić D and Lukić M 2009. Application of alternative growth promoters in broiler production. Biotechnology in Animal Husbandry 25, 387-397.
12. Perić L, Milošević N, Žikić D, Bjedov S, Cvetković D, Markov S, Mohnl M and Steiner T 2010. Effects of probiotic and phytogenic products on performance, gut morphology and cecal microflora of broiler chickens. Archiv fur Tierzucht 53, 350-359.
13. Wallace RJ, Oleszek W, Franz C, Hahn I, Baser KHC, Mathe A and Teichmann K 2010. Dietary plant bioactives for poultry health and productivity. British Poultry Science 51, 461–487.
14. Windisch W, Schedle K, Plitzner C and Kroismayr A 2008. Use of phytogenic products as feed additives for swine and poultry. Journal of Animal Science 86, 140–148.
15. Zou J, Zheng P, Zhang K, Ding and Bai S 2013. Effects of exogenous enzymes and dietary energy on performance and digestive physiology of broilers. Journal of Animal Science and Biotechnology 4, 14.

Review paper

BLACK PEPPER (*Piper nigrum* L.) AND HOT RED PEPPER (*Capsicum annum* L.) IN BROILER CHICKEN NUTRITION

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Abstract

Antibiotics have been widely used in animal production for decades in past. Although some are used therapeutically to improve the health and well-being of animals, most were given for prophylactic purposes and to improve growth rate and feed conversion ratio efficiency. After the ban of nutritive antibiotic usage in animal nutrition in the European Union, many alternative substances have been investigated for growth promoter potential as their replacement. A large number of experiments have confirmed a wide range of activities of phytoadditives such as black pepper (*Piper nigrum* L.) and hot red pepper (*Capsicum annum* L.) in poultry nutrition for feed intake stimulation, antimicrobial, antioxidative, anticoccidiostatic effects. Besides these effects, black pepper and hot red pepper also have a strong influence on lowering the cholesterol level in edible tissuses as well as on reducing deposition of abdominal fat in carcass. The aim of this review is to present characteristics and effects of phytoadditives, especially black pepper and hot red pepper and their bioactive components in broiler chicken nutrition.

Key words: *black pepper, chickens, nutrition, phytoadditives, red pepper*

Introduction

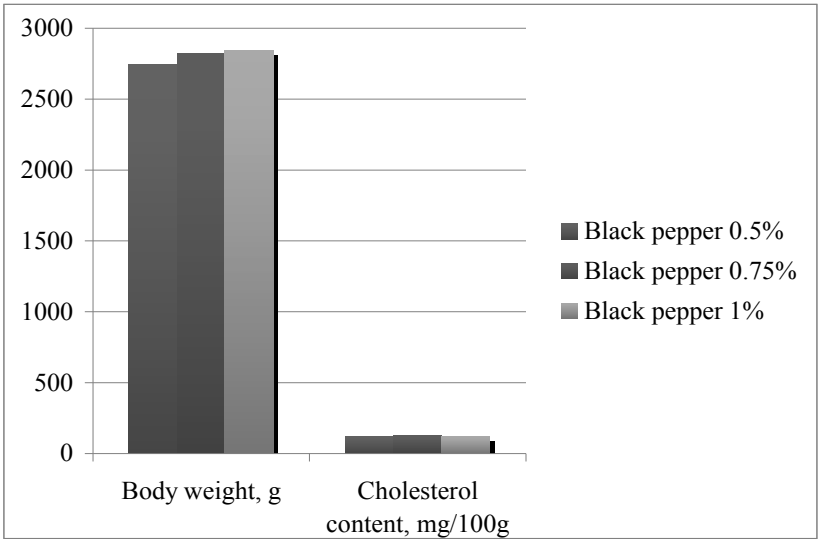
Antibiotics have been widely used in animal production for decades. Although some are used therapeutically to improve the health and well-being of animals, most were given for prophylactic purposes and to improve growth rate and feed conversion ratio efficiency. However, due to the emergence of microbes resistant to antibiotics which are used to treat human and animal infections, the European Commission has banned the use of antibiotics as growth promoters in animal feed. In many countries and in Serbia as well, consumer pressure is pushing the poultry industry to rear animals without antibiotics as growth promoters (Dibner and Richards, 2005; Castanon, 2007). The removal of antibiotics as growth promoters has led to animal performance problems, feed conversion ratio increase and a rise in the incidence of certain animal diseases (Wierup, 2001). The alternatives to antibiotics as growth stimulators are numerous, amounting to finding an adequate non-pharmacological products from the group of prebiotics, probiotics, organic acids, essential oils, medicinal plants or parts of plants such as thyme, basil, oregano, pepper and plenty of others (Simon, 2005; Kostadinović and Lević, 2012; Puvača et al., 2013). Black pepper

(*Piper nigrum* L.) is a flowering vine of Piperaceae family and has been a prized spice in many cultures all over the world. This herb is a known spice which improves digestibility (Moorthy et al., 2009). It is a common medicinal herb used in human diet. The volatile oil of pepper has been shown to have antimicrobial activity (Dorman and Deans, 2000). Black pepper has many medicinal properties for treatment of vertigo, asthma, indigestion, congestion, fever, paralytic, diarrhoea (Ravindran, 2000; Turner, 2004). When used in broiler chicken nutrition it has been found that very small addition of black pepper in the diet, about 0.5 to 1.0%, significantly reduces cholesterol levels in meat (Al-Kassie et al., 2011). Hot red pepper (*Capsicum annum* L.) is one of the most important herbs widely used in human nutrition. Besides its pungent effect, in poultry nutrition it is added in small amounts between 0.25 and 1.0% because it plays an important role in increasing the ability analyser and deposition of cholesterol and fat in the body and contributes to decrease levels of triglycerides and works to support the vascular system in the body. Hot red pepper is also rich in vitamin C which has a considerable impact on improving production through contributing the reduction of heat stress taking into account that poultry consumption of hot red pepper induces a considerable change in energy balance (Yoshioka et al., 2001; Al-Kassie et al., 2012). The aim of this paper is to present the effects of herbal plants such as black pepper and hot red pepper in poultry nutrition as a phytobiotics and possible alternative to antibiotics.

Black pepper (*Piper nigrum* L.) in broiler chicken diets

Black pepper is known as spice due to its pungent quality. Black pepper is a flowering vine in the family Piperaceae, genus piper and species *piper nigrum*. Black pepper is found to improve digestibility (Moorthyet et al., 2009). Pepper efficiency compounds consist of cupsaeesin, cupsisin and cupsantine and some of them can allay rheumatic aches. Piperine is one of the compounds of black pepper which has antiache effect (Mahadyet al., 2008). In addition, the bioactive molecule, piperine, present in pepper, has a major pharmacological impact on nervous and neuromuscular system and it can help in digestion (Great, 2003). Black pepper was found to be rich in glutathione peroxidase and glucose-6-phosphate dehydrogenase while it has been shown that piperine can dramatically increase absorption of selenium, vitamin B complex, beta carotene and curcumin as well as other nutrients (Khalafet et al., 2008; Taziet et al., 2014). Piperine enhances the thermogenesis of lipid and accelerates energy metabolism in the body and also increases the serotonin and β-endorphin production in the brain (Al-Kassieet et al., 2011). Pepper has been found to have antioxidant properties and anticarcinogenic effect, especially when compared to chilli (Naliniet al., 2006). The outer fruit layer contains important odour contributing terpenes, including pinene, sabinene and limonene which give tasty properties. The consumption of these spices exerts several health beneficial effects by the virtue of their innumerable therapeutic potentials in fever, asthma, cold, cough and other general health disorders (Rakesh and Sushil, 2003). Phytogenic feed additives are plant derived products used in animal feeding to improve the performance of agricultural livestock and may satisfy the increasing concerns of consumers, since they prove to be safe and effective. Piperine (1-piperoyl-piperidine) is a major alkaloid component of black pepper (*Piper nigrum* L.) and is responsible for its pungent and biting taste (Dograet et al., 2004). Among its chemical and biological activities, piperine exhibits antimicrobial (Reddy et al., 2004), anti-inflammatory (Pradeep and Kuttan, 2004) and antioxidant (Mittal and Gupta, 2000) properties. It also increases the bioavailability of certain drugs in the organism (Karan et al., 1999) and acts as a chemo preventive factor (Reenet et al., 1997). According to Kohlertet et al. (2000), the active principles of phytogenic additives are absorbed in the

intestine by enterocytes and are quickly metabolized by the body. Piperine induces alterations in membrane dynamics and permeation characteristics, as well as the synthesis of proteins associated with cytoskeletal function, resulting in an increase in the small intestine absorptive surface (Khajuriaet et al., 2002). The rapid metabolism and the shortlife of piperine indicate a low risk of accumulation in the tissue. Cardoso et al. (2009), while working on broiler chickens, found that orally administered piperine did not interfere in weight gain or liver relative weight. However, liver hystopathological changes were observed in a dose dependent manner, indicating that 1.0 mg/kg of piperine, with oral route of administration, is nontoxic for broiler chickens, as previously reported for rats and mice (Dograet et al., 2004; Gaginiet et al., 2010). The effect of feeding broiler chicks on diets containing different levels of black pepper as natural feed additive on productive performance, carcass characteristics and economic efficiency were studied by Taziet et al. (2014). A total of one hundred and sixty, one day old broiler chicks were randomly divided into four experimental groups. Each group was further subdivided into five replicates at the rate of eight chicks per pen in a complete randomized design. The chicks were fed on two basal diets. The black pepper was added to the basal diets at several levels (0.0, 0.5, 0.75 and 1%). The experimental diets were fed for six weeks duration. The result indicated that the group supplemented with black pepper in amount of 1% had significantly ($P<0.05$) highest values for body weight gain, feed intake, dressing, improved feed conversion ratio, and commercial cuts percentages (breast, drumstick and thigh). The birds fed on the control group produced significantly ($P<0.05$) highest abdominal fat percentage. The mortality rate and the percentages of edible giblets (liver, heart and gizzard) were not affected significantly ($P>0.05$) by the addition of the black pepper in broiler diets. Birds fed on the highest level of black pepper (1%) yielded the highest net profit as compared to other experimental groups. A study was also conducted by Al-Kassieet et al. (2011) to determine the performance of broilers fed diets with black pepper. A total of 250 one day old chicks were used. Four levels of black pepper at the rate of 0.25%, 0.50%, 0.75% and 1% were incorporated into the basal diet of broilers for six weeks. The results revealed that the inclusion of black pepper at the levels of 0.50%, 0.75% and 1% in the diets improved body weight gain, feed intake and conversion ratio. At the same time the black pepper of 0.50 %, 0.75% and 1% depressed the cholesterol ratio concentration. It was concluded that the use of black pepper as feed additive at 0.50%, 0.75% and 1% enhanced the overall performance of broiler chicks. Graph 1 shows the effect of different levels of black pepper powder on final body weight of chickens and total cholesterol levels in tissues. Besides being a natural compound that does not produce detected residues in the animal or in their derived products, piperine is easily isolated in great amounts and has shown interesting biological effects in studies on animals.



Graph.1. *Final body weight of chickens fed with black pepper powder and total cholesterol levels in tissues (Al-Kassieet et al., 2011; Akbarinet et al., 2012; Valiollahiet et al., 2013).*

Hot red pepper (*Capsicum annum* L.) in broiler nutrition

It has been proven that some of the phytobiotic components have different active substances (Al-Kassie and Witwit, 2010). In spite of that the poultry do not sense the effect of hot spice, because of the lack of the receptors specific to capsaicin binding (Mason and Maruniak, 1983; Geisthovel et al., 1986), or they have receptors that are insensitive to capsaicin (Szolcsangi, 1976), but it increases the appetite that the addition of hot red pepper to the diet influence on the feed consumption of broilers (Yoshioka et al., 1999). A recent study involved in the poultry performance showed that blends of active compounds of hot red pepper can cause chemo preventive and chemotherapeutic effects. As for hot red pepper the capsaicin is the main active compound responsible for the pungent effects of various species of hot pepper (Jancso et al., 1997) and the main component of hot red pepper including hot taste, capsaicin has been shown to have a protective function in the gastric mucosa as a stimulant of afferent nerve endings. Hot red pepper plays an important role in increasing the ability analyser and deposition of cholesterol and fat in the body and contributes to decreasing levels of triglycerides and acts to support the vascular system in the body. Hencken, (1991) explained that hot red pepper is rich in vitamin C which has a considerable impact on improving production through contributing the reduction of heat stress taking into account that birds consumption of hot red pepper induces a considerable change in energy balance when individuals are given free access to feed (Yoshioka et al., 2001). Although it is well known that plant extracts improve the digestibility of the feeds in broilers, Hernandez et al. (2004) revealed that the effect of different additives on digestibility has slightly improved performance and the differences were not significant. Capsinoids are a family of compounds that are analogues of capsaicin, which is the pungent component in hot red peppers. Capsinoids are widely present at low levels in red pepper fruit, they include capsiate, dihydrocapsiate and have a very favourable safety profile (Kobata et al., 1999). Hot red pepper is known as the herb crucial for stimulating the healing effects of the body organs such as kidney, lungs, stomach and heart. Al-Kassie

et al. (2012) conducted the study to investigate the efficiency of utilization of feed mixture supplemented with hot red pepper to broiler on its productive performance and some haematological traits. A total of 300 one day old chicks were divided into five groups of 60 birds each and were allocated to five feeding treatments, a control group free from any additions, and groups with addition of 0.25, 0.5, 0.75 and 1 % of hot red pepper respectively. The results showed a highly significant ($P<0.05$) average in (live weight gain, feed consumption, feed conversion ratio and dressing percent) with no significant difference in the edible giblet. At the same time the above mixture with addition of 0.75 and 1.0% of hot red pepper depressed the cholesterol ratio concentration. It was concluded that using a mixture as feed additive at levels 0.75 and 1 % enhanced the overall performance of broiler chicks, and improved haematological traits. Shahverdi et al. (2013) conducted the study with objective to determine the effect of the use of red pepper, black pepper and their mixture powder on performance of broiler chicks. Chicks were fed basal diet as control, 0.02% red pepper, 0.02% black pepper and with the mixture of these two powders. Feed intake, body weight gain and feed conversion ratio were determined. Cholesterol, triglyceride, glucose levels and antibody titter against new castle vaccine were investigated. The results revealed that the inclusion of red and black pepper in broiler diet improved body weight gain, feed intake and conversion ratio. In addition, the use of red and black pepper depressed the cholesterol, triglyceride and glucose concentration and decreased H/L ratio concentration in broiler blood plasma ($P<0.05$). Data from the study showed that the use of red and black pepper powder on broiler diets can cause increase in total diameter of small intestine parts ($P<0.05$). It was concluded that the use of red and black pepper as feed additive at 1% enhanced the overall performance of broiler chicks.

Conclusion

Based on the available data it can be concluded that phytobiotics can be used as natural non-antibiotic growth promoters in broiler nutrition. The efficacy of phytobiotic applications in poultry depends on many factors. The most important consideration seems to be the differences in composition of the active components and feed inclusion levels, poultry genetics and overall diet composition. The advancement of knowledge and understanding of the complex poultry gut ecosystem in order to be able to fully explore the precise modes of action of phytogenic compounds represents a clear prerequisite for the design of highly efficacious phytogenic products. From the obtained data and field results it can be concluded that phytobiotics such as black pepper and hot red pepper can be successfully used in poultry nutrition for the improvement of overall productive performances, reduction of cholesterol levels in edible tissues and meat quality improvement. In general, phytobiotics have positive effects but the knowledge of their use in poultry nutrition is still limited and requires further research.

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References

1. Akbarian A, Golian A, Kermanshahi H, Gilani A and Moradi S 2012. Influence of turmeric rhizome and black pepper on blood constituents and performance of broiler chickens. African Journal of Biotechnology 11, 8606-8611.

2. Al-Kassie GAM and Witwit NM 2010. A comparative study on diet supplementation with a mixture of herbal plants and dandelion as a source of prebiotics on the performance of broilers. Pakistan Journal of Nutrition 9, 67-71.
3. Al-Kassie GAM, Al-Nasrawi MAM and Ajeena SJ 2011. Use of black pepper (*Piper nigrum*) as feed additive in broilers diet. Research Opinions in Animal and Veterinary Sciences 1, 169-173.
4. Al-Kassie GAM, Ghassan Y, Butris SJA and Ajeena J 2012. The potency of feed supplemented mixture of hot red pepper and black pepper on the performance and some hematological blood traits in broiler diet. International Journal of Advanced Biological Research 2, 53-57.
5. Cardoso VS, Lima CAR, Lima MEF, Dorneles LEG, Tei Xeira Filho Wl, Lisboa RS, Guedes Junior DS, Direito GM and Danelli MGM 2009. Administração oral de piperina em frangos de corte. Ciência Rural 39, 1521‑1526.
6. Castanon JIR 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poultry Science 86, 2466-2471.
7. Dibner JJ and Richards JD 2005. Antibiotic growth promoters in agriculture: History and mode of action. Poultry Science 84, 634-643.
8. Dorman HJ and Deans SG 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology 88, 308-316.
9. Dogra RKS, Khanna S and Shanker R 2004. Immunotoxicological effects of piperine in mice. Toxicology 196, 229-236.
10. Gagini TB, Silva RE, Castro IS, Soares BA, Lima MEF, Brito MF, Mazur, C, Direito GM and Danelli MGM 2010. Oral administration of piperine for the control of aflatoxin intoxication in rats. Brazilian Journal of Microbiology 41, 345‑348.
11. Geisthovel E, Ludwig O and Simon E 1986. Capsaicin fails to produce disturbances of autonomic heat and cold defense in avain species (Anas platy rhynchos). Pflügers Archiv - European Journal of Physiology 406, 343-350.
12. Great H 2003. Plants and plant extracts for improving animal productivity. Proceedings of Nutritional Society 62, 279-290.
13. Hencken H 1991. Cooling the burn from hot peppers. Journal of the American Medical Association 266, 2766.
14. Hernandez F, Madrid J, Garcia V, Orengo J and Megias MD 2004. Influence of two plant extracts on broiler performance digestibility and digestive organ size. Poultry Science 85, 1466-1471.
15. Jancso G, Kiraly E and Jansco-Gabor A 1997. Pharmacologically induced selective degeneration of chemosensitive primary sensory neurons. Nature 270, 741-743.
16. Karan RS, Bhargava VK and Garg SK 1999. Effect of trikatu, an Ayurvedic prescription, on the pharmacokinetic profile of rifampicin in rabbits. Journal of Ethnopharmacology 64, 259‑264.
17. Khalaf AN, Shakya AK, Al-Othman A, El-Agbar Z and Farah H 2008. Antioxident activity of some common plants. Turkish Journal of Biology 32, 51-55.
18. Khajuria A, Thusu N and Zutshi U 2002. Piperine modulates permeability characteristics of intestine by inducing alterations in membrane dynamics: influence on brush border membrane fluidity, ultrastructure and enzyme kinetics. Phytomedicine 9, 224-231.
19. Kobata K, Todo G, Yazawa S, Iwa I and Watab T 1999. Novel capsacicinoid-like subatances, capsiate and dihydrocapsiate, from the fruits of a nonpungent cultivar, CH-19

sweet of pepper (*Capsicum annuum* L.). Journal of Agricultural Food Chemistry 46, 1695-1697.

20. Kostadinović LJ and Lević J 2012. Use of phytogenic products for pig and broiler diseases. Proceedings of 15th International Feed Technology Symposium, Novi Sad, Serbia, pp, 64-74.
21. Mahady, G.B., Pendl, S.L., Yun, G.S., Lu, Z.Z. and Stoia, A. (2008) Ginger (Zingiber officinale) and the gingerols inhibit the growth of Cag A+ strains of Helicobacter pylori. Anticancer Research 23: 3699-3702.
22. Mason JR and Maruniak JA 1983. Behavioral and physiological effects of capsaicin in red winged blackbirds. Pharmacology Biochemistry and Behavior 19, 857-862.
23. Mittal R and Gupta RL 2000. In vitro antioxidant activity of piperine. Methods and Findings in Experimental and Clinical Pharmacology 122, 271-274.
24. Nalini N, Manju Y and Menon V 2006. Effect of spices on lipid metabolism in 1, 2-dimethylhydrazine-induced rat colon carcinogenesis. Journal of Medicinal Food 9, 237-45.
25. Moorthy M, Ravikumar S, Viswanathan K and Edwin SC 2009. Ginger, pepper and curry leaf powder as feed additives in broiler diet. International Journal of Poultry Science 8, 779-782.
26. Pradeep CR and Kuttan G 2004. Piperine is a potent inhibitor of nuclear factor‐kB (NF‐kB), c‐Fos, CREB, ATF‐2 and pro‐inflammatory cytokine gene expression in B16F‐10 melanoma cells. International Immunopharmacology 4, 1795‐1803.
27. Puvača N, Stanaćev V, Glamočić D, Lević J, Perić L, Stanaćev V and Milić D 2013. Beneficial effects of phytoadditives in broiler nutrition. World's Poultry Science Journal 69, 27-34.
28. Rakesh L and Sushil KK 2003. Bronchial asthma in scientific basis for ayurvedic therapies. Analytical Biochem 47, 389-394.
29. Ravindran PN 2000. Black pepper: Piper nigrum series: Medicinal and aromatic plants. Centre for Medicinal Plant Research, Kerala, India.
30. Reddy SV, Srinivas PV, Praveen B, Kishore KH, Raju BC, Murthy US and Rao JM 2004. Antibacterial constituents from the berries of Piper nigrum. Phytomedicine 11, 697‐700.
31. Reen RK, Wiebel FJ and Singh J 1997. Piperine inhibits aflatoxin B1-induced cytotoxicity and genotoxicity in V79 Chinese hamster cells genetically engineered to express rat cytochrome P4502B1. Journal of Ethnopharmacolology 58, 165-173.
32. Shahverdi A, Kheiri F, Faghani M, Rahimian Y and Rafiee A 2013. The effect of use red pepper (*Capsicum annum* L) and black pepper (*Piper nigrum* L) on performance and hematological parameters of broiler chicks. European Journal of Zoological Research 2, 44-48.
33. Simon O 2005. Micro-organisms as feed additives-probiotics. Advances in Pork Production 39, 161-167.
34. Szolcsangi J 1976. On the specificity of pain producing and sensory neuron blocking effect of capsaicin. Symposium on Analgesics, Akademiai kiad, Budapest, pp, 167-172.
35. Tazi SME, Mukhtar MA, Mohamed K and Tabidi MH 2014. Effect of using black pepper as natural feed additive on performance and carcass quality of broiler chicks. International Journal of Pharmaceutical Research and Analysis 4, 108-113.
36. Turner J 2004. Spice: Black pepper and White pepper. The History of a Temptation. London, Vintage Books.

37. Valiollahi MR, Rahimain Y, Miri Y and Rafiee A 2013. Effect use ginger (*Zingiber officinale*), black pepper (*Piper Nigrum* L) powders on performance, some blood parameters and antibody titer against new castle vaccine on broiler chicks. Scholarly Journal of Agricultural Science 3, 535-540.

38. Yoshioka M, St-Pierre S, Drapeau V, Dionne I, Doucet E, Suzuki M and Tremblay A 1999. Effects of red pepper and caffeine consumption on energy balance in subjects given free access to foods. British Journal of Nutrition 80, 503-510.

39. Yoshioka M, Doucet E, Drapeau V, Dionne I and Tremblay A 2001. Combined effects of red pepper and caffeine consumption on energy balance in subjects given free access to foods. British Journal of Nutrition 85, 203-211.

40. Wierup M 2001. The Swedish experience of the 1986 ban of antimicrobial growth promoters, with special reference to animal health, disease prevention, productivity, and usage of antimicrobials. Microbial Drug Resistance 7, 183–190.

Original paper

LINSEED OIL AS A SOURCE OF POLYUNSATURATED FATTY ACIDS IN BROILER CHICKEN NUTRITION

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Abstract

The aim of the paper was to examine the effect of polyunsaturated fatty acids from linseed oil on productive performances and fatty acid composition of lipids of broiler chicken abdominal fat, as well as correlation and regression between consumed amount of oil and possibilities of their incorporation in the tissues. For the need of experimental research, three groups of 40 day old chicken, hybrid line Cobb 500 were formed. Every group had five replicates, in total amount of 200 chickens per treatment. Control group were fed with diet in addition of 4% soybean oil, while the experimental groups were fed with addition of 4 and 8% of linseed oil in the diet. Addition of linseed oil had a statistically high ($P<0.01$) influence on chicken body weight reduction in treatments T2 and T3, compared to the body weights of chicken in control group T1. Feed conversion ratio in chicken in experimental groups T2 and T3 was 1.84 and 1.82 kg of feed/kg of gain. Introduction of linseed oil in amounts of 4 and 8% in chicken nutrition led to statistically high ($P<0.01$) differences in content of linoleic acid (C18:2) in treatments T2 and T3, compared to control group T1, which leads to a conclusion that linseed oil significantly reduces linoleic acid in tissue. Compared to linoleic acid, content of α-linolenic acid (C18:3) was statistically significantly increased ($P<0.05$) in treatment T2 and highly significantly ($P<0.01$) in treatment T3, compared to control. Negative values of regression (b) and correlation (r) also confirm the fact that introduction of linseed oil in the diet significantly reduces content of linoleic acid in fat tissue of chickens (b=-0.551; r=-0.79), while the same coefficients show positive dependence between added linseed oil in the diet and deposited linolenic acid in tissues (b=1.081 and r=0.87). Based on the gained results it can be concluded that addition of linseed oil in the amount of 4 and 8% could be successfully used in chicken nutrition for fatty acid composition improvement and for lowering the ratio between n-6/n-3 polyunsaturated fatty acid in tissues of broilers.

Key words: *fatty acids, linseed oil, regression, correlation, nutrition, chickens*

Introduction

Poultry production is the fastest way for obtaining high-quality food of animal origin which meets the high demands of modern human nutrition. Improving the quality of meat is reflected among other things in a favorable balance of fatty acids and a reduced content of undesirable and increased participation of desirable fatty acids in muscle lipids of

broilers. For normal metabolic processes in the human body essential fatty acids are necessary (Ryan et al., 2010; Wall et al., 2010). Since body is unable to synthesize them, they have to be inserted in sufficient quantity, usually by consuming food of animal origin. Studies have shown that the composition of lipids in food can significantly affect the fatty acid composition of lipids in chicken meat that can be used freely in the diet of cardiovascular patients and people with high blood cholesterol levels (Adkins and Kelley, 2010; Stanaćev et al., 2013; Puvača et al., 2013). The main sources of polyunsaturated long chain fatty acids are oils of sea and river fish (Ljubojević et al., 2013; Ljubojević et al., 2013a). However, the addition of fish oil in feed for chickens violates the organoleptic characteristics of the product, so we resorted to the enrichment of chicken meat in polyunsaturated fatty acids with 18 carbon atoms by the addition of vegetable oils rich in these acids. Linseed (*Linum usitatissiumum* L.) belongs to this nutrient because it contains oil rich in n-3/n-6 polyunsaturated fatty acid which is used in animal nutrition to improve the fatty acid composition of meat and eggs (Hall et al., 2007; Bassett et al., 2009; Grashorn, 2013) and thus provide a functional food for consumers (Vukelić et al., 2012). Linseed oil contains a high level of α-linolenic acid of the total amount of polyunsaturated fatty acids contained in the oil (Ahmad et al., 2013). Since the poultry is unable to synthesize the same acid it can be successfully incorporated in the edible tissues of the chicks by introducing the linseed oil diet (Shen et al., 2005; Vukmirović et al., 2012). It is well known that the quality of broiler meat is a complex concept that can be evaluated from several aspects. Thus, from the point of slaughter industry and consumer interests it is very important that fattened chickens have a good yield, the preferred conformation, as much meat on the carcass, the optimal distribution of adipose tissue rich in polyunsaturated fatty acids and the appropriate skin color. Representation of certain tissues in more valuable parts of the carcass is an important element that determines the quality of meat (Grashorn and Serini, 2006; Eleroglu et al., 2013; Moujahed and Haddad, 2013). For proper interpretation of the results we used the correlation and regression analysis of the data showing the extent and severity of dependence between variables, which is of great importance when it comes to fatty acids and their possible incorporation into tissue lipids depending on their level in feed mixtures (Vidović, 2009; Vidović, 2013; Lukač and Vidović, 2013).

The aim of this study was to investigate the effect of polyunsaturated fatty acids originating from linseed oil in the diet of broiler chickens on production performance and fatty acid composition of lipids in adipose tissue, as well as correlation and regression dependence between the entered quantity of oil through the diet and the possibility of their incorporation in the tissues of chickens.

Material and methods

Biological tests were carried out under production conditions at the experimental farm "Pustara" in property of the Faculty of Agriculture, Department of Animal Science. At the beginning of experiment, three groups of 40 day-old broiler chickens of Cobb 500 line in five reps on a total of 200 chickens per treatment were formed. For the chicks nutrition three mixtures were used, with 21, 20 and 18% of crude protein (Table 1). The first 14 days, during the preparatory period, the chicks were fed starter mixture. Following the preparation period, the chicks were divided into three groups and fed the next 21 days with Grover mixture, and then the last 7 days of fattening period with finisher mixture. The control group (T1) was fed a standard commercial feed mixture for chicken, while the experimental groups were fed a diet supplemented with 4% (T2) and 8% (T3) of linseed oil. During the experiment, which lasted 42 days, the chicks were fed and watered ad

libitum, and microclimate conditions were regularly monitored. Chickens were on the floor holding system. Control of body weight and feed consumption was performed every 7 days.

Table 1. *The structure and chemical composition of feeds mixtures for broiler chickens, %*

Feedstuffs	Starter	Grover		Finisher	
	T1	T2	T3	T2	T3
	0%	4%	8%	4%	8%
Corn flour	50.00	56.50	44.00	60.60	51.00
Bran	4.00	1.00	6.00	5.00	5.50
Linseed oil	0.00	4.00	8.00	4.00	8.00
Soybean oil	4.00	0.00	0.00	0.00	0.00
Soybean meal	17.40	34.00	33.00	26.00	23.00
Sunflower meal	0.00	0.00	4.50	0.00	8.00
Soybean grits	21.00	0.00	0.00	0.00	0.00
Yeast	3.00	0.00	0.00	0.00	0.00
Monocalcium phosphate	1.50	1.50	1.40	1.50	1.50
Salt	0.30	0.30	0.30	0.25	0.30
Limestone	1.50	1.50	1.50	1.50	1.50
Lysine	0.10	0.00	0.10	0.00	0.00
Methionine	0.20	0.20	0.20	0.15	0.00
Premix	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00
ME, MJ/kg	12.88	13.13	13.59	13.30	13.73

For the purpose of determining the fatty acid composition of lipids, at the end of the experiment, 10 chicks of each group, 5 male and 5 female of an average body weight were sacrificed. After sacrifice, all supporting operation was carried out, and then samples of the abdominal fat were taken for analysis of fatty acid content. For a proper interpretation of the results analysis of variance (ANOVA), LSM and Fisher's LSD post-hoc test were performed and also analysis of the regression coefficient and Pearson's correlation coefficient within the statistical software STATISTICA 12.

Results and discussion

Based on the obtained results it can be concluded that the addition of linseed oil at a concentration of 4 and 8% in the diet of broiler chickens did not statistically significantly ($p>0.05$) affected the achieved body weight of chickens in the first fattening period, until the end of the experiment when was observed a statistically highly significant ($p<0.01$) decrease in body weight of chicks in experimental treatments T2 and T3 compared to the final weights of the chickens on control treatment T1, which amounted to a row 2636.06 g 2648.51 g and 2701.44 g (Table 2).

In their research, Lopez-Ferrer et al. (2001) have come to similar conclusions that the addition of linseed oil in the amount of 2 to 4% in the mixture for feeding broiler chickens affected slightly a depression in the final body weight of chickens, as well as the addition of 8% fats of animal origin. In later studies, addition of linseed oil in the amount of 6% by Leonard et al. (2004) also demonstrated a depressing effect on the body weight of chicks at the end of the experiment. When it comes to the efficiency of feed utilization, it can be

seen that the chickens in the experimental treatment had lower feed conversion ratio, compared to chickens of the control group, which was statistically highly significant difference ($p<0.01$). The achieved conversion ratio of chickens in the control treatment T1, at the end of the sixth week of the experiment amounted to 1.87 kg of feed per kg of gain, while the conversion ratio in the experimental groups T2 and T3 was 1.84 and 1.82 kg feed/kg gain (Table 3).

Table 2. *Body weight of chickens (LSM), g*

Treatments and weeks of experiment	Control (T1) 0%		Linseed oil (T2) 4%			Linseed oil (T3) 8%		
	LSM	SE_{Lsm}	LSM	SE_{Lsm}	p	LSM	SE_{Lsm}	p
End of preparatory period	468.00	5.91	468.12	5.91	0.98^{ns}	468.00	5.91	1.00^{ns}
End of III week	994.72	10.04	970.33	10.04	0.75^{ns}	984.44	10.04	0.25^{ns}
End of IV week	1552.11	18.49	1599.11	18.49	0.00**	1607.33	18.49	0.00**
End of V week	2139.57	18.80	2164.78	18.86	0.00**	2082.17	18.31	0.06^{ns}
End of VI week	2701.44	28.48	2636.06	28.56	0.00**	2648.51	27.73	0.00**

$p<0.05$ - *; $p<0.01$ - **; $p>0.05$ - ^{ns}

Table 3. *Feed conversion ratio ($\bar{x}$), kg feed/kg gai*n.

Treatments and weeks of experiment	Control (T1) 0%	Linseed oil (T2) 4%		Linseed oil (T3) 8%	
	$\bar{x}$	$\bar{x}$	p	$\bar{x}$	p
End of preparatory period	1.34	1.29	0.00**	1.34	0.76^{ns}
End of III week	1.37	1.39	0.02*	1.42	0.00**
End of IV week	1.48	1.41	0.00**	1.49	0.04*
End of V week	1.62	1.59	0.00**	1.68	0.00**
End of VI week	1.87	1.84	0.00**	1.82	0.00**

$p<0.05$ - *; $p<0.01$ - **; $p>0.05$ - ^{ns}

By analyzing the fatty acid composition of the linseed oil lipids and adipose tissue lipids using gas chromatography, 6 to 11 fatty acids in total were detected (Table 4 and 5).

Table 4. *Fatty acid composition of linseed oil (LSM), g/100 g*

Oil source	Fatty acids											
	C16:0		C18:0		C18:1		C18:2		C18:3		C20:0	
Lan	LSM	SE_{Lsm}	LSM	SE_{Lsm}	LSM	SE_{Lsm}	LSM	SE_{Lsm}	LSM	SE_{Lsm}	LSM	SE_{Lsm}
	2.63	28.3	4.94	21.4	19.97	14.5	16.74	26.8	55.53	15.9	0.18	18.3

From the results shown in Table 4 it can be seen that the most common member of the oil is α-linolenic acid (C18: 3 n-3), with 55.53%, followed by oleic (C18: 1 n-9) to 19.97%, and linoleic acid (C18: 2 n-6) to 16.74%.

Table 5. *Fatty acid composition of abdominal fat (LSM), g/100 g*

Fatty acids		Control (T1) 0%		Linseed oil (T2) 4%			Linseed oil (T3) 8%		
		LSM	SE_{Lsm}	LSM	SE_{Lsm}	p	LSM	SE_{Lsm}	p
myristic	C14:0	0.04	0.01	0.06	0.01	0.27^{ns}	0.06	0.01	0.31^{ns}
palmitic	C16:0	17.74	0.38	18.39	0.38	0.26^{ns}	16.35	0.38	0.02*
palmitoleic	C16:1	3.27	0.25	3.83	0.25	0.14^{ns}	3.45	0.25	0.61^{ns}
stearic	C18:0	5.23	0.17	5.06	0.17	0.52^{ns}	4.71	0.17	0.06^{ns}
oleic	C18:1	35.45	1.01	35.72	1.01	0.85^{ns}	34.36	1.01	0.46^{ns}
linoleic	C18:2	29.22	0.55	25.13	0.55	0.00**	24.80	0.55	0.00**
linolenic	C18:3	5.88	0.96	9.61	0.96	0.01*	14.53	0.96	0.00**
arachidic	C20:0	0.07	0.02	0.07	0.02	0.97^{ns}	0.08	0.02	0.73^{ns}
gadoleinic	C20:1	0.45	0.06	0.42	0.06	0.70^{ns}	0.32	0.06	0.20^{ns}
behenic	C22:0	0.06	0.02	0.01	0.02	0.18^{ns}	0.08	0.02	0.52^{ns}
lignoceric	C24:0	0.00	0.00	0.00	0.00	0.24^{ns}	0.00	0.00	0.24^{ns}

$p<0.05$ - *; $p<0.01$ - **; $p>0.05$ - ^{ns}

In Table 5, the results of fatty acid composition of abdominal fat show statistically significant and highly significant differences in the content of the individual fatty acids in the experimental treatment compared to the control treatment. Introduction of linseed oil at concentrations of 4 and 8% in the diet of chickens has led to a statistically significant difference ($p<0.01$) in the content of linoleic acid (C18:2) in the treatment T2 (25.13 g/100 g), and T3 (24.80 g/100 g) compared to the control treatment T1 (29.22 g/100 g), what indicates a significant reduction of linoleic acid in the tissue. The content of α-linolenic acid (C18:3) was significantly increased ($p<0.05$) in the treatment T2 (9.61 g/100 g), and highly significantly ($p<0.01$) in the treatment T3 (14.53 g/100 g) in response than the content of α-linolenic acid in the chickens tissue of treatment T1, which amounted to 5.88 g/100 g. From the results shown in the same table it can be seen that the addition of linseed oil at both concentrations was not statistically significant ($p>0.05$) affected on the presence of oleic acid (C18:1). The research of Nyquist et al. (2013) with the linseed oil in the diet of chickens showed an increased content of n-3 EPA, DPA and DHA and reduced content of arachidonic acid (AA) of the n-6 family of fatty acids. These authors noted that the relationship between AA/EPA to 19/1 in the group with addition of the soybean oil in the mixture decreased to 1.7/1 in the treatment of chickens with the linseed oil in the feed. A number of authors (Bou et al., 2005; Haug et al., 2007; Smink et al., 2008; Wongsuthavas et al., 2011) in their studies with the addition of linseed oil to feed for chickens came to similar results with increasing concentrations of α -linolenic acid, EPA, DPA and DHA in comparison with the group with addition of the soybean oil, which had higher concentrations of linoleic and arachidonic acid in the tissues.

In the case of regression and correlation coefficients, from the results in Table 6, it can be observed dependence with statistically significant differences.

By the means of the regression coefficient and Pierson correlation coefficient we came to the conclusion that there is a positive and a negative correlation between the added linseed oil to the chicken diet and deposit of fatty acids in lipids. The negative value of the

regression (b) and correlation (r) show that the introduction of linseed oil statistically ($p<0.01$) significantly affected the content of linoleic acid in adipose tissue of chickens (b=-0.551, r=-0.79), while the regression coefficients and correlations show a positive correlation between the added linseed oil to diet and deposited α-linolenic acid in the tissues (b=1.081 and r=0.87) with a statistically high significance ($p<0.01$). Table 7 also shows the regression equation which shows, or predicts, how the content of certain fatty acids in adipose tissue of chickens will increase or decrease by a certain amount of linseed oil (x) supplement in the diet mixture.

Table 6. *The coefficient of regression and correlation for the observed properties of fatty acid composition*

Fatty acids		a	b	The regression equation	r	r^2	p
myristic	C14:0	0.048	0.002	y=0.048+0.002 x	0.28	0.08	0.30^{ns}
palmitic	C16:0	18.196	-0.174	y=18.196-0.174 x	-0.49	0.25	0.06^{ns}
palmitoleic	C16:1	3.431	0.023	y=3.431+0.023 x	0.14	0.02	0.63^{ns}
stearic	C18:0	5.262	-0.064	y=5.262-0.064 x	-0.51	0.26	0.05^{ns}
oleic	C18:1	35.723	-0.135	y=35.723-0.135 x	-0.21	0.04	0.45^{ns}
linoleic	C18:2	28.595	-0.551	y=28.595-0.551 x	-0.79	0.62	0.00**
linolenic	C18:3	5.686	1.081	y=5.686+1.081 x	0.87	0.77	0.00**
arachidic	C20:0	0.071	0.001	y=0.071+0.001 x	0.09	0.01	0.72^{ns}
gadoleinic	C20:1	0.467	-0.016	y=0.467-0.016 x	-0.36	0.13	0.19^{ns}
behenic	C22:0	0.043	0.003	y=0.043+0.003 x	0.16	0.02	0.57^{ns}
lignoceric	C24:0	0.005	-0.000	y=0.005-0.000 x	-0.33	0.11	0.23^{ns}

$p<0.05$ - *; $p<0.01$ - **; $p>0.05$ – ^{ns}; x – variable amount of oil

Conclusion

Based on the obtained results it can be concluded that the introduction of linseed oil in the amounts of 4% and 8% had mild depressant effect on the final body mass of fattening chickens and a positive effect on the reduction of feed conversion ratio. Regarding the fatty acid composition of lipids in adipose tissue, it can be concluded that the introduction of the oil in diet affects highly significantly the increased incorporation of n-3 polyunsaturated fatty acids, in particular α-linolenic fatty acids, and highly significantly the reduction of the content of n-6 fatty acid, linoleic acid. From the obtained results it can be concluded that linseed oil in the amount of 4% and 8% can be successfully used in the diet of broiler chickens to improve fatty acid composition of edible tissues of chickens and balance the ratio of n-6/n-3 polyunsaturated fatty acids, which would also improve products and make them suitable in cardiovascular patients nutrition and in the prevention of atherosclerosis and other diseases related to disorders of the digestive tract. Also, the application of these regression and corelation models may be one of the practical solutions on the ground in the formulation of diet mixtures for poultry when it comes to improving the fatty acid composition of the final product.

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References

1. Adkins Y and Kelley DS 2010. Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. Journal of Nutritional Biochemisty 21, 781-792.
2. Ahmad S, Yousaf M, Kamran Z and Sohail MU 2013. Effect of feeding whole linseed as a source of polyunsaturated fatty acids on performance and egg characteristics of laying hens kept at high ambient temperature. Revista Brasileira de Ciência Avícola 15, 21-25.
3. Bassett CMC, McCullough RS, Edel AL, Maddaford TG, Dibrov E, Blackwood D.P, Austria JA and Pierce GN 2009. Trans-Fatty acids in the diet stimulate atherosclerosis. Metabolism 58, 1802-1808.
4. Bou R, Guardiola F, Barroeta AC and Codony R 2005. Effect of dietary fat sources and zinc and selenium supplements on the composition and consumer acceptability of chicken meat. Poultry Science 84, 1129-1140.
5. Eleroglu H, Yıldırım A, Isıkl ND, Şekeroglu A and Duman M 2013. Comparison of meat quality and fatty acid profile in slow-growing chicken genotypes fed diets supplemented with Origanum vulgare or Melissa officinalis leaves under the organic system. Italian Journal of Animal Science 12, 64-70.
6. Grashorn MA 2013. Functional eggs and poultry meat – A niche production or a future chance? Proceedings of International Symposium 23, 1-3.
7. Grashorn MA and Serini C 2006. Quality of chicken meat from conventional and organic poultry. Proceedings of European Poultry Conference 12, 268-273.
8. Hall MN, Campos H, Li H, Sesso HD, Stampfer MJ, Willett WC and Ma J 2007. Blood levels of long-chain polyunsaturated fatty acids, aspirin, and the risk of colorectal cancer. Cancer Epidemiology, Biomarkers and Prevention 16, 314-321.
9. Haug A, Eich-Greatorex S, Bernhoft A, Wold JP, Hetland H, Christophersen O.A and Sogn T. Effect of dietary selenium and omega-3 fatty acids on muscle composition and quality in broilers. Lipids in Health and Diseases 6, 29-37.
10. Leonard AE, Pereira SL, Sprecher H and Huang YS 2004. Elongation of long-chain fatty acids. Progress in Lipid Research 43, 36–54.
11. Lopez-Ferrer S, Baucells MD, Barroeta AC and Grashorn MA 1999. n-3 enrichment of chicken meat using fish oil: alternative substitution with rapeseed and linseed oils. Poultry Science 78, 356–365.
12. Lopez-Ferrer S, Baucells MD, Barroeta AC, Galobrat J and Grashorn MA 2001. n-3 enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated fatty acids: Linseed oil. Poultry Science 80, 753-761.
13. Lukač D and Vidović V 2013. Parameters of genetic and phenotypic type in pigs mating in pure breed and crossbreeding on litter size. African Journal of Agricultural Research 8, 4664-4669.
14. Ljubojević D, Ćirković M, Đorđević V, Puvača N, Trbović D, Vukadinov J and Plavša N 2013. Fat quality of marketable fresh water fish species in the Republic of Serbia. Czech Journal of Food Science 31, 445-450.
15. Ljubojević D, Ćirković M, Novakov N, Jovanović R, Janković S, Đorđević V and Mašić Z, Productivity and meat nutrient in fish: the diet effect. Kafkas Universitesi Veteriner Fakultesi Dergisi 19, 43-49.
16. Moujahed AR and Haddad B 2013. Performance, livability, carcass yield and meat quality of tunisian local poultry and fast-growing genotype (Arbor Acres) fed standard diet and raised outdoor access. Journal of Animal Science Advances 3, 75-85.

17. Nyquist NF, Rødbotten R, Thomassen M and Haug A 2013. Chicken meat nutritional value when feeding red palm oil, palm oil or rendered animal fat in combinations with linseed oil, rapeseed oil and two levels of selenium. Lipids in Health and Diseases, 12, 69-82.

18. Puvača N, Stanaćev S.V, Stanaćev Ž.V, Đukić-Stojčić M, Milić D and Beuković D 2013. Fatty acid composition of edible chicken tissues enriched with fatty acids from linseed. Proceedings of International Symposium 23, 201-203.

19. Ryan AS, Astwood JD, Gautier S, Kuratko CN, Nelson EB and Salem NJ 2010. Effects of long-chain polyunsaturated fatty acid supplementation on neurodevelopment in childhood: a review of human studies. Prostaglandins Leukotrienes and Essential Fatty Acids 82, 305-314.

20. Salamatdoustnobar R 2010. Turkey breast meat change of EPA and DHA fatty acids content during fed canola oil. Global Veterinary 5, 264-267.

21. Shen Y, Feng D, Oresanya T and Chavez E 2005. Fatty acid and nitrogen utilization of processed flaxseed by adult chickens. Journal of the Science of Food and Agriculture 85, 1137-1142.

22. Smink W, Gerrits WJJ, Hovenier R, Geelen MJH, Lobee HWJ, Verstegen MWA and Beynen AC 2008. Fatty acid digestion and deposition in broiler chickens fed diets containing either native or randomized palm oil. Poultry Science 87, 506-513.

23. Stanaćev SV, Stanaćev ŽV, Milošević N, Puvača N, Milić D and Beuković D 2013. Effect of different oil sources in broiler chicken nutrition. Proceedings of International Symposium 23, 229-231.

24. Stanaćev V, Kovčin S, Stanaćev V, Pucarević M and Puvača N 2011. Extruded canola seed in improving chicken fattening and fatty acid composition. Kuwait Journal of Science and Enginering 38, 71-80.

25. Tres-Oliver A, Nuchi CD, Magrinya-Navarro N, Guardiola-Ibarz F, Bou-Novensa R and Codony-Salcedo R 2012. Use of palm-oil by-products in chicken and rabbit feeds: effect on the fatty acid and tocol composition of meat, liver and plasma. Animal 6, 1005-1017.

26. Vidović V 2013. Linear models in animal breeding (In Serbian). Monograph, Stylos, Novi Sad (Serbia).

27. Vidović V 2009. Principals and methods of animal breeding (In Serbian). University book, Faculty of Agriculture, Novi Sad (Serbia).

28. Vukelić N, Živković J, Okanović Đ and Puvača N 2012. Consumer perception of animal feed in relation to food safety. Proceedings of International Feed Technology Symposium 15, 22-23.

29. Vukmirović Đ, Ivanov D, Čolović R, Lević J, Jovanović R, Đuragić O and Spasevski N, Improving the nutritive value of chicken meat by the addition of kompo omega sun as functional component enriched with linseed oil. Proceedings of International Feed Technology Symposium 15, 238-245.

30. Wall R, Ross RP, Fitzgerald GF and Stanton C 2010. Fatty acids from fish: the anti-inflammatory potential of long-chain omega fatty acids. Nutre Review 68, 280-289.

31. Wongsuthavas S, Yuangklang C, Vasupen K, Mitchaotha J, Alhaidary A, Mohamed HE and Beynen AC 2011. Fatty acid metabolism in broiler chickens fed diets either rich in linoleic or alpha-linolenic acid. Asian Journal of Animal and Veterinary Advances 6, 282-289.

Review paper

SUPPLEMENTARY FEEDING OF GRAZING DAIRY COWS

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Abstract

The objective of this paper was to consider the effect of supplementary feeding of grazing dairy cows on dry matter intake (DMI), milk production and milk composition. Accurate estimations of total DMI and pasture DMI are important to the management of dairy grazing systems. The intakes of dry matter (DM) and net energy-NE_L are lower on the pasture-only diet compared with cows supplementary fed with concentrate. Many pasture factors affect DMI, including pregrazing pasture mass and pasture allowance. Milk production of high producing grazing dairy cows in early lactation increases linearly as the amount of concentrate increases to 10 kg DM/day with a milk response of 1 kg milk/kg concentrate. In late lactation, increases are with a lower milk response per kilogram of supplemented concentrate. With the amount of concentrate supplementation, milk fat and protein yield increase while milk fat percentage decreases. Supplementation with rumen undegradable protein (RUP) is important for meeting requirements of grazing dairy cows, because the pasture has high ruminal crude protein (CP) degradability. Corn silage supplementation to grazing cows may increase milk production if pasture offered is restricted, but if pasture is offered ad libitum milk production does not change or can decrease. Supplementation of ruminally inert fat could have positive effect on milk production with concentrate supplemented at a lower rate.

Key words: *cows, intake, pasture, performances*

Introduction

The use of pasture for dairy cows is considered as lower-cost feeding system because grazed forage is the cheapest source of nutrients. Milk production per cow is usually lower on grazing-based than on confinement-based farms. Milk production of grazing cows is limited by the inability to consume enough pasture DM to meet the nutrient requirements for higher milk production, or an imbalance of rumen fermentable carbohydrate and rumen degradable protein-RDP (Stojanović et al., 2006a; Stojanović et al., 2009). The duodenal protein supply of a dairy cow grazing pasture is adequate to meet the production requirements of approximately 25 kg of milk per day (Reis and Combs, 2000). Supplementation-concentrate as a source of starch provides the energy for more efficient utilization of the RDP in pasture (Grubić and Adamović, 2003; Stojanović et al., 2007).

According to Clark and Kanneganti (1998) pasture used for dairy cows is characterized as high quality pasture with the content of 18 to 24% DM, 18 to 25% CP, 40 to 50% NDF, and 6.40 to 7.0 MJ/kg DM of NE_L. The main objective of supplementation of grazing dairy cows is to increase total DMI and energy intake relative to that achieved with pasture-only

diets (Stojanović et al., 2004). Supplemental nutrition of grazing cows improves the use of pasture with the possible higher stocking rate per unit of land, increases milk production per cow, improves body condition score-BCS and reproduction performances of grazing cows (Kellaway and Porta, 1993).

Appropriate strategies for supplementation of high producing dairy cows require an understanding of the effect of different types of supplements on DMI, digestion and animal performance and imply providing nutrients that complement the nutrient content of pasture and meet the nutrient requirements of dairy cows (Stojanović et al., 2006).

The objective of this study was to consider the impact of supplemental concentrate and forage nutrition of grazing cows on production performances.

Dry matter intake of grazing cows

Low pasture DMI is identified as a major factor limiting milk production of high producing cows with a grazing system (Grubić et al., 2003). Mayne and Wright (1988) reported that pasture DMI of high yielding dairy cows might reach 3.25-3.5% of BW, with no pasture quantity and quality restrictions. The intake of DM and NE_L is lower on the pasture-only diet compared with cows fed total mixed ration (TMR), (Kolver and Muller, 1998). Total DMI of high producing cows fed pasture-only diets is lower than for cows fed pasture diets plus concentrates. This may be explained by physical constraints, rate of forage removal from the rumen, and water consumption associated with pasture.

Many pasture factors affect DMI, including pregrazing pasture mass (kgDM/ha) and pasture allowance - PA (amount of pasture offered per cow, kg DM/cow/day). Over a range of PA from 20 to 70 kg DM/cow/day, pasture DMI increased 0.19 kg/kg of increased PA (Bargo et al. 2003). In order to maximize pasture DMI of high producing dairy cows, unrestricted pasture quality and quantity must be ensured. Unrestricted pasture conditions (high PA) also imply low pasture utilization (pasture DMI/PA < 50%). The use of very high PA might result in deterioration of pasture quality because of the increase in residual pasture height. Even under unrestrictive pasture conditions, total DMI of high producing dairy cows is lower compared with cows consuming TMR or pasture plus supplements (Bargo et al., 2002). Because of low pasture utilization and deterioration of pasture quality at high PA, a practical recommendation is to provide a PA of 2 times the expected pasture DMI or 25 kg DM/cow/day when cows are fed supplements (Bargo et al., 2002). Grazing cows are characterized by low grazing efficiencies - 45.5% of the pasture allowance (Vazquez and Smith, 2000).

Regression equations for prediction of pasture DMI (PDMI) for grazing dairy cows:

PDMI, kg/day = 4.47 + 0.14 * FCM + 0.024 * BW + 2.00 * CBW + 0.04 * PA + 0.022 * PASUP − 0.90* SUP − 0.13* NDFp − 0.037 * LEG, (Vazquez and Smith 2000)

FCM - 4% fat corrected milk (kg/day), BW – body weight (kg), CBW - change in BW (kg/day), PA – pasture allowance (kgDM/cow/day), PASUP - pasture allowance and total supplementation interaction (PA * SUP), SUP - total supplementation (kg DM), NDFp - NDF in pasture available (% DM), LEG - percentage of legumes in pasture (%);

PDMI = –0.61 + 0.981 * PA + 0.479 * CI – 0.039 * (PA * CI) – 0.014 * PA^2, (Meijs and Hoesktra, 1984)

PA – pasture allowance (kg DM/cow/day), CI – intake of concentrates (kg/day);

For low pasture allowance, NDF intake as a percentage of the BW is 1.2%, whereas for high PA, intake of NDF is significantly higher than 1.3% (1.5% of BW in grazing dairy cows consuming only pasture), (Kolver and Muller, 1998).

Arriaga-Jordan and Holmes (1986) reported that concentrate supplementation of dairy cows reduced grazing time 11 min/kg of concentrate in continuous grazing and 8 min/kg of concentrate in rotational grazing. When the amount of concentrate was increased from 5 to 10 kg/day, grazing time decreased 16 and 20 min/kg of fiber-based or starch-based concentrate, respectively (Sayers, 1999). Bargo et al. (2002) reported that supplementation with 7.9 kg/day of a corn-based concentrate reduced grazing time by 75 min/day at low PA and by 104 min/day at high PA.

Effect of supplementation on production performances of grazing dairy cows

Supplementary feeding of grazing cows decreases pasture DMI while increases total DMI. Decreasing of pasture DMI is indicated by value of substitution rate – SR. Substitution rate is defined as the decrease in pasture intake per kilogram of supplemental feed.

SR (kg pasture DM/kg supplement DM) = (pasture DMI in unsupplemented grazing cows, kg - pasture DMI in supplemented grazing cows, kg) / supplement DM, kg

A substitution rate < 1 kg/kg means that total DMI of the supplemented grazing cows is higher than total DMI of the unsupplemented grazing cows. Bargo et al. (2003) reported that decreasing of pasture DMI was 13%, when pasture was supplemented with 1.8 to 10.4 kgDM of concentrate, while the increasing of total DMI was 24%. Milk response – MR (kg of additional milk/kg supplement) is one of the main indicators that determine whether supplementation is profitable.

The type of supplement influences substitution rate and milk yield and response. Supplementation with forages, such as hay or corn silage, decreases pasture DMI more than concentrates and results in higher SR than supplementation with concentrates. Mayne and Wright (1988) found SR ranged from 0.84 to 1.02 kg/kg for grass silage supplementation and from 0.11 to 0.50 kg/kg for concentrate supplementation. Meijs (1984) reported that SR was reduced from 0.45 kg pasture/kg high-starch concentrate to 0.21 kg pasture/kg fiber-based concentrate when cows grazed a ryegrass pasture.

There is usually a negative relationship between substitution rate and milk response, indicating that the lower SR the higher MR is expected. At high producing grazing dairy cows with increasing of pasture allowance, substitution rate also increased and milk response decreased. Bargo et al. (2002) in study with dairy cows (101 days in milk – DIM, milk yield of 45.8 kg/day) grazed orchardgrass (*Dactylis glomerata*) with corn as supplement (7.9 kg/day), with pasture allowance 25 and 40 kg DM/cow/day, reported SR as 0.26 and 0.55 kg pasture/kg concentrate, and MR as 1.36 and 0.96 kg milk/kg concentrate, respectively. Robaina et al. (1998) in the research on grazing dairy cows (pasture: perennial ryegrass - *Lolium perenne* and white clover - *Trifolium repens*) 180 DIM and yielding 20.5 kg milk/day, with 4.3 kg/day concentrate (barley/lupin) and pasture

allowance 21.1 and 42.3 kg DM/cow/day, found that SR was 0.31 and 0.57 kg pasture/kg concentrate, and MR was 0.98 and 0.54 kg milk/kg concentrate, respectively. Higher substitution rate found with higher pasture allowance may be partially explained by the higher quality of pasture actually consumed, because cows have the opportunity to be more selective.

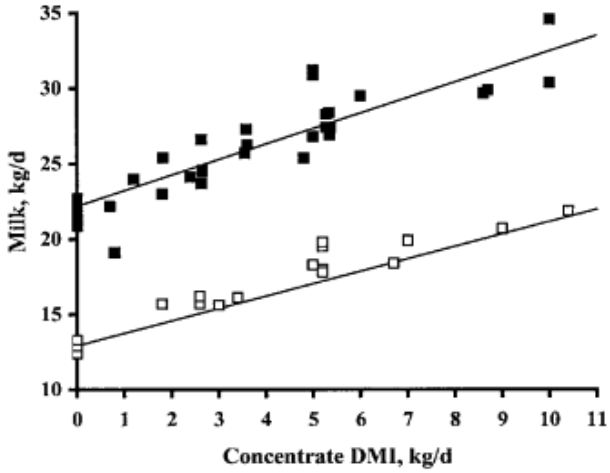

Figure 1. *Relationship between milk production and concentrate DMI by grazing dairy cows supplemented with different amounts of concentrate: ■-studies with cows < 90 DIM or > 28 kg milk/day; □-studies with cows > 160 DIM or < 23 kg milk/day (Bargo et al., 2003).*

Milk production of high producing grazing dairy cows in early lactation increases linearly as the amount of concentrate increases from 1.8 to 10 kg DM/day with milk response of 1 kg milk/kg concentrate, whereas in late lactation, milk response is lower. In early lactation, cows partition more nutrients toward milk production thus MR to supplementation may be higher than in late lactation, when more nutrients are directed to BW. To avoid metabolic health problems such as subclinical acidosis, it is not recommended to supplement more than about 10 kg DM/day or >50% of DMI. The high fiber intake (pasture NDF >50% DM) may allow for feeding high amounts of concentrate (Bargo et al., 2003).

Milk production increases with the amount of concentrate supplementation, as well as the milk fat and protein yield, while milk fat percentage decreases. Supplementation of 8.7 kg of corn for the grazing dairy cows (orchardgrass - *Dactylis glomerata*) with pasture DMI of 19 kg/day, decreased pasture DMI – 15.8 kg/day while increased total DMI – 24.5 kg/day, increased milk yield (20.7 to 29.8 kg/day) and milk fat yield (0.79 to 0.97 kg/day) and milk protein yield (0.60 to 0.90 kg/day), while milk fat percentage was decreased (3.81 to 3.31%) with increased milk protein percentage (2.96 to 3.10%), (Bargo et al., 2002). Supplementing grazing dairy cows (perennial ryegrass - *Lolium perenne*) with 1.8 or 3.6 kg/day of concentrate (corn/dry beet pulp), decreased pasture DMI (17.1 to 16.5 and 16.8 kg/day) while total DMI was increased (17.1 to 18.3 and 20.4 kg/day), as well as milk yield (24.0 to 25.0 and 26.6 kg/day), milk fat yield (0.88 to 0.90 and 0.93 kg/day) and milk protein yield (0.77 to 0.82 and 0.86 kg/day), while milk fat content was decreased (3.71 to 3.68 and 3.55%) with increased milk protein content (3.25 to 3.28 and 3.26%), (Dillon et al., 1997). Reis and Combs (2000) found that using 5.0 and 10.0 kg of corn in diets for

grazing cows (alfalfa - *Medicago sativa*, red clover - *Trifolium pratense*, perennial ryegrass - *Lolium perenne*) decreased pasture DMI (13.9 to 12.7 and 9.8 kg/day) increasing at the same time total DMI (13.9 to 17.7 and 19.8 kg/day) with increase in milk production (21.8 to 26.8 and 30.4 kg/day) and decrease in milk fat yield (0.88 to 0.83 and 0.75 kg/day) while milk protein yield was increased (0.62 to 0.79 and 0.93 kg/day), content of milk fat was reduced (3.89 to 3.50 and 3.08%) whereas higher values for milk protein were determined (2.85 to 2.95 and 3.05%).

Supplementation with rumen undegradable protein-RUP could be important for meeting requirements of grazing dairy cows, because the pasture has a high ruminal CP degradability (>70%), and therefore provides smaller amounts of RUP compared with cows on TMR diets (Grubić et al., 2003a; Stojanović et al., 2010). Providing necessary dietary content of RUP is possible by using high-quality protein feeds - sources of undegradable (protected) protein: heat-treated soybean and soybean meal (extruded, toasted), soybean expeller, corn gluten meal, heat or chemical treated rapeseed expeller and meal (Stojanović et al., 2010a). Ruminal ammonia concentration of grazing Holstein cows decreased linearly with the inclusion of supplement (corn-based concentrate 5 and 10 kg DM/day) in the diet. Reduction in ruminal ammonia (from 13.2 to 10.4 and 8.13 mmol) is most likely due to the ability of the ruminal bacteria to utilize larger amounts of ammonia because of an increased supply of ruminally fermented organic matter (Reis and Combs, 2000). The pasture species have a large impact on the RUP concentration in consumed pasture. A winter oats pasture contains 18.4% CP and 19.3% RUP in CP, while an orchardgrass pasture contains 24.8% CP and 39.1% RUP in CP. An increase in milk yield (6 to 18%) with supplementation of high RUP concentrates is reported in the study by Schor and Gagliostro (2001).

Corn silage supplementation to grazing cows may increase milk production if pasture offered is restricted - low pasture allowance, but if pasture is offered ad libitum - high PA, milk production does not change or can decrease. Different forms and amounts of hay supplementation reduced pasture DMI, whereas the effect on total DMI depended on the substitution rate values: with lower SR (0.33) total DMI was increased, and higher SR (0.81-0.97) resulted in similar total DMI (Bargo et al., 2003).

Supplementation of rumen-protected fat positively affects milk production with concentrate supplemented at a rate lower than 4 kg DM/day (milk production less than 30 kg/d). Milk fat percentage was higher (4.02 to 4.36%) when 0.5 kg/day of ruminally inert fat was added to 3.3 kg DM/day of concentrate-barley for grazing cows (King et al., 1990).

Conclusion

Low pasture DMI is a major factor which can limit the milk production of high producing grazing cows. The pasture factors that mostly affect DMI are pregrazing pasture mass and pasture allowance. Supplementary feeding of grazing cows decreases pasture DMI while increases total DMI. Increasing in milk yield, milk response, is one of the main positive effects of supplementary feeding, followed with improvement in pasture utilization, possible increase in stocking rates on the pasture and positive effects on BCS and reproduction. Milk production increases with the amount of concentrate supplementation (up to 10 kg DM/day), as well as milk fat and protein yield, while milk fat percentage decreases. Dietary crude protein utilization is improved by inclusion of concentrate in diets for grazing cows. Corn silage supplementation to grazing cows may increase milk production if pasture offered is restricted.

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References

1. Arriaga-Jordan CM and Holmes W 1986. The effect of concentrate supplementation on high yielding dairy cows under two systems of grazing. J. Agric. Sci. (Camb.) 107, 453–461.
2. Bargo F, Muller LD, Kolver ES and Delahoy JE 2003. Invited Review: Production and Digestion of Supplemented Dairy Cows on Pasture. J. Dairy Sci. 86, 1–42.
3. Bargo F, Muller LD, Delahoy JE and Cassidy TW 2002. Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. J. Dairy Sci. 85, 1777–1792.
4. Clark DA and Kanneganti VR 1998. Grazing management systems for dairy cattle. In Grass for Dairy Cattle (Cherney JH, Cherney DJR) p. 331. CAB International, Oxon, UK.
5. Dillon P, Crosse S and O'Brien B 1997. Effect of concentrate supplementation of grazing dairy cows in early lactation on milk production and milk processing quality. Irish J. Agric. Food Res.36, 145–159.
6. Grubić G and Adamović M 2003. Ishrana visokoproizvodnih krava. Institut PKB Agroekonomik, Beograd.
7. Grubić G, Adamović O and Stojanović B 2003. Ishrana domaćih životinja u uslovima organske proizvodnje. Proizvodnja i sertifikacija organskih proizvoda, Mataruška banja, str. 29-30.
8. Grubić G, Adamović O, Stojanović B and Đorđević N 2003a. Contemporary aspects in assessment of protein needs in dairy cows. Vet. Glasnik 57 (3-4), 101-112.
9. Kellaway R and Porta S 1993. Feeding concentrates supplements for dairy cows. Dairy Research and Development Corporation, Melbourne, Australia.
10. King KR, Stockdale CR and Trigg TE 1990. Influence of high energy supplements containing fatty acids on the productivity of pasture-fed dairy cows. Aust. J. Exp. Agric. 30, 11–16.
11. Kolver ES and Muller LD 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 81, 1403–1411.
12. Mayne CS and Wright IA 1988. Herbage intake and utilization by the grazing dairy cow. In Nutrition and Lactation in the Dairy Cow (Garnsworthy PC) p. 280. Butterworths, London.
13. Meijs JAC and Hoekstra JA 1984. Concentrate supplementation of grazing dairy cows. 1. Effect of concentrate intake and herbage allowance on herbage intake. Grass Forage Sci.39, 59–66.
14. Mertens DR 1992. Nonstructural and structural carbohydrates. In Large Dairy Herd Management (Van Horn HH and Wilcox CJ) p. 219–235. American Dairy Science Association, Savoy, IL.
15. Reis RB and Combs DK 2000. Effects of increasing levels of grain supplementation on rumen environment and lactation performance of dairy cows grazing grass-legume pasture. J. Dairy Sci. 83, 2888–2898.

16. Robaina AC, Grainger C, Moate P, Taylor J and Stewart J 1998. Responses to grain feeding by grazing dairy cows. Aust. J. Exp. Agric. 38, 541–549.
17. Sayers HJ 1999. The effect of sward characteristics and level and type of supplement on grazing behaviour, herbage intake and performance of lactating dairy cows. Ph.D. Thesis. Queen's University of Belfast, The Agricultural Research Institute of Northern Ireland, Hillsborough.
18. Schor A and Gagliostro GA 2001. Undegradable protein supplementation to early-lactation dairy cows in grazing conditions. J.Dairy Sci. 84, 1597–1606.
19. Stojanović B, Grubić G, Đorđević N, Božičković A and Ivetić A 2010. Effects of protein sources in high-yielding dairy cows nutrition. 15. Conference on Biotechnology, Faculty of Agronomy, Čačak, vol. 15 (17), p. 567-572.
20. Stojanović B, Grubić G, Đorđević N and Božičković A 2010a. Efficient protein nutrition of high-yielding dairy cows – the possibility of improvement nitrogen utilization in diet. 14. International Eco-Conference „Safe food", Ecological movement of Novi Sad, Novi Sad, pp. 265-271.
21. Stojanović B, Grubić G, Đorđević N, Božičković A and Ivetić A 2009. Chemical and Physical Quality of Forages for Dairy Cows Nutrition. 13. Symposium Feed Technology, Institute for food Technology in Novi Sad, International Feed Industry Federation. Novi Sad, pp.217-228.
22. Stojanović B, Grubić G and Đorđević N 2007. Milk urea nitrogen (MUN) as dairy cattle protein feeding evaluation parameter. 21. Conference of agronomist, veterinarians and technologists, Institute PKB-Agroekonomik Belgrade, vol.13, (3-4), pp. 33-40.
23. Stojanović B**,** Grubić G and Đorđević N 2006. Animal nutrition strategy improvement regarding to reduction of environment nitrogen emission. 4. International Eco-Conference «Safe Food» Novi Sad, 69-74 pp..
24. Stojanović B, Grubić G and Đorđević N 2006a. Milk urea as a diagnostic of optimal protein content in lactating dairy cow diets. Symposium „Milk and milk products". Faculty of Agriculture Belgrade, Animal Husbandry Association Belgrade, Tara, pp. 27-30.
25. Stojanović B, Adamović O and Grubić G 2004. Unapređenje strategije ishrane domaćih životinja u cilju smanjenja negativnih uticaja na životnu sredinu. 3. Međunarodna Eko-Konferencija, Zdravstveno Bezbedna Hrana, Novi Sad, pp. 22-25.
26. Vazquez OP and Smith TR 2000. Factors affecting pasture intake and total dry matter intake in grazing dairy cows. J. Dairy Sci. 83, 2301–2309.

Original paper

PRIMARY ACTIVE SEED SUBSTANCES FROM MEDICINAL PLANTS AS A POSSIBLE SUPPLEMENT TO LIVESTOCK NUTRITION

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Abstract

Some of the active substances from the seeds of medicinal plants have a beneficial effect on human and animal health. The representatives of this group of plants, in addition to the secondary (biologically) active substances significant for their therapeutic properties and the flavours in the content of their seeds, have a high percentage of primary active substances (carbohydrates, oils, proteins and the like). These components are essential for plant growth and reproduction, but they are also very important as constituent substances.

The objective of this study was to determine the effect of storage duration (from one to five years) on oil and protein content of the seeds of various medicinal plants. The seeds of 9 cultivated plant species namely: white mustard (*Sinapis alba* L.), flax (*Linum usitatissimum* L.), fenugreek (*Trigonella foenum graecum* L.), evening primrose (*Oenothera biennis* L.), marigold (*Calendula officinalis* L.), milk thistle (*Silybum marianum* Gaertn.), black cumin (*Nigella sativa* L.), borage (*Borago officinalis* L.) and castor oil plant (*Ricinus communis* L.) were analyzed. Laboratory studies have been performed in three replications in the course of 2014 according to accredited methods.

On average, the highest oil content was obtained from castor seed (50.44%), whereas the lowest average oil content was recorded in fenugreek seed (5.35%). The greatest statistically significant variation of the oil content was observed in the seeds of white mustard. In spite of that, the highest number of medicinal plants had a statistically considerable reduction in oil contents between seeds of different ages. The highest protein content was recorded in the seeds of white mustard (31.96%), which was by about 2.3 times higher than the lowest protein content in seeds of evening primrose (13.80%). The largest statistically significant variation in protein content was observed with the seeds of white mustard, marigold and flax.

The high content of oil and protein, with their beneficial effects, can positively affect the proper functioning of the immune system of bred animals, and can also work as an

antioxidant, as a blood purifier, it destroys intestinal parasites, stops diarrhea, and cures foot-and-mouth disease, and the like.

Key words: *medicinal plants, oil and protein content, seed storage duration*

Introduction

In the modern approach to livestock breeding, practical and research experiences with medicinal plants are also used for the treatment of animals. The possibilities of the application of selected medicinal plants and spice plants in the prophylaxis and treatment of various diseases of farm animals, particularly, their use in the production of fodderphytoadditives, as a safe alternative to synthetic antibiotics, growth promoters and other xenobiotics, can be used as an inexhaustible resource of animal health for the purpose of the production of safe food for humans (Kostadinović, 2013). In addition to the medicinal and the protective function in the utilisation of this group of plants, animals receive the necessary proteins, oils and carbohydrates for their growth and development.

In the production of fodder, plants provide a real opportunity to increase the value of food using a variety of functional accessories. In developed European countries, there is a very strong trend of replacing synthetic antibiotic preparations with medicinal herbal preparations. These compositions have antibacterial activity, antiinflammatory activity, they stimulate digestion, have laxative activity, antidiarrhoeal activity, choleretic activity, and the like, which are already used in human medicine, and which can be added to animal feed in the treatment of various disorders of animal health (Runjaić-Antić et al., 2010). A complete ban on antibiotics in poultry feeds was brought into force on 1st January 2011 by the European Union (EU); thus, all of the antibiotics used at sub-therapeutic doses for growth promotion were withdrawn (Toghyani et al., 2010).

The aim of this study was to determine the effect of storage duration (from one to five years) on the content of oil and protein in the seeds of several medicinal plants.

Material and methods

As the material, the seeds of plant varieties and domesticated plant populations were used: white mustard (*Sinapis alba* L.), flax (*Linum usitatissimum* L.), fenugreek (*Trigonella foenum graecum* L.), evening primrose (*Oenothera biennis* L.), marigold (*Calendula officinalis* L.), milk thistle (*Silybum marianum* Gaertn.), black cumin (*Nigella sativa* L.), borage (*Borago officinalis* L.) and castor oil plant (*Ricinus communis* L.), which had been produced in the production plots of the Institute for Medicinal Plant Research "Dr Josif Pančić" in Pančevo. The processing and preparation for the analysis of oil and protein content was performed in the seed testing laboratory of the Institute in Pančevo. The research was conducted with seeds ranging from five years of age (the seeds from the year 2009) to one year of age (the seeds from the year 2013).

The content of oil and protein was determined in the chemical laboratory of the Institute "Tamiš"–"Tamiš Agrolab" in Pančevo according to the methods prescribed by the Ordinance on the methods of sampling and the methods of physical, chemical and microbiological analysis of animal feed (the Official Gazette of the Socialist Federal Republic of Yugoslavia, Number 15/87). For the determination of protein and oil content was used by 1 g, and 10 g of seed in three replications.

The obtained experimental data were statistically analyzed using the method of variation statistics: the mean value (I) and the coefficient of variation (Cv). The statistical significance of the difference between the calculated mean values was obtained by applying the analysis of variance models (ANOVA), using the statistical software package "Statistica 10 for Windows".

Results and discussion

The highest protein content was recorded in three-year-old seeds (22.25%), and the lowest content was in the oldest ones – five-year old seeds (19.50%). The highest protein content was achieved with the seed of white mustard (31.96%), fenugreek (29.06%) and flax (25.74%). Twice as low protein content was recorded in the seeds of evening primrose, marigold and milk thistle (tables 1 and 2). Both tested factors, as well as their interactions, had a statistically significant variation between the obtained values of protein content ($p < 0.01$).

Table 1. *Protein content (%) in medicinal plant seeds*

Seed maturity	Year					Average
	V	IV	III	II	I	
White Mustard (*Sinapis alba* L.)	22.97	29.13	39.00	33.39	35.31	31.96
Flax (*Linum usitatissimum* L.)	24.45	27.17	26.75	24.71	25.66	25.74
Fenugreek (*Trigonella foenum graecum* L.)	27.79	27.78	28.66	30.53	30.53	29.06
Evening Primrose (*Oenothera biennis* L.)	13.18	13.91	14.66	14.95	14.66	14.27
Marigold (*Calendula officinalis* L.)	11.88	15.06	13.39	14.60	14.07	13.80
Milk Thistle (*Silybum marianum* Gaertn.)	15.25	15.88	15.22	15.53	15.97	15.57
Black Cumin (*Nigella sativa* L.)	22.66	23.41	24.54	24.34	24.50	23.89
Borage (*Borago officinalis* L.)	17.41	18.22	17.19	17.75	17.92	17.70
Castor Oil plant (*Ricinus communis* L.)	19.94	20.95	20.90	22.00	19.83	20.72
Average	19.50	21.28	22.25	21.98	22.05	21.41

Table 2. *Analysis of variance of protein content (%)*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant species	8	5301.8230	662.7279	3208.18**	<.001
Years of age	4	129.6687	32.4172	156.93**	<.001
Interaction	32	429.8970	13.4343	65.03**	<.001
Residual	89	18.3851	0.2066		
Total	133	5824.2179			
Cv= 2,1%, * P≤5%, ** P≤1%					
	Plant species		Years of age		Interaction
LSD 5%	0.3298		0.2458		0.7374
LSD 1%	0.4368		0.3256		0.9768

The highest content of oil and protein was recorded in two-year-old seeds (23.17%), and the lowest content was in the oldest ones – five-year-old seeds (19.68%). The highest oil content was achieved with the seeds of castor oil plant (50.44%), flax (35.88%) and black

cumin (33.47%), whereas the oil content in white mustard was slightly lower than expected, amounting to 19.20% (tables 3 and 4). The obtained values of oil content were less than the significance level, 1 and 5%, so it can be concluded that the F-ratio was highly significant ($p<0.01$), or, in other words, that there was a significant effect of plant age and species on oil content.

Table 3. *Oil content (%) in medicinal plant seeds*

Seed maturity	Year					Average
	V	IV	III	II	I	
White Mustard (*Sinapis alba* L.)	14.53	23.91	21.00	17.67	18.91	19.20
Flax (*Linum usitatissimum* L.)	32.73	34.65	36.23	41.96	33.83	35.88
Fenugreek (*Trigonella foenum graecum* L.)	5.57	5.01	5.38	5.11	5.72	5.35
Evening Primrose (*Oenothera biennis* L.)	5.10	5.57	7.42	5.91	5.25	5.85
Marigold (*Calendula officinalis* L.)	12.59	12.20	11.25	11.29	11.29	11.72
Milk Thistle (*Silybum marianum* Gaertn.)	24.04	24.25	24.12	24.29	25.06	24.35
Black Cumin (*Nigella sativa* L.)	28.10	30.53	35.77	36.11	36.88	33.47
Borage (*Borago officinalis* L.)	10.17	12.23	10.49	10.89	11.33	11.02
Castor Oil plant (*Ricinus communis* L.)	44.33	47.14	48.86	55.34	56.54	50.44
Average	19.68	21.72	22.28	23.17	22.75	21.92

Table 4. *Analysis of variance of oil content (%)*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant species	8	28636.2660	3579.5333	22204.73	<.001
Years of age	4	203.6588	50.9147	315.84	<.001
Interaction	32	658.9649	20.5927	127.74	<.001
Residual	90	14.5085	0.1612		
Total	134	29513.3983			
Cv= 1,8%, * P≤5%, ** P≤1%					
	Plant species		Years of age		Interaction
LSD 5%	0.2913		0.2171		0.6513
LSD 1%	0.3858		0.2876		0.8627

The following section of the paper presents the results of some former research. Depending on the cultivated variety, better results than our results of the seeds of certain field genotypes of white mustard contain from 25.6% to 28.1% of oil (Piętka et al., 2004). Some of the selected genotypes of white mustard contain even up to 52.8% of oil. An increase in the protein content results in the reduction of the content of p-hydroxybenzyl glucosinolate and erucic acid (Katepa-Mupondwa et al., 1999). According to the statements by Carter (2002), the seeds of flax oil plant contain from 26.9 to 31.6% of protein, and from 31.9 to 37.8% of oil. Similar results in the conditions of South Banat, depending on the variety and agro-ecological conditions of cultivation, the average oil content of about 41.0% was achieved (Filipović et al., 2014). A higher content of oil in flax was achieved on chernozem soils with the signs of gleisation in relation to chernozem on sandy loess (Filipović et al., 2010). The content of protein and oil in the seeds of evening primrose varied depending on the mineral nutrition with nitrogen nutrients and the time of sowing.

The several times higher values than our values of oil content had an interval from 23.5 to 30.1%, whereas the content of protein ranged from 12.5 to 16.2% received Ghasemnezhad and Honermeier (2008). According to Hulan et al. (1987) and Christie (1999), the oil content of evening primrose seeds varies with such factors as the age of the seeds, the cultivar and growth conditions, and typically varies between 18 and 25%, with occasional outliers to 12 or 28%. The seeds of pot marigold have a significant around 20% oil content (Saleem et al., 1986), which is better in comparison with our results. Similar results of the oil content of pot marigold were reported by Dulf et al. (2013). The lipid seeds varied between 13.6 and 21.7 g oil per 100 g of seeds. Also, similar results with our results had the seeds of milk thistle, in addition to secondary metabolites, which are rich in vegetable oil, in the amount from 26.67 to 27.16%, and in protein, in the amount from 23.4 to 24.2% (Khan et al., 2007). Black cumin, depending on agro-ecological conditions, achieves higher or lower oil content. The above results also point to the high content of two primary metabolites. For example, in the conditions of Italy (D'Antuono et al., 2002), from 13 to 23% of oil was achieved, whereas, in the climate of Iran (Cheikh-Rouhou et al., 2007), the seeds of black cumin had as much as 40% of oil. The level of synthesized protein in the seeds of black cumin depends on the variety cultivated, so, in the pieces of research by Cheikh-Rouhou et al. (2007), an interval was recorded from 22.6% to 26.7%.

Conclusion

The age of the seeds of the tested medicinal plant species significantly influenced the content of the two primary metabolites: protein and oil. The highest protein content was recorded in the seeds three years of age (22.25%), and the lowest in five-year-old seeds (19.50%). The highest protein content was achieved in the seeds of white mustard (31.96%), fenugreek (29.06%) and flax (25.74%). The highest oil content was achieved, as expected, in oil plant species (castor oil plant (50.44%), flax (35.88%) and black cumin (33.47%)), whereas the oil content in the seeds of white mustard was somewhat lower than expected, totaling 19.20%.

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References

1. Barry TN, McNeill DM and McNabb WC 2001. Plant secondary compounds; their impact on nutritive value and upon animal production, in: Proceedings of the XIX International Grass Conference, Sao Paulo, Brazil, 445-452.
2. Carter J 2002. Flaxseed as Functional Food for People and as Feed for Other Animals, Department of Plant Sciences, North Dakota State University, 1-12.
3. Cheikh-Rouhou S, Besbes S, Hentati B, Blecker C, Deroanne C and Attia H 2007. *Nigella sativa* L. Chemical composition and phytochemical characteristics of lipid fraction. Food Chem. 101, 673–681

4. Christie WW 1999. The analysis of evening primrose oil. Industrial Crops and Products 10, 2, 73–83.
5. D'Antuono LF, Moretti A and Lovato AF 2002. Seed yield, yield components, oil content and essential oil content and composition of *Nigella sativa* L. and *Nigella damascena* L. Industrial crops and products 15, 1, 59-69.
6. Dulf FV, Pamfil D, Baciu AD and Pintea A 2013. Fatty acid composition of lipids in pot marigold (*Calendula officinalis* L.) seed genotypes. Chemistry Central Journal 7, 1, 1-11.
7. Filipović V, Radivojević S, Kuzevski J and Stanković S 2010. Uticaj agroekoloških uslova na produktivnost i kvalitet semena uljanog lana. Poljoprivredne aktuelnosti 9, 1-2, 37-48.
8. Filipović V, Popović V, Glamočlija Đ, Jaramaz M, Jaramaz D, Anđelović S and Tabaković M. 2014. Genotype and soil type Influence on morphological characteristics, yield and oil content oil-flax. Bulgarian Journal of Agricultural Science 20, 1, 89-96.
9. Ghasemnezhad A and Honermeier B 2008. Yield, oil constituents, and protein content of evening primrose (*Oenothera biennis* L.) seeds depending on harvest time, harvest method and nitrogen application. Industrial crops and products 28, 1, 17-23.
10. Hulan HW, Hall IV, Nash DM and Proudfoot FG 1987. Composition of native evening primrose seeds collected from Western Nova Scotia. Crop Res. (Hortic. Res.) 27, 1 – 9
11. Katepa-Mupondwa F, Rakow G and Raney Ph 1999. Meal quality characteristics yellow mustard (*Sinapis alba* L.). 10th International Rapeseed Congress. Canberra Australia, Canberra, Australia 26-29.09.1999-CD.
12. Khan I, Khattak HU, Ullah I and Bangash FK 2007. Study of the Physicochemical Properties of *Silybum marianum* Saeed Oil. Journal oh the Chemical Society of Pakistan 29, 6, 545-548.
13. Kostadinović LJ 2013. Uticaj lekovitog bilja na zdravlje životinja. Naučni institut za prehrambene tehnologije. Novi Sad.
14. Piętka T, Krótka K and Krzymański J 2004. Gorczyca biała podwójnie ulepszona – alternatywna jara roślina oleista (Double low white mustard (*Sinapis alba* L.) – alternative spring oilseed crop). Rośliny Oleiste, 2, 403-414.
15. Runjaić-Antić D, Pavkov S and Lević J 2010. Biljke u održivoj ishrani životinja. Biotechnology in Animal Husbandry 26, 3-4, 203-214.
16. Saleem M, Zaka G, Shakir N and Khan SA 1986. Studies on marigold seed oil and seed meal. Fette, Seifen, Anstrichm. 88, 178–180.
17. Toghyani M, Toghyani M, Gheisari A, Ghalamkari G and Mohammadrezaei M 2010. Growth performance, serum biochemistry and blood hematology of broiler chicks fed different levels of black seed (*Nigella sativa*) and peppermint (*Mentha piperita*). Livestock Science 129, 1, 173-178.

Original paper

THE ENERGY VALUE AND ENERGY YIELDS OF ALFALFA FORAGE DEPENDING ON THE CUTTING TIME IN FORAGE-SEED PRODUCTION SYSTEM

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Abstract

Economic importance of alfalfa (*Medicago sativa L.*) is reflected in the production of high-quality forage, however, the seeds of alfalfa are valuable commodity in the domestic and international markets. In Serbia, the seed is produced in forage-seed mode of exploitation where the second or third growth is used for seed production, and the remaining growths are used for fodder. The aim of this work was to determine the influence of cuttings and time of cutting on total energy value of hay in a model of forage and seed production. The second and third cuts were used as seed cut. In the variant where the second cut was used as seed cut, the first cut was harvested at different times (A_1-early, A_2-medium early, A_3-late and A_4- very late). In the model where the third cut was used for seed, two pre-cuts were used for forage production (A_5). In all the following variants, seed cut was followed by one more cutting (final cut), used for the production of fodder. The highest average total production (from pre-cut and final cut) of NE_L (34606 $MJha^{-1}$) and NE_M (33811 $MJha^{-1}$) was realized in the system of cutting with two pre-cuts. In the variant with single pre-cut, the highest yield was formed in the early system. Later cutting of the first cut causes decline of NE_L production and it was the lowest in the system of very late cutting or 15.1 % less than in the early system and 38% lower than in the system of cutting with two pre-cuts. Delaying of utilization of alfalfa to the later stages of exploitation has contributed to the lower yield of NE_M and in the medium early system and late system and especially in the very late cutting system, where by 22.2 % less was realized than in the early production system. Studies have shown that late cutting of first cut resulted in the reduction of nutritional value expressed in the amount of net energy per unit area.

Key words: *alfalfa, cutting, net energy, yield*

Introduction

Alfalfa is the most important forage legume in Serbia. Its importance is reflected primarily in production of fodder, while the seed production is less important to the national economy. The local seed production satisfies the domestic demand for alfalfa seed, and in some years the alfalfa seed is even exported. In the agro-ecological conditions of Serbia, the alfalfa seed is most commonly produced from the second and sometimes the third growth of alfalfa. Time of cutting and number of pre-cuts significantly influence the yield

of alfalfa seed (Vučković et al., 2004; Karagić, 2004; Karagić et al., 2006; Terzić, 2011). Karagić (2004) and Karagić et al. (2006) and Terzić (2011) have established that by late cutting of the first cut in the average yield of seed from the second growth is higher compared to yields of pre-cut carried out at the beginning of May.

Cutting at certain phase of development significantly influences the yield and quality of alfalfa. Cutting of alfalfa in later development phases ensures higher forage yield and sustainability of alfalfa **(**Undersander et al., 2004**).** The best quality and the highest yield of nutrients are achieved in early phases of alfalfa development - budding stage and before blossoming (Mejakić et al., 1997**).**

In the research of Terzić et al. (2013), in the same dual model of production (hay-seed), the highest average production of NE_L and NE_M in pre-cuts was realized in a harvesting system with two pre-cuts (31981 $MJha^{-1}$ and 31163 $MJha^{-1}$). In the variant with a single pre-cut the highest yield from the first growth was formed in the early system (20988 and 20508 $MJha^{-1}$). Later first cutting led to decrease in production of NE_L and NE_M, with the lowest values recorded in the very late cutting system (18199 and 16017 $MJha^{-1}$).

In addition to the production of hay from pre-cuts, in the dual model of production, the last cut is used for production of hay.

The aim of this study was to examine the total production of the NE_L and NE_M of the final cut and total production of NE_L and NE_M in the seed-forage model of production.

Materials and methods

Study was carried out on experimental field of the Institute for Forage Crops in Kruševac in 2005, 2006 and 2007. The size of the basic plot was 10.5 m^2. Treatments were different time of pre-cut of alfalfa seed crop: A_1- early cut (around May 5th), A_2- medium early cut (around May 15th), A_3- late cut (around May 25th), A_4- very late cut (around June 5th), A_5- cut (A5 I- around May 5th and II - around June 5th). Contrary to the harvest procedure in the A_1 to A_4 systems, where the second cut was used for seed production, in the A_5 harvest procedure the third cut was used for seed production. The final cut was done at the end of October. At the moment of cutting the plants were on average in the early system (A_1) in the stage of budding, in the medium early system (A_2), in the stage of the beginning of flowering, in the late (A_3), in the stage of full flowering and in very late system (A_4) in the stage of post-flowering. In a system with two pre-cuts (A_5), at the time of cutting, plants were in the budding stage and in final cut plants were in various stages of intensive growth. Cutting of growth for forage was done manually at height of about 5 cm. All results related to chemical composition are expressed through absolute dry matter. The quantity of nutrients was calculated based on the chemical composition and yield in absolute dry matter. The total energy value Ne_L and NE_M is calculated as the sum of the energy values of pre-cut in the seed cut and energy value of the last cut. Net energy value of crop residues after harvest of seed was not calculated.

Chemical analyses were carried out in the laboratory of the Institute for Forage Crops in Kruševac. The NE_L and NE_M values were calculated ($NE_L=k_1$ x ME, q=ME/UE x 100; $NE_M=k_{mf}$ x ME, q= ME/UE x 100). The calculation used the following digestibility coefficients (Obračević, 1990) CP-80%, SC-53%, DM-46%, NFE-78%. Statistical processing of obtained data was done by variance analysis. Testing of the significance of differences was done by LSD test.

Results and discussion

The paper presents the content of crude protein and crude fiber, energy value of the last cut and total energy value of pre-cut and the final cut in the production model forage-seed production system. Contents of crude protein and crude fibre in the final cut are shown in Table 1.

Table 1. *Contents of crude protein and crude fibre in the final cut (gkg^{-1}DM)*

Cutting term	Year						Average conteint	
	2005		2006		2007			
	Crude proteins	Crude fibre	Crude proteins	Crude fibre	Crude proteins	Crude fibre	Crude proteins	Crude fibre
A_1	203.0	256.7	210.5	276.8	195.1	249.9	202.9	261.1
A_2	214.4	229.9	216.1	285.7	200.3	226.9	210.3	247.5
A_3	219.4	229.4	227.3	252.6	205.5	232.0	217.4	238.0
A_4	222.8	242.9	228.9	234.6	215.7	224.4	222.5	234.0
A_5	221.9	239.5	230.1	232.4	216.4	224.8	222.8	232.2

The content of crude protein in the final cut increases going from A_1 to A_5 system of cutting, while the average content of crude fiber decreases. The results of the final cut in the present study show contradiction in relation to changes in the content of crude protein and crude fiber in the first cut in studies by Mejakić et al. (1997), Undersander et al. (2004) and Terzić et al. (2012) who state that crude protein content in the first cut decreases with the delay in cutting. At the moment of cutting, in the final cut, the plants are in the various stages of intensive growth. Plants from the previous system of cutting, due to earlier cutting of pre-cuts and earlier harvesting of seed production had more time to regenerate in the final cuts. More time for regeneration contributed to the plants from earlier cutting periods (A_1 and A_2) to be developed in relation to the plants from late harvesting of the first cut A_3 and A_4 system, which has influenced the treatments with earlier pre-cuts (A_1 and A_2) to have a lower protein content in the final cut relative to the later harvesting periods (A_3 and A_4).

The energy value of alfalfa last-cut depending on the cutting system is shown in Table 2.

In the last cut, the lowest average values of NE_L (5.61 MJkg^{-1}DM) and NE_M (5.63 MJkg^{-1}DM) were realized in the early system (A_1) of cutting of the first growth. Slightly higher energy value was generated in the very late (A_4) cutting system (5.78 MJkg^{-1}DM and 5.83 MJkg^{-1}DM) as well as in system (A_5) with two pre-cuts (5.79 MJkg^{-1}DM and 5.83 MJkg^{-1}DM). Realized energy value in this study is higher than the value recorded for the first growth in research by Terzić et al. (2013) in the forage-seed model of production, where the energy value of NE_L ranged from 5.40 MJkg^{-1}DM in early cutting to 3.88 MJkg^{-1}DM in very late cutting, and of NE_M 5.28 MJkg^{-1}DM for early system of cutting to 3.41 MJkg^{-1}DM in very late cutting system. In the results stated by Terzić et al. (2013) in the first cut, NE_L and NE_M MJkg^{-1}DM values have declined going from early to late cutting system, while in this research, in case of the last cut, values increased going from early to late system of the first cut.

Table 2. *Contents of NE_L and NE_M of final alfalfa cut (MJ kg^{-1} DM)*

Cutting term	Year						Average content	
	2005		2006		2007			
	NE_L	NE_M	NE_L	NE_M	NE_L	NE_M	NE_L	NE_M
A_1	5.65	5.68	5.51	5.50	5.67	5.71	5.61	5.63
A_2	5.79	5.76	5.54	5.53	5.75	5.82	5.69	5.70
A_3	5.74	5.81	5.74	5.77	5.81	5.88	5.76	5.82
A_4	5.77	5.80	5.75	5.82	5.81	5.87	5.78	5.83
A_5	5.81	5.88	5.70	5.74	5.80	5.87	5.79	5.83

Differences in tendencies between the first and the last cut are the result of harvesting at different stages of development of the first cut. Earlier harvest deadlines of first growth have contributed to the slightly earlier seed harvests, which later, in the final cut, caused, that swaths that have been harvested earlier have a greater number of days to regenerate and plants in the earlier first harvest later are more developed (in the last cut), which caused the plants harvested at earlier periods of the first cut to have lower contents of NE_LMJkg^{-1}DM and NE_M MJkg^{-1}DM compared to plants that are harvested at later periods of the first cuts.

Reduction of the energy value of alfalfa in the later stages of exploitation is consistent with the results of Obračević (1990), Dinić (1997) and Đorđević et al. (2003). The values obtained in the present study are slightly lower than the values reported by Macgregor (1994) cit. by Glamočić (2002), where the values of NE_L and NE_M in the stage before flowering were 5.96 and 5.89 MJkg^{-1}DM. The values were higher than the values stated by Dinić (1997) where the average values of NE_L and NE_M in alfalfa silage were 5.25 MJkg^{-1} and 5.34 MJkg^{-1}DM in budding stage. Higher values are consequence of harvesting the alfalfa in the final cut in the stage before the bud stage when the leaf and stem ratio is more favourable.

In Table 3 is given the total net energy in MJha^{-1} calculated from pre-cut and final cut together.

Total NE_L energy ranged from 17798 MJha^{-1} (very late period in 2007) to 37380 MJha^{-1} (in the system with two pre-cuts in 2007). In regard to the total production of NE_L and NE_M in MJha^{-1} (Table 3) it can be seen that the highest yield of NE_L was generated in the system (A_5) with two pre-cuts, NE_L (34 606 MJkg^{-1}) and NE_M (33811MJha^{-1}). In the single pre-cut harvesting system, the highest total net energy in MJha^{-1} in milk production was recorded in the system with early harvesting (A_1) of the first growth 25105 MJha^{-1} and 24 637 MJha^{-1} in the production of meat. Postponing of the first cut reduces the yield decreases and the lowest values are recorded in very late (A_4) harvest period.

In earlier harvest periods, lower net energy MJkg^{-1}DM has been realized in the earlier cutting but with higher production of dry matter, which contributed to more MJha^{-1} formed in the total production of NE_L and NE_M per unit area in earlier harvest periods of first cut. Studies have shown that late harvest results in the reduction of yield of net energy per unit area. Later first cut reduces the production of NELMJ$ha^{-1}$and the lowest value is realized in the system of very late (A_4) harvest or 15.1% less than in the early system (A_1) and 38% less than in the system of harvesting with two pre-cuts (A_5).

Table 3. *Total net energy from pre-cut and final cut (MJ ha $^{-1}$)*

Cutting schedule	Year						Average content	
	2005		2006		2007			
	NE_L	NE_M	NE_L	NE_M	NE_L	NE_M	NE_L	NE_M
A_1	27674	27189	24847	24377	22794	22344	25105	24637
A_2	26324	25126	23952	22914	21139	20180	23805	22740
A_3	25323	23430	22303	20727	20668	19277	22765	21145
A_4	24180	21756	21996	19937	17790	15809	21322	19167
A_5	33617	33007	32821	31944	37380	36482	34606	33811
F- test	**	**	**	**	**	**	Level	
LSD	972	925	2415	2339	1241	1182	5%	
	1414	1347	3514	3403	1805	1719	1%	

Delaying the utilization of alfalfa from the early period to the later stages of exploitation has contributed to the formation of lower total yield of NE_MMJha^{-1}in the medium early and late harvesting systems and especially in the very late harvesting system, where 22.2% less is formed than in the early production system and 43% less than in production with two pre-cuts. The highest net energy value is realized in the system with two pre-cuts.

Analyses showed that between the years the highest production was realized in 2005, which was characterized with a lot of rainfall in the summer and the lowest production in 2007, when less rainfall was recorded in summer.

Overall, the total production of NE_L and NE_M, which is given in this paper and the production of NE_L and NE_M in pre-cuts A_1, A_2, A_3, A_4 and A_5 system is reported by Terzić et al. (2013). It can be observed that in the last harvest, the share of production from the last cut is considerably lower than the production of the first cut. Compared with the production in the first cut, the final cut has participated with 15-17% in the total production, and in the system with two pre-cuts, last swath participated with only 8% in the total production of NE_L and NE_M in MJha^{-1}.

Conclusion

Earlier harvesting of the first growth in forage-seed production influenced forming of slightly lower value of NE_L and NE_M MJkg^{-1} DM in final cut.

Later first cut reduces the yield of NE_LMJha^{-1} and the lowest value is realized in the system of very late harvest (A_4).

Delaying of utilization of alfalfa from the early period (A_1) to the later stages of exploitation has contributed to the formation of lower yield of NE_MMJha^{-1} in the medium early (A_2) and late (A_3) harvesting systems and especially in the very late (A_4) harvesting system.

The highest net energy value MJha^{-1} is realized in the system with two pre-cuts (A_5) and seed production in the third cut.

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References

1. Dinić B 1997. Uticaj faze razvića biljaka, nivoa suve mase i hemijskog konzervansa na kvalitet i hranljivu vrednost silaže lucerke. PhD, Belgrade Univ.
2. Đorđević N, Grubić G and Jokić Ž 2003. Osnovi ishrane domaćih životinja (praktikum). Univezitet u Beogradu. Poljoprivredni fakultet, pp. 99-160.
3. Erić P 1988. Uticaj načina i gustine setve na prinos i kvalitet semena lucerke (*Medicago sativa* (L) em Wass.) sorte "NS Banat ZMS II" na zemljištu tipa černozem. PhD, Novi Sad Univ
4. Glamočić D 2002. Ishrana preživara-praktikum. Poljoprivredni fakultet u Novom Sadu, SYMBOL, Novi Sad.
5. Karagić Đ 2004. Komponente prinosa, prinos i kvalitet semena u zavisnosti od sistema kosidbe. PhD, Novi Sad Univ.
6. Karagić Đ, Katić S, Milić D and Vasiljević S 2006. Effect of cutting sheldule on seed yeild in alfalfa. XXVI EUCARPIA. Breding and seed production for convetional and organic agriculture. Perugia, Faculty of Agriculture 3-7 sep., pp. 124-128.
7. Mejakić V, Stojčić B, Nedović B and Vojin S 1997. Optimalne faze razvoja novostvorene sorte lucerke (*Medicago sativa* L.) "Biljana" za korišćenje. Agroznanje, Banja Luka, God I, 241-255.
8. Obračević Č 1990. Tablice hranljivih vrednosti stočnih hraniva i normativi u ishrani preživara. Naučna knjiga, Beograd.
9. Terzić D 2011. Uticaj vremena kosidbe, đubrenja, mikroelementima i regulatorima rasta na prinos i kvalitet semena lucerke (*Medicago sativa* L.). PhD, Belgrade Univ.
10. Terzić D, Dinić B, Stanisavljević R, Đokić D, Marković J, Anđelković S, Milenkvić J 2012. The effect of time of cutting of first alfalfa growth on content and yield of crude proteins. Proceedings of The First International Symposium on Animal Science, November 2012, Belgrade, 537-545.
11. Terzić D, Dinić B, Stanisavljević R, Đokić D, Marković J, Milenković J, Anđelković S 2013. The Effect of cutting schedule on energy value of first alfalfa growth**.** 10th International Symposium Modern Trends in Livestock Production. October 2-4, Institute for Animal Husbandry, Autoput 16, P. Box. 23, 11080, Belgrade-Zemun, Serbia, 2013, pp. 1165-1171.
12. Undersander D, Becker R, Cosgorve D, Cullen Eileen, Doll J, Grau C, Kelling K, Rice EM Schmitt M, Sheaffer C, Shewmaker G and Sulc M 2004. Alfalfa menagement guide. American Society of Agronomy, Inc, Crop Sciene Sociaty of America. pp. 2-55.
13. Vučković S, Simić A, Ćupina B, Stojanović Ivana, Stanisavljević R, Vojin S and Dubljević R 2004. Uticaj vremena kosidbe prvog otkosa na prinos semena lucerke u agroekološkim uslovima Zapadnog Srema. Acta Agriculturae Serbica. Vol. IX, 123-127.

Original paper

INFLUENCE OF ENERGY AND PROTEIN LEVEL IN LACTATING SOWS DIET ON THE MOBILIZATION OF RESERVES FROM INTERNAL ORGANS

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Abstract

The aim of this paper was to investigate the effect of feeding sows in lactation, in diets with different levels of energy and protein to mobilization of reserves from internal organs. The experiment was conducted on 240 sows divided into two groups of 120 sows each, with two sub-groups of 60 sows. Experimental period lasted 65 days, and during the last 30 days of pregnancy and lactation, until weaning. For sows feeding a mixture of the standard composition and the quality, with the 15% crude protein, and 14 to 12 MJ ME / kg and 19% crude protein, and 14 to 12 MJ ME / kg of the mixture were used. Sows were divided into two groups and four subgroups consumed 2.20 and 3.30 kg of food per day. Based on the statistical analysis of the data it can be concluded that the different amounts of feeds with different levels of protein and energy in the diet resulted in the mobilization of the reserves from the internal organs of lactating sows. When we talk about the content of protein in the liver of lactating sows, statistically significant effect ($P>0.05$) to diets with 15% crude protein (19.04%) and 19% crude protein (19%) was not recorded. The same trend in the content of crude protein in the kidney (14.61 and 14.84%) was also observed, but with no statistically significant difference ($P>0.05$). Analysis of variance was established as statistically significant effects ($P<0.01$) protein content in the diet on protein content in the muscle of the heart muscle (17.59 and 18.71%). Effect of different energy level in mixture used for the diet of lactating sows, the protein content in the liver, kidneys and heart was missing.

Key words: *energy, nutrition, protein, sows*

Introduction

In intensive pig production important place takes the nutrition of sows, whose main goal is the cost-effective production. This set of goals is not easy to achieve because sow reproductive efficiency is influenced by many factors of which diet take a significant role. Success in the modern conditions of pig production greatly depends on the efficiency of the breeding herd (Rupić et al., 2005; Rosendo et al., 2007). It is believed that during the exploitation, sows should raise a total of 70-75 piglets (Smits 2003; Close and Turnley, 2004). Achieving this goal can be defined only if the optimal housing conditions and adequate nutrition of sows were in all phases of the reproductive cycle. The problem of optimal nutrition of sows is complex due to the very different needs of sows in the food and nutrients in different stages of the reproductive cycle (Kovčin et al., 2005). In

pregnancy, the needs of sows in nutrients are not great and come down primarily to meet basic maintenance requirements, which are the largest item in the structure needs. In addition to their maintenance requirements necessary to provide part of the nutrients for the development and creation of reserves, then the intrauterine development of piglets and to prepare the mammary glands for milk secretion, this begins immediately after farrowing (Beuković, 1999). Needs of lactating sows are extremely high which can be a serious problem when it comes to feed consumption, namely, insufficient consumption of sowing order, the secretion of milk will be satisfactory (Kim and Wu, 2008), and at the same time, should not lose too much reserves of nutrients from the body, in order to avoid delayed estrus after weaning piglets (Whittemore, 1998; Trottier and Johnston, 2000, Boyd et al. 2000; Kongsted, 2005). The secretion of milk requires an appropriate amount of protein in the diet, so it is necessary to increase the participation of protein nutrients of plant or animal origin (Trottier and Johnston, 2005; Dean, 2005). The protein needs for sows, during lactation depends mainly of milk secretion amount, and much less of the weight of the sows (Beuković, 1999, McNamara and Pettigrew, 2002). In order to satisfy the needs in nutrients during lactation via feed which sows consumed during this stage, it would be necessary to consume 6-7 kg of feed, which is much higher than the actual consumption (Beuković, 1999). Engagement body reserves of nutrients from the body during the period of lactation is a regular occurrence (Foxcroft et al., 1997; Eissen et al., 2000), which is hard to miss and is not a big problem if it is not too long and big (Eissen et al., 2003; Thaker and Bilkei, 2005). In the body there are reserves of protein, so it comes to engaging body protein from muscle and vital internal organs (Aherne and Williams, 1992, Yang et al., 2000; Clowes et al., 2003). Previous studies have indicated that increasing the amount of protein and energy in pregnancy has a negative effect on embryonic survival (Whittemore, 1987), and recent studies point to the positive effect of increasing amounts of protein and energy on litter size, especially when it comes to young sows (Wu et al., 2006). Increasing amounts of protein and energy in pregnancy aims to provide the best possible preparation for the next lactation sows and possibly increase the body weight of newborn piglets.

The aim of this study was to investigate the effect of feeding sows in lactation diets with different levels of energy and protein to mobilization of reserves from internal organs.

Materials and methods

The experiment was conducted on 240 sows, divided into two groups of 120 sows each, with two sub-groups of 60 sows. Experimental period with sows lasted 65 days, respectively during the last 30 days of pregnancy and lactation, until weaning. The sows were fed a mixture with a standard composition and the quality of the 15% crude protein, and 14 to 12 MJ ME / kg and 19% crude protein, and 14 to 12 MJ ME / kg of feed (Table 1 and 2). Sows were divided into two groups and four subgroups consumed 2.20 and 3.30 kg of food per day. In the last thirty days of experiment sows were housed in group pens. Feed is performed automatically, twice a day. Water supply was from automatic drinkers. Micro-climatic conditions, the relative humidity and temperature were automatically controlled. Fresh air flow was enabled by perforated ceiling, while drawing air was through a vertically mounted vent with running on the roof. During the experimental period sow body mass was controlled 30 days before farrowing and immediately before farrowing and weaning. The results regarding gain of sows in gestation and weight loss in lactation were also accompanied in the trial. After farrowing the sows in all groups were fed diets for lactating sows, which differed by the amount of crude protein and energy levels. At this stage, feeding is performed on two occasions in an amount of 5 kg. Amount

of consumed food in this phase of the experiment was not registered. Piglets were weaned in 28 days age. After the end of the experimental period, sows were sacrificed, and analysis of nutrients in the liver, kidney and heart was determined. The results obtained in this experiment are shown in the tables as well as an average or relative value. Statistical significance was determined by measurement obtained by using analysis of variance (ANOVA) and t-test. The level of statistical significance of differences between groups was expressed as a statistically highly significant, statistically significant or the difference that is not statistically significant. Software package Statistica for Windows ver. 8.0 (StatSoft Inc., USA) was used for statistical data processing.

Table 1. *Structure of diet mixtures used in experiment, %*

Feedstuffs	In mixture, %				
	Sows	Lactating sows			
		15/12 (CP/ME)	15/14 (CP/ME)	19/12 (CP/ME)	19/14 (CP/ME)
Corn	48.38	23.56	56.62	26.56	44.50
Soybean meal	3.48	6.20	13.70	19.58	25.50
Wheat bran	30.00	15.00	8.00	15.00	8.00
Barley	10.00	41.00	10.00	25.00	10.00
Soybean grits		1.00	5.00		5.00
Sunflower meal	5.00	10.00		10.00	
Vegetable oil			3.30		3.80
Lysine	0.06	0.17	0.03		
Lime stone	1.17	1.70	1.42	2.02	1.44
Monocalcium phosphate		0.07	0.60	0.50	0.43
Salt (NaCl)	0.31	0.30	0.33	0.30	0.33
Premix	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00

Table 2. *Structure of diet mixtures used in experiment, %*

	Sows		Lactating sows							
			15/12 (CP/ME)		15/14 (CP/ME)		19/12 (CP/ME)		19/14 (CP/ME)	
Nutrients	ND	DM	ND	DM	ND	DM	ND	DM	ND	DM
Dry matter	88.93	100.00	88.01	100.00	88.14	100.00	87.31	100.00	87.60	100.00
Moisture	11.07	-	11.99	-	11.86	-	12.69	-	12.40	-
Crude proteins	13.47	15.15	15.56	17.68	15.47	17.55	19.11	21.89	19.15	21.86
Crude fat	2.87	3.23	2.71	3.08	3.60	4.08	2.49	2.85	6.85	7.82
Crude fiber	3.92	4.41	3.65	4.15	3.39	3.85	3.49	4.00	3.69	4.21
Ash	4.16	4.68	4.82	5.48	4.88	5.54	5.87	6.72	5.19	5.92
NEM	64.51	72.53	61.27	69.62	60.80	68.98	56.35	64.54	52.72	60.18
Ca	0.67	0.75	0.73	0.83	0.80	0.91	1.00	1.15	0.79	0.90
P	0.47	0.53	0.45	0.51	0.50	0.57	0.58	0.66	0.49	0.56

Results and discussion

The results shown in Table 3 indicate that the protein content in the liver was under the influence of the protein level in the diet for sows. When the level of protein in the diet was 750 g, the protein content in the liver was 19.04%, while the increase of protein to 950 g in diet leads to an increase in the protein content of 19.31% in the liver. Statistical analysis of the results shows that the level of protein in the liver was not significantly (P>0.05) different between groups fed with different levels of protein in the diet.

Table 3. *Protein level in liver*

Protein level (%)	15		19		Mean:
Energy level (MJ)	12	14	12	14	
2.20	18.85	18.89	19.1	19.66	19.12
3.30	18.87	19.54	18.96	19.5	19.22
Mean:	18.86	19.22	19.03	19.58	
Protein effect	19.04^{ns}		19.31^{ns}		
Energy effect	18.94		19.4		

In contrast to the liver, changes in the protein content in the kidney are not affected by the protein in a meal, and it can be concluded that the protein from the kidneys is less involved in the mobilization of protein to meet the needs of the protein during lactation. The content of protein in the kidneys of affected levels of protein in the diet is shown in Table 4 in addition to the protein content of kidney which marked a significant difference (P>0.05).

Table 4. *The level of protein in kidneys*

Protein level (%)	15		19		Mean:
Energy level (MJ)	12	14	12	14	
2.20	14.01	14.49	15.1	15.22	14.71
3.30	14.37	15.57	14.41	14.61	14.74
Mean:	14.19	15.03	14.755	14.915	
Protein effect	14.61		14.84		
Energy effect	14.48		14.97		

Table 5. *Level of protein in the hearth muscle*

Protein level (%)	15		19		Mean:
Energy level (MJ)	12	14	12	14	
2.20	17.49	17.61	18.5	19.07	18.14
3.30	17.50	17.78	18.38	18.90	18.17
Mean:	17.50	17.70	18.44	18.99	
Protein effect	17.59^{B}		18.71^{A}		
Energy effect	17.97		18.34		

The protein content in the heart muscle was not affected by protein meals so it can be concluded that the protein of the body was less involved in the mobilization of protein to

meet the needs of the protein during lactation. The protein content in the heart muscle is shown in Table 5. To the basis of Statistical analysis of results it can be noted that the level of protein in the muscle of the heart had a significant effect ($P<0.01$) to the levels of protein in sows feed while the energy levels in sow feed had no significant effect ($P> 0.05$).

Conclusion

On the basis of the tests and the results it can be concluded that the level of protein and energy in the diet of lactating sows can influence the mobilization of protein from vital internal organs, especially the liver. The content of protein level in the liver was higher in sows fed with high levels of protein and energy. Sows that were fed diet with 750 g of protein and 60 MJ ME had a protein content in the liver of 18.86%, 14.19% in the kidney, and 17.50%. in the heart. Increasing the energy level of the food with 70 MJ ME causes increase of the protein content in vital internal organs, the liver 19.22%, kidney 15.03% and heart 17.70%. The content of protein in the internal organs was increased in diet with increased protein in the diet. At the protein level of 950 g and 60 MJ ME protein content amounts to 19.03% in liver, 14.41% in kidney and 18.44%. in the heart. With the increase of the energy level to 70 MJ ME the same level of protein leads to an increase of protein in internal organs, in the liver 19.58%, in the kidney 14.91% and 18.99% in the heart. Increasing the amount of food in the last stage of pregnancy had no significant impact on the content of protein levels of vital internal organs.

References

1. Aherne FX and Williams IH 1992. Nutrition for optimizing herd performance. Vet. Clinics N. America: Food Animal Practice 8, 589–608.
2. Beuković M 1999. Efekat nivoa proteina u ishrani mladih krmača tokom laktacije. Doktorska disertacija. Univerzitet u Novom Sadu, Poljoprivredni fakultet.
3. Boyd RD, Touchette KJ, Castro GC, Johnston ME, Lee KU and Han IK 2000. Recent advances in amino acid and energy nutrition of prolific sows- An Invited Review. Asian-Aus J. Anim. Sci. 13:1638
4. Close WH, Turnley K 2004. Creating technical and educational forums that help pig producers meet performance and economic goals: the Premier Pig Program™.In: Biotechnology in the Feed Industry, Proceedings of the 20™ Annual Symposium (eds.T.P. Lyons and K.A. Jacques) Nottingham University Press, Loughborough,UK. pp. 113-119.
5. Clowes EJ, Aherne FX, Foxcroft GR and Baracos VE 2003. Selective protein loss in lactating sows is associated with reduced litter growth and ovarian function. J. Anim. Sci. 81, 753–764.
6. Dean J, Nortey T and Anil L 2005. How should we compare levels of sow attrition. University of Minnesota Group.
7. Eissen JJ, Kanis E and Kemp B 2000. Sow factors affecting voluntary feed intake during lactation. Livestock Production Science 64, 147-165.
8. Eissen JJ, Apeldoorn EJ, Kanis E, Verstegan MWA and de Greef KH 2003. The importance of a high feed intake during lactation of primiparous sows nursing large litters. Journal of Animal Science 81, 594-603.
9. Foxcroft GH, Zak L, Aherne FX and Kirkwood RN 1997. Management of the early weaned sow. In: Proceedings of the Banff Pork Seminar, Advances in Pork Production. Banff, Canada. 8, 117-135.

10. Kim SW and G Wu 2008. Regulatory role for amino acids in mammary gland growth and milk synthesis. Amino Acids doi:10.1007/s00726-008-0151-5
11. Kongsted AG 2005. Effect of energy intake on pregnancy rate and litter size with particular reference to group housed non-lactating sows-a review. Department of Agroecology, Danish Institute of Agricultural Sciences, P.O. Box 50, DK-8830 Tjele.
12. Kovčin S, Stančić B, Gagrčin M, Radović I 2005. Faktori koji utiču na polno sazrevanje nazimica. Simpozijum: »Stočarstvo, veterinarstvo i agroekonomika u tranzicio-nim procesima» Herceg Novi 19–24. juni. Zbornik radova, strana 30.
13. McNamara JP and Pettigrew JE 2002. Proteins and fats used in lactating sows: I. Effects on milk production and body composition. J. Anim. Sci. 80, 2442-2451.
14. Rosendo A, Druet T, Gogue J, Canario L and Bidanel JP 2007. Correlated responses for litter traits to six generations of selections for ovulation rate or prenatal survival in French Large White pigs. J. Anim.Sci. 85, 1615-1624.
15. Rupić V 2005. Reprodukcija domacih životinja, Hrvatska mljekarska udruga, Zagreb.
16. Smits R 2003. Sow reproductive performance- a snapshot of the present With a view to the future. In: Biotechnology in the Feed Industry, Proceedings of the 19™ Annual Symposium (eds. T.P. Lyons and K.A.Jacques) Nottingham Universit Press, Loughborough, UK. pp. 237-246.
17. Thaker MYC and Bilkei G 2005. Lactation weight loss influences subsequent reproductive performance of sows. Animal Reproduction Science. 88, 309-318.
18. Trottier LN and Johnston LJ 2000. Feedeng gilts during development and sows during gestation and lactation. In: Lewis J.A., L.L. Southern: Swine nutrition.
19. Trottier NL and Johnston 2005. Feeding gilts during development and sows during gestation and lactation. In:Swine Nutrition (Ed. A. J. Lewis and L. L. Southern), 2nd Ed. CRC Press, New York. pp. 726-760.
20. Whittemore CT 1987. Tactics and strategies for the nutrition of breeding sows. In: Elements of Pig Science. Longman Hanbook in Agriculture, London. pp. 105-117.
21. Whittemore CT 1998. Influence of pregnancy feeding on lactation performance. In: Verstegen MWA, Moughan PJ and Scharma JW (editors): The Lactating Sow. Wageningen Pers, PO Box 42, The Netherlands.
22. Wu G, Bazer FW, Wallace JM and Spencer TE 2006. Intrauterine growth retardation: Implications for the animal sciences. J. Anim. Sci. 84, 2316–2337.
23. Yang H, Pettigrew JE, Johnson LJ, Shurson GC, Wheaton JE, White ME, Koketsu Y, Sower SF and Rathmacher JA 2000. Effects of dietary lysine intake during lactation on blood metabolites, hormones, and reproductive performance in primiparous sows. J. Anim. Sci. 78, 1001–1009.

Original paper

EFFECT OF CONSECUTIVE CUT AND VEGETATION STAGE ON CNCPS PROTEIN FRACTIONS IN ALFALFA (*Medicago sativa* L.)

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Abstract

Crude protein (CP) of forages can be separated into fractions of differentiated abilities to provide available amino acids in the lower gut of ruminants. This knowledge is critical to develop feeding systems and to predict animal responses. The objective of this research was to asses whether CP concentrations and the relative proportion of CP fractions by CNCPS in alfalfa (*Medicago sativa* L.) *cv* K-28 were affected by different cuts and vegetation stages. Fraction B_2, which represents true protein of intermediate ruminal degradation rate, was the largest single fraction in all cuts except in the third cut. Soluble fraction A was less than 400 g kg^{-1} CP in all cuts except in the third cut, while the unavailable fraction C ranged from 56 g kg^{-1} CP in the first cut to 134.8 g kg^{-1} CP in the fourth cut. The remaining fraction B_3 (true protein of very low degradation rate) only represented less than 60 g kg^{-1} of total CP. Results showed that undegraded dietary protein represented a small proportion of total CP in alfalfa from the first to the fourth cut.

Key words: *alfalfa, CNCPS, cut, protein*

Introduction

Livestock enterprises are significant contributors to nonpoint sources of environmental N pollution because of their contributions to ammonia emissions and nitrate contamination of surface and ground water (NRC, 1993). Purchased feed, especially protein supplements, is a major source of imported nutrients and farm expenses on dairy farms (Klausner et al., 1998). Under these economic and environmental constraints, improving the efficiency of N utilization and thus reducing N excretion are very important to maintain the sustainability of dairy farms, and nutrition models became an effective farm management tool to accomplish these tasks (Dinn et al., 1998; Wattiaux and Karg, 2004).

Milk production will be reduced when protein supplied by the diet is below energy-allowable milk production, which is affected by protein degradation rates (Fox et al., 2004). Feed protein fractionation systems have been integrated into nutrition models to account for differences in protein availability and utilization. The in situ techniques and schemes based on solubility in buffers and detergent solutions have been adopted by the NRC (2001) and the

Cornell Net Carbohydrate and Protein System (CNCPS; Fox et al., 2004) to measure protein fractions in feeds.

In the CNCPS the CP (Crude Protein) of feeds for ruminants is divided into three fractions: the non-protein nitrogen (NPN, PA), the true protein (PB) and the combined protein (PC) (Sniffen et al., 1992), of which the true protein (PB) is further divided into PB_1, PB_2 and PB_3. The PA and PB_1 are soluble in buffer and highly degradable, whereas PB_3 and PB_2 are combined with plant cell wall with different degradabilities in the rumen. Although the calculation of PA, PB_1, PB_2, PB_3 and PC is based on the chemical analysis of the CP, the soluble crude protein (SCP), the neutral detergent insoluble crude protein (NDICP) and the acid detergent insoluble crude protein (ADICP) of feeds, PA, PB_1, PB_2, PB_3 and PC have been closely related to the rumen degradation characteristics of feeds (Sniffen et al., 1992).

The objective of this study was to determine the effect of cutting alfalfa at different periods of vegetation on protein fractions determined as CNCPS.

Materials and methods

The experiment was designed as a factorial trial, by randomized block system in three replicates. Samples of alfalfa, *cv* K 28 was taken on May 4th at mid-bud stage in the first cut, on June 6th at early flowering in the second cut, on July 6th at mid-bloom in the third cut and on August 8th at full bloom in the fourth cut. Dry matter was determined by drying out samples at 65º C and grinding and sieving them to 1 mm particle size. The samples were dried in an oven at 105º C for 6 h for dry matter determination.

The CP of the samples was determined using Kjeldahl method. The NPN, NDICP, ADICP, SolP, TP (True protein) and IP (Insoluble protein) were determined by Licitra et al. (1996). The CP, NPN, SolCP, NDICP, ADICP, TP and IP were calculated as follows:

CP = Total N x 6.25

NPN = (Total CP – Residual CP_{NPN})/CP x 1000

SolCP = (Total CP – Residual CP $_{SolCP}$)/CP x 1000

ADICP = Residual CP_{ADICP}/CP x 1000

NDICP = Residual CP_{NDICP}/CP x 1000

TP = Residual CP_{NPN}/CP x 1000

IP = Residual CP_{SolCP}/CP x 1000

NPN_{SolCP} = NPN/SolCP x 1000

Where, CP is the crude protein, NPN - non-protein nitrogen (g kg^{-1} CP); SolCP, the soluble crude protein (g kg^{-1} CP); NDICP, the neutral detergent insoluble crude protein (g kg^{-1} CP); ADICP, the acid detergent insoluble crude protein (g kg^{-1} CP); TP – true protein (g kg^{-1} CP); IP – insoluble crude protein (g kg^{-1} CP) and NPN_{SolCP}, (g NPN kg^{-1} $SolCP^{-1}$).

The CNCPS crude protein fractions of the samples, PA, PB, PB_1, PB_2, PB_3 and PC were calculated based on CP, NPN, SolCP, NDICP, ADICP contents of samples according to Sniffen et al. (1992).

PA = NPN

PB_1 = SolCP – NPN

PB_2 = CP – SolCP – NDICP

PB_3 = NDICP – ADICP

PB = 1000 – PA – PC

PC = ADICP

Where, PA refers to the non-protein nitrogen (g kg^{-1} CP); PB_1, the rapidly degraded crude protein (g kg^{-1} CP); PB_2, the intermediately degraded crude protein (g kg^{-1} CP); PB_3, the slowly degraded crude protein (g kg^{-1} CP) and PC, the bound crude protein (g kg^{-1} CP).

Data were processed by the analysis of variance in a randomized block design. Effects were considered different based on significant ($P< 0.01$) F ratio.

Results and discussion

The analyses of variance (Table 1) revealed a statisticaly significant effects of the cut on crude protein content in dry matter of alfalfa. The cut was an important source of variability for all investigated protein fractions. Alfalfa had the highest content of crude protein at mid-bud stage in the first cut (199.1 g kg^{-1} DM) and the lowest content of CP at mid-bloom stage in the third cut. This is in agreement with Taylor and Quesenberry (1996) who reported that in early spring young plants of alfalfa have a large proportion of leaves, a high moisture content, protein and minerals.

The highest contents of NDICP and ADICP were in full-bloom stage in the fourth cut. Yari et al. (2012) concluded that alfalfa at the flowering stage had higher content of NDF, ADF and NDICP compared to alfalfa at early and late bud stage. The contents of IP were similar in the first and the second cut of alfalfa, but the lowest content was observed at the mid-bloom stage in the third cut. The values for buffer soluble CP in alfalfa were slightly lower than those reported by Sniffen et al. (1992) and Yari et al. (2012) for spring growth in alfalfa. Results in this investigation showed that all content of SolCP is represented by NPN.

Table 1. *Content of crude protein fractions in alfalfa, cv K 28, g kg^{-1} CP*

Cut	CP	NDICP	ADICP	IP	SolCP	TP	NPN	NPN_{SolCP}
I	199.1^{a}	104.0^{c}	55.9^{d}	653.6^{a}	346.3^{c}	588.1^{b}	411.8^{c}	1000.0^{a}
II	198.3^{ab}	120.6^{b}	90.7^{b}	656.8^{a}	343.5^{c}	675.4^{a}	324.5^{d}	942.8^{b}
III	182.2^{c}	77.5^{d}	70.6^{c}	496.8^{c}	503.1^{a}	471.1^{d}	528.9^{a}	1000.0^{a}
IV	198.0^{b}	194.6^{a}	134.8^{a}	629.1^{b}	370.8^{b}	553.3^{c}	454.9^{b}	1000.0^{a}

Different letters denote significantly different means ($P< 0.01$)

In models designed to asses utilization of dietary protein by ruminants, it is assumed that most of the soluble protein (PA and PB_1) is completely degraded in the rumen, and varying proportions of insoluble fractions (PB_2, PB_3 and PC) escape ruminal degradation depending on the interactive effects of digestion and passage (Sniffen et al., 1992). Because various

protein fractions differ in rate and extent of ruminal degradation, the proportions of these different protein fractions in alfalfa are believed to influence the amounts of ruminally degraded and escape protein consumed by animals (Elizalde et al., 1999). The results of these protein fractions by CNCPS are presented in Table 2.

Table 2. *Content of crude protein fractions in alfalfa, cv K 28 by CNCPS, g kg^{-1} CP*

Cut	PA	PB	PB_1	PB_2	PB_3	PC
I	346.3^{c}	597.7^{a}	0.0^{b}	549.5^{a}	48.1^{a}	55.9^{d}
II	323.8^{c}	585.7^{a}	19.3^{a}	536.1^{a}	29.9^{b}	90.7^{b}
III	503.1^{a}	426.3^{c}	0.0^{b}	419.4^{b}	7.0^{c}	70.4^{c}
IV	370.8^{b}	494.3^{b}	0.0^{b}	434.5^{b}	59.9^{a}	134.7^{a}

Different letters denote significantly different means (P< 0.01)

The results indicate that alfalfa cut at mid-bloom stage in third cut had the highest rapidly degradable NPN fraction-PA fraction of crude protein. The highest content of undegradable PC fraction, associated with the lignin and cell wall was observed at full-bloom stage in the fourth cut. The slowly degradable PB_3 fraction associated with the plant cell wall was the lowest at mid-bloom stage of alfalfa in the third cut. PB_2 fraction, which is intermediately degradable in the rumen was the highest protein fraction in all the cuts of alfalfa. Values for this fraction were similar in the first and the second cut, but higher than values for PB_2 fraction in the third and the fourth cut.

The protein fractions of alfalfa in this study differed from tabular values in NRC (2001). Fraction PA was higher, fraction PB ($PB = PB_1 + PB_2 + PB_3$) was lower and fraction PC was higher that the tabular value in NRC (2001), except for the value of PC fraction in the first cut. Sniffen et al. (1992) found that the fraction PB_2 was the largest CP fraction in alfalfa, with a mean value of 41% of the total CP, which is in agreement with our results in the third and the fourth cut. Elizalde et al. (1999) reported a PB_2 value of 51.6% of the total CP in alfalfa, which is in agreement with our results in the first and the second cut. The values for PB_3 fraction of alfalfa in this study were similar to the results obtained by Elizalde et al. (1999) who reported that this value amounted to 3% of CP in alfalfa. From a nutritive point of view, PC fraction appears to be essentially indigestible and the amount apparently digested is poorly used by ruminant animal (Sniffen et al., 1992). In this study, the proportion of fraction PC averaged approximately 9% of the total CP which is higher than the results obtained by Cherney et al. (1992) for alfalfa (1.8-4.6% CP).

Conclusion

Cutting alfalfa at different cuts and different vegetation stages had profound influence on the protein fractions as determined by CNCPS. The present data indicate that alfalfa from different cuts and different vegetation stages differ in proportions of protein fractions, which account for different rumen degradation characteristics.

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References

1. Cherney DJR., Volenec JJ and Cherney JH 1992. Protein solubility and degradation *in vitro* as influenced by buffer and maturity of alfalfa. Anim. Feed Sci. technol, 37, 9-20.
2. Dinn NE, Shelford JA and Fisher LJ 1998. Use of the Cornell Net Carbohydrate and Protein System and rumen-protected lysine and methionine to reduce nitrogen excretion from lactating dairy cows. Journal of Dairy Science, 81, 229-237.
3. Elizalde JC, Merchen NR and Faulkner DB 1999. Fractionation of fiber and crude protein in fresh forages during the spring growth. J. Anim. Sci., 77, 476-484.
4. Fox DG, Tedeschi LO, Tylutki TP, Russell JB, Van Amburgh ME, Chase LE, Pell AN and Overton TR 2004. The Cornell Net carbohydrate and protein System model for evaluating herd nutrition and nutrient excretion. Animal Feed Science and Technology. 112, 29-78.
5. Klausner SD, Fox DG, Rasmussen CN, Pitt RE, Tylutki TP, Wright PE, Chase LE and Stone WC 1998. Improving dairy farm sustainability I: An approach to animal and cop nutrient management planning. J. prod. Agric, 11, 225-233.
6. Licitra G, Hernandez TM and Van Soest PJ 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science Technology, 57, 347-358.
7. NRC 1993. Soil and water quality: An agenda for agriculture. National Academic Press, Washington, DC.
8. NRC 2001. Nutrient Requirements for Dairy Cattle. National Academic Press, Washington, DC.
9. Sniffen CJ, O'Connor DJ, Van Soest PJ, Fox DG and Russell JB 1992. A net carbohydrate and protein system for evaluating cattle diets. II: Carbohydrate and protein availability. J. Anim. Sci., 70, 3562-3577.
10. Taylor NL and Quesenberry KH 1999. Red clover science. Kluwer, Academic Publishers.
11. Wattiaux MA and Karg KL 2004. Protein level for alfalfa and corn silage-based diets: II. Nitrogen balance and manure characteristics. Journal of Dairy Science. 87, 3492-3502.
12. Yari M, Valizadeh R, Naserian AA, Ghorbani GR, Moghaddam PR, Jonker A and Yu P 2012. Botanical traits, protein and carbohydrate fractions, ruminal degradablity and energy contents of alfalfa hay harvested at three stages of maturity and in the afternoon and morning. Anim. Feed Sci. Technol., 172, 162-170.

Original paper

EFFECT OF DIFFERENT LEVELS OF *LAVANDULA STOECHAS* ESSENCE ON PRODUCTION PERFORMANCE AND EGG QUALITY OF LAYING HENS

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Abstract

The objective of this study was to evaluate the effects of different levels of *Lavander stoechas* essence on performance and egg quality of laying hens for 8 weeks. This experiment was conducted with 160 Hy-Line (W36) laying hens (30 wks of age), randomly divided into 4 experimental groups, 5 replicates and 8 birds per each (2 cages for each replicate and 4 birds in each cage). Treatments were control (without *Lavander* essence), and levels of 200, 400, and 600 ppm *Lavander* essence. All hens were provided the layer diets (2870 kcal/kg ME and 15.5 % CP) ad- libitum and received 16 h of light/ 8 h of dark. Performance parameters including egg weight and egg production were recorded daily and feed intake, feed conversion ratio (grams of feed:grams of egg) and egg quality were measured weekly. The following characteristics were recorded for the individual eggs: weight, shape index, eggshell breaking strength, shell thickness, yolk color, and Haugh unit. Levels of 200 and 400 ppm of *Lavander* essence showed a significant increase in production percentage and egg weight but these parameters decreased at level of 600 ppm. Feed intake was highest for control, 200 and 400 ppm essence dietary levels but level of 600 ppm showed significant decrease. Addition of essence to the diet had no effect on FCR. Shape index and Haugh unit were not significantly affected by the treatments. Higher yolk color score was observed in treatments containing *Lavander* essence in comparison with control ($p<0.05$). Levels of 200 and 400 ppm essence increased significantly egg shell quality including thickness, weight and shell breaking strength. The results showed that the addition of essences of *Lavender* up to 400 ppm increased production performance and improved egg quality.

Key words: *egg quality, Lavender essence, laying hens, performance*

Introduction

In the commercial egg type chicken industry profits depend on the cost and nutritive value of the feed. One of the promoting enhances productive performance of layers are antibiotics. Antibiotics have been used as growth promoting substance. However, the use of antibiotics as feed additives is risky due to not only cross-resistance but also to multiple resistances in pathogens (Bach Knudsen, 2001; Schwarz et al., 2001). Consequently, the animal feed

industry is under increasing consumer pressure to reduce the use of antibiotics as a feed additive and to find substitutes for antibiotics in the diet (Hertrampf, 2001; Humphrey et al., 2002). Many scientists have searched for alternatives to antibiotics (Langhout, 2000; Mellor, 2000; Wenk, 2000; Kamel, 2001). Recently, it has been found that natural additives such as herbs and edible plants have some properties as growth enhancers to replace antibiotics. These additives are given to animals or birds to improve their physiological and productive performance. The antimicrobial effect of the medicinal plants is well documented (Valero and Salmeron, 2003). Lavander stoechas is a flowering plant in the family of *Lamiaceae*. The medicinal parts are the essential oil from the fresh flowers and/or the inflorescence, the flowers collected just before opening and dried, the fresh flowers and the dried flowers. Lavender oil has been reported to contain more than 100 components. The essential oil (1 to 3%) of *Lavandula* is rich in linalool and linalyl acetate. Further aroma components are β-ocimene, cineol, camphor and caryophyllene epoxide. Linalyl acetate is the major compound found in flowers. The plant contains also rosmarinic acid, and coumarin. Because no information is available about the administration of *Lavandula* in laying hens diet, this experiment was conducted to investigate the effect of using Lavander stoechas as additive on productive traits and egg quality traits of laying hens.

Material and methods

Birds and housing

One hundred and sixty Hy-Line (W36) laying hens (30 wks of age) were individually weighed and randomly housed in cages and allotted for four dietary treatment groups of five replicates and six birds in each replicate for ten weeks (three birds in each cage and two cages for each replicate). Two weeks were for adaptation and eight weeks for sampling. The birds were maintained under commonly 16 h light:8 h dark cycle throughout the experimental period. Hen house temperature was 17- 20°C during the experiment. Feed and water were offered ad-libitum. Treatments were basal diet (Corn-Soybean diet with 2870 kcal/kg ME and 15.5 % CP) and increasing levels of Lavander stoechas essence (200, 400 and 400 ppm) added to basal diet. The experimental diets were in mash form and formulated to meet or exceed NRC (1994) recommendations.

Productive and egg quality traits

Egg production performance was expressed as a percentage of hen-day egg production. During the entire experimental period, eggs from each replicate were collected daily and weighed to determine average egg weight. Feed intake was recorded on a weekly basis. Egg mass and feed conversion ratios were calculated as below:

Daily egg mass (g/hen) = hen-day egg production (%) × egg weight (g)/100.

Feed conversion ratio (g feed/g egg) = daily feed intake (g/hen)/daily egg mass (g/hen).

To evaluate egg quality, 10 eggs from each treatment every week were randomly collected. Eggs were weighed individually, then broken and the inner contents were placed on a leveled glass surface to determine the inner egg quality.

Eggshell thickness, eggshell strength, yolk colour score, Haugh unit (HU) and albumen height as egg quality parameters were examined. When determining egg quality characteristics, the

samples of eggs were individually weighed at initiation, and then egg shell breaking strength was measured using egg shell tester equipment (Futura resistant tool), and expressed as unit of compression force exposed to unit egg shell surface area (kg/cm^2). Then, eggs were cracked and carefully separated from the shells. Egg shell thickness (with membrane) was measured at three different points (i.e. the top, middle and bottom) using a micrometer (model IT-014UT, Mitutoya, Kawasaki, Japan). The average of three different thickness measurements from each egg was used to describe egg shell thickness. The shell was weighted and the relative proportion of shell was determined. Albumen height was measured by Futura equipment. A Haugh unit was calculated according to the formula given as follows:

Haugh unit = 100 log [albumen height (mm) + 7.57 - 1.7 egg weight 0.37 (g)]

Yolk colour was determined according to the Roche Yolk Colour fan.

All the results were statistically analyzed by General Linear Models (GLM), one way analysis of variance, using SAS software (SAS Institute, 1999). Differences among means were separated using Duncan's multiple range test (Duncan, 1955).

Results and discussion

Productive traits

Table 1. *Effect of treatments on performance parameters of laying hens during the experiment*

Level of essence (ppm)	Egg Weight (g)	Production Percentage (d/h)	Feed Intake (g/h/d)	Feed Conversion Ratio
0	57.30^{b}	87.18^{ab}	100.4^{a}	2.01
200	58.47^{a}	88.03^{a}	100.2^{a}	1.92
400	58.44^{a}	87.12^{ab}	99.4^{a}	1.96
600	57.25^{b}	85.58^{b}	94.6^{b}	1.92
SEM	0.41	2.16	1.87	0.05

^{a,b} Column means with different superscripts differ significantly ($P < 0.05$)

The productivity data of laying hens are summarized in Table 1. Data from Table 1 indicate that egg weight during the experiment was significantly affected by treatments. It can be observed from this table that hens fed 200 and 400 *ppm* of *Lavandula* essence had higher egg weight when compared with other treatments. Supplementation layer diet with different levels of *Lavandula* essence had no significant effect on production percentage compared to the control. But as numerical, the highest production percentage was found in 200 *ppm* essence that had significant effect with 600 *ppm*.

Supplementation layer diet with different levels of *Lavandula* essence had a significant effect on the amount of feed intake through the experiment. Hens fed the levels of 200 and 400 *ppm* of essence and control diet had significantly ($p<0.05$) greater feed intake than hens fed diet supplemented with 600 *ppm* essence. However, there was no significant ($p>0.05$) difference in feed intake between hens fed control and essence up to 400 *ppm* (Table 1). Feed conversion ratio was not affected by the treatments ($p>0.05$). The results of this experiment are in accordance with the results of Hassan *et al.* (2011) that observed increase in egg weight, feed intake and production percentage by using Eucalyptus powder in the diets of quail. Previous studies reported that plant extract supplementation to diet showed different effect on egg production and egg quality of laying hens. This might stem from the amount of plant extract

and the source of it. Our findings related to egg production traits did not confirm some earlier works that indicated beneficial effects from essential oils in the diets for laying hens. In our study, egg weights of 200 and 400 *ppm* essence were significantly higher compared to control treatment. Bozkurt *et al*. (2009) showed that mixture of essential oil supplementation in diet did not affect egg production and egg weight of broiler breeders. In another study (Botsoglou *et al*., 2005), the effects of dietary aromatic plant extracts on the laying performance of hens from 32 to 40 wk of age were investigated and the results showed no significant differences in egg production and egg weight among the treatment groups.

Egg quality traits

Table 2. *Effects of treatments on egg quality of laying hens during the experiment*

Level of essence (ppm)	Haugh unit	Egg yolk colour score[1]	Breaking strength (kg/cm^2)	Eggshell thickness (mm)	Eggshell weight (g)	Eggshell weight ratio (%)
0	79.84	6.06^{b}	1.67^{c}	0.341^{b}	5.66^{b}	9.87^{b}
200	80.76	7.46^{a}	2.22^{a}	0.374^{a}	6.20^{a}	10.60^{a}
400	81.36	7.31^{a}	2.04^{ab}	0.368^{a}	5.97^{ab}	10.20^{ab}
600	79.70	7.03^{a}	1.88^{bc}	0.353^{b}	5.86^{b}	10.23^{ab}
SEM	1.18	0.17	0.094	0.120	0.12	0.35

[a,b] Column means with different superscripts differ significantly ($P < 0.05$); [1] Roche yolk colour score: 1 light yellow; 15 orange

Supplementation layer diet with different levels of *Lavandula* essence had no significant effect on Haugh unit (Table 2). Egg yolk colour was affected by dietary treatments. Yolk colour of the essence groups was higher compared to the control. The outer egg quality parameters (eggshell weight, eggshell thickness, eggshell weight ratio and eggshell strength) were affected by dietary treatments ($p<0.05$) (Table 2). Layers fed diets supplemented with 200 *ppm* essence had highest eggshell weight among treatments. Eggshell weight ratio was significantly increased by increasing levels of essence in the diet ($p<0.05$). Layers fed diets supplemented with *Lavandula* essence (200 and 400 *ppm*) had thicker egg shell, while the lowest one was detected for control diet and 600 *ppm* essence. Layers fed diets supplemented with 200 *ppm* essence had highest breaking strength among treatments. The results of our study showed significant differences in eggshell weight, eggshell weight ratio, eggshell thickness, eggshell strength and egg yolk colour among treatments. However, there were no significant differences in Haugh unit score. Since there is not enough information about *lavandula* essence on egg quality of layers we use the results of other plant extracts in poultry nutrition. Results reported herein are in agreement with those reported by Bacha *et al*. (1997) saying that anise is rich in mineral elements what plays an important role in increased egg shell intensities. In contrast with the result of our study, there are several studies that oregano essential oil supplementation in diet did not affect eggshell thickness (Botsoglou *et al*., 2005). On the other hand, Haugh unit results of our study agree with the results of Botsoglou *et al*. (2005) who reported that Haugh unit score was not affected by oregano essential oil supplementation of diet.

Conclusion

Lavandula essence showed a significant positive effect on performance of layers up to 400 *ppm* in diet. Moreover, *Lavandula* essence supplementation in diet increased eggshell quality

of layers. Collectively, these findings suggest that *Lavandula* essence supplementation in commercial layer diets might be beneficial up to 400 *ppm*. More detailed studies are still needed to determine the function of *Lavandula* essence supplementation to laying hen diets.

References

1. Bach Knudse KE 2001. Development of antibiotic resistance and options to replace antimicrobials in animal diets. Proceedings of the Nutrition Society 60, 291-299.
2. Bacha MA, Ali MA and Farahat FA 1997. Chemical composition of pollen grains of some date palm males grown in Riyadh, Saudi Arabia. Arab Gulf Journal of Scientific Research 15, 783-803.
3. Botsoglou NA, Florou-Paneri P, Botsoglou E, Datos V, Giannenas I, Koidis A and Mitrakos P 2005. The effect of feeding rosemary, oregano, saffron and α-tocopheryl acetate on hen performance and oxidative stability of eggs. South African Journal of Animal Science 35, 143-151.
4. Bozkurt M, Alçiçek A, Çabuk M, Küçükyilmaz K and Çatli AU 2009. Effect of an herbal essential oil mixture on growth, laying traits, and egg hatching characteristics of broiler breeders. Poultry Science 88, 2368-2374.
5. Duncan DB 1955. Multiple range and multiple F-Test. Biometrics 11, 1-42.
6. Hassan M S, El Sanhoury H, Ali MHWAH and Ahmed AMH 2011. Effect on using Eucalyptus levels as natural additives on productive, physiological, immunological and histological performance of laying Japanese quail. Egyptian Poultry Science 31, 305-329.
7. Hertrampf JW 2001. Alternative antibacterial performance promoters. International Journal of Poultry Science 40, 50-52.
8. Humphrey BD, Huang N and Klasing KC 2002. Rice expressing lactoferrin and lysozyme has antibiotic-like properties when fed to chicks. Journal of Nutrition 132, 1214-1218.
9. Kamel C 2001. Tracing modes of action and the roles of plant extracts in non-ruminants. In: Recent advances in animal nutrition. Eds. Garnsworthy, PC, Wiseman J, Nottingham University Press, Nottingham pp. 135-150.
10. Langhout P 2000. New additives for broiler chickens. World Poultry-Elsevier 16(3), 22-27.
11. Mellor S 2000. Nutraceuticals–alternatives to antibiotics. World Poultry-Elsevier 16(2), 30-33.
12. NRC 1994. Nutrient Requirements of Poultry. ^{9}th revised Edition. National Academy Press. Washington, DC.
13. SAS Institute 1999. SAS User1's Guide. SAS Inst. Inc., Cary, NC.
14. Schwarz S, Kehrenberg C, and Walsh TR 2001. Use of antimicrobial agents in veterinary medicine and food animal production. International Journal of Antimicrobial Agents, 17, 431-437.
15. Valero M and Salmeron M C 2003. Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth. International Journal of Food Microbiology 85, 73-81.
16. Wenk C 2000. Recent advances in animal feed additives such as metabolic modifiers, antimicrobial agents, probiotics, enzymes and highly available minerals. Review. Asian - Australasian Journal of Animal Sciences 13, 86-95

Original paper

EFFECTS OF CADMIUM ON RUMEN FERMENTATION AND NUTRIENT DIGESTIBILITY USING DUAL FLOW CONTINUOUS CULTURE SYSTEM

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Abstract

This experiment was conducted to investigate the effects of different doses of cadmium on fermentation characterization and nutrient digestibility using dual flow continuous culture system. Eight dual-flow, continuous culture fermenters were used in 2 replicated periods of 10 d (7 d of adaptation and 3 d of sampling). Fermenters were inoculated with a composited ruminal fluid from 3 beef steers fed a high concentrate diet for at least 2 mo before the beginning of the trial. Anaerobic conditions were maintained by the infusion of N_2 at a rate of 40 mL/min. Temperature (38.5°C) and liquid (10%/h) and solid (5%/h) dilution rates were kept constant. Fermenters were fed daily with 120 g dry matter in three equal portions. Treatments arranged as complete randomized block design with control and addition three levels of cadmium (0.1, 1 and 10 mg/l) to the high concentrate diet (15:85 forage to concentrate ratio). Organic matter, dry matter, NDF and ADF digestibility significantly decreased with the addition of 1 and 10 mg/l cadmium to the culture media compered to control treatment ($P<0.05$). Cadmium significantly decreased total volatile fatty acids concentration, acetate proportion and acetate to propionate ratio. Propionate and butyrate proportion increased significantly in response to cadmium addition ($P<0.05$). Concentration of NH3-N was significantly decreased in 1 and 10 mg/l cadmium treatments compared with control treatment. The results of this study suggest that cadmium in doses of 1 mg/l or more had strong inhibitory effect on mixed rumen microorganisms.

Key words: *Cadmium, Digestibility, Rumen fermentation, Toxicity.*

Introduction

Heavy metals are well known to be toxic to most organisms when present in excessive concentrations (Giller et al., 1998). Cadmium, a heavy metal, is a member of group IIb in the periodic table of elements which is present in soils, sediments, air and water (Stoeppler, 1991). Cadmium (Cd) is a non-essential heavy metal which accumulates in mammals and is potentially toxic to both humans and animals. It accumulates in many agricultural crops, mainly as a result of phosphate fertilizer and sewage sludge use, and presents a significant

risk to humans consuming high levels of food products from hyper accumulators, such as offal products derived from grazing animals (Wilkinson et al., 2003). Cadmium is transported into microorganisms by the energy-dependent manganese or magnesium transport systems (Nies and Silver, 1999). Cd is highly toxic (Babich and Stotzky,1977). It competes with and replaces other functional metals inside cells (Hughes and Poole, 1989). It also brings about the denaturation of proteins, inhibits bacterial respiration and proton–solute cotransport, and causes single-stranded breaks in cellular DNA (Cunningham and Lundie, 1993). Many microorganisms have evolved mechanisms to tolerate and overcome Cd toxicity. A major mechanism for heavy metal resistance involves alterations in the membrane transport system of an organism, resulting in the reduction or denial of entry of Cd into the organism (Laddaga et al., 1985). Alternatively, the intracellular or extracellular sequestration of heavy metals by adsorption to cell walls (Mullen et al., 1989) or by binding to a specific biopolymer results in tolerance to heavy metal toxicity (Kurek et al., 1991). Heavy metals can be stimulatory, inhibitory, or even toxic for biochemical reactions, depending on their concentrations (Gikas and Romanos, 2006). The presence of many metals is required in trace amounts for the activation or functioning of many enzymes and coenzymes. Excessive amounts, however, can lead to inhibition or toxicity (Juliastuti et al., 2003). The various mechanisms of metal toxicity in microorganisms are (1) substitutive ligand binding, (2) redox reactions with sulfur groups, (3) Fentontype reactions, (4) inhibition of membrane-transport processes, and (5) electron siphoning (Harrison et al., 2007). The aim of this study was to detect the toxic effect of cadmium on rumen microbial fermentation and nutrient digestibility using dual flow continuous culture system.

Material and methods

Apparatus and experimental design

Eight dual-flow continuous culture fermenters (Hoover et al., 1976) were used in 2 repeated periods of 10 d each (7 d of adaptation, 3 d of sampling). Fermenters were inoculated with a composited ruminal fluid taken after slaughter from 3 beef cattle fed a high-concentrate diet. Donor animals were therefore different in each period. Temperature (38.5°C) and liquid (0.10/h) and solid (0.05/h) dilution rates were maintained constant. Anaerobic conditions were maintained by the infusion of N2 at a rate of 40 ml/min. Artificial saliva (Weller and Pilgrim, 1974) was continuously infused into flasks and contained 0.4 g/l of urea to simulate recycled N. Fermenters were fed 97g of DM/d in 3 equal portions, at 08:00, 16:00, and 24:00 h.

Treatments arranged as complete randomized block design with control and addition three levels of cadmium (0.1, 1 and 10 mg/l) to the high concentrate diet (15:85 forage to concentrate ratio).

Sample collection, processing and chemical analysis

During sampling days, collection vessels were maintained at 4°C to impede microbial action. Solid and liquid effluents were mixed and homogenized for 1 min, and a 500-ml sample was removed via aspiration. Upon completion of each period, effluent from the 3 d of sampling was composited and mixed within fermenter and homogenized for 1 min. Subsamples were taken for total N, ammonia-N, and VFA analyses. The remainder of the sample was dried. Dry samples were analyzed for DM, ash, NDF and ADF.

Dry samples were ashed overnight at 550°C in a muffle furnace (AOAC 1990; ID 942:05). Ether extract was analyzed by Soxhelet, and total N was determined as described by AOAC (1990; ID 976.05) procedures. The NDF were analyzed sequentially by the

detergent system (Van Soest et al., 1991). Samples for VFA were prepared using 4-methylvaleric acid (Aldrich Chemical Company, Milwaukee, WI, USA) as the internal standard. The analysis was performed by GLC using a polyethylene glycol nitroterephthalic acid-treated capillary column. A 4-ml subsample of filtered fluid was acidified with 4 ml of 0.2 N HCl and frozen. Samples were centrifuged at 25000 × g for 20 min, and the supernatant was analyzed for ammonia-N (Chaney and Marbach, 1962).

Statistical analysis

Data were analyzed as a randomized block design using PROC MIXED of SAS (version 8.1; SAS Inst., Inc., Cary, NC). Differences in average between treatments were declared at P <0.05 using Tukey's multiple comparison test, and least squares means for treatments are shown.

Results and discussion

Table 1. *Effects of Cadmium on Apparent nutrient digestibility in continuous culture*

Parameters	Control	0.1 mg Cd/l	1.0 mg Cd/l	10 mg Cd/l	SEM	P value
DM	53.2[a]	52.1[a]	50.8[b]	22.6[c]	1.40	0.001
OM	47.8[a]	46.4[a]	45.3[b]	18.4[c]	1.01	0.001
NDF	40.1[a]	39.6[a]	36.2[b]	14.3[c]	2.12	0.001
ADF	38.4[a]	38.2[a]	34.9[b]	11.6[c]	2.33	0.001

[a,b,c] Means within a row with different superscripts differ (P < 0.05)

Addition of 1 and 10 mg Cd/l significantly decreased apparent dry matter, organic matter, NDF and ADF digestibility. Low level of Cd (0.1 mg Cd/l) had no significant effect on nutrient digestibility. It has been reported that sensitivity of ruminal bacteria to heavy metals is depends on bacterial species and conditions which affect their metabolic activity (Faixová and Faix, 2002; Mihaliková et al., 2009). Individual species of rumen bacteria differed from one another in their sensitivities to various heavy metals (Forsberg, 1978). Salem et al (2010) reported when the inhibitory effect of Cd was examined on separated bacterial and protozoal fractions, it was more inhibitory to bacteria.

Although Cd is extremely toxic, some bacteria can overcome its adverse effects or resist its presence at low concentrations. Divalent ion transport systems are normally required to transport essential metals such as magnesium, phosphate, and sulfate (Laddaga et al., 1985). Nutrient metal transport systems are often up-regulated in times of need or starvation. An adverse consequence of this is the co-transport of other cations that may be toxic to the organism. Sensitive bacteria can accumulate 3 to 15 times more Cd(II) than resistant bacteria (Laddaga et al., 1985). Cadmium resistance occurs through all of the biochemical resistance mechanisms with the exception of enzymatic detoxification. This metal will not move out of the cell; instead they will remain to undergo oxidation back to its original form (Nies, 1992). Resistance to Cd(II) can be mediated by chromosomes, plasmids, or transposons (Lebrun et al., 1994). The most prominent metal resistance system for Cd(II) is by effux pumps. The reduction in nutrient digestibility at levels of 1 and 10 mg Cd/l concentration showed that mixed rumen anaerobic microorganisms could not tolerate these levels of heavy metal and the digestion process corrupted by addition of Cd. The results is supported by the investigation conducted by Yue et al., (2007) reported severe decrease in anaerobic digestion of cattail by the 1.6 mg/l Cd addition to the culture medium.

Table 2. *Effects of Cadmium on total and individual volatile fatty acids (VFA) and ammonia nitrogen in continuous culture*

Parameters	Control	0.1 mg Cd/l	1.0 mg Cd/l	10 mg Cd/l	SEM	P value
Total VFA, m*M*	112.1[a]	105.2[b]	103.6[b]	41.3[c]	5.3	0.001
VFA, mol/100 mol						
Acetate	52.5[a]	44.6[b]	41.3[bc]	36.9[c]	2.35	0.004
Propionate	24.4[d]	28.9[c]	30.5b[c]	33.4[a]	1.97	0.009
Butyrate	17.2[c]	19.9[b]	21.2[b]	24.0[a]	1.38	0.021
Valerate	2.35	3.44	3.88	3.49	0.71	0.039
Isovalerate	3.61[a]	3.07[a]	3.14[a]	2.14[b]	0.58	0.019
C2:C3[1]	2.15[a]	1.54[b]	1.36[bc]	1.11[c]	0.17	0.001
NH3-N, mg/100 ml	13.1[a]	10.5[b]	5.45[c]	4.12[c]	1.39	0.001

[a,b,c] Means within a row with different superscripts differ ($P < 0.05$); [1]C2:C3 = acetate to propionate ratio

Addition of Cd affected total VFA concentration, and the molar proportions of acetate and propionate ($P < 0.05$; Table 2). Total VFA concentration decreased with increasing level of Cd in the culture medium. Molar proportion of acetate (mol/100mol) was greatest in the control diet (52.5) followed by 0.1 mg Cd/l (44.6), 1 mg Cd/l (41.3) and 10 mg Cd/l (36.9) having the lowest acetate proportion among treatments. Propionate concentration was lowest in the control treatment and greatest in 10 mg Cd/l, and consequently acetate to propionate ratio was highest and lowest in control and 10 mg Cd/l treatment, respectively.

Data are lacking investigating the effects of Cd on VFA concentration and proportion. The results are probably due to the effects on different microorganisms fermenting different substrates. Forsberg (1977) reported resistance in *Streptococcus bovis* and *Megasfera elsdenii* to mercury. These microorganisms are able to ferment glucose to propionate. However at low concentrations, Cd had a stimulatory effect on some ruminal enzymes (Faixová and Faix, 2002). Nies (1999) showed that resistance to Cd in bacteria is based on Cd efflux and our results suggest that, due to their high toxic effects, Cd has limited beneficial effects on rumen bacteria. More research in needed for conclusion.

Cd addition significantly decreased NH3-N concentration. Reduction in urease activity of rumen microorganisms in the presence of Cd reported by Faixova and Faix (2002). In the conditions of the present experiment, it is likely that the reduced protein degradation may be related to the reduction in the digestibility of fiber associated with the protein within feeds. The undigested fiber within feeds will reduce the access of bacteria and enzymes to the protein, and therefore reduce protein degradation (Devant et al., 2000).

Conclusion

Cadmium addition to the culture medium significantly decreased nutrient digestibility and VFA production. The results of this study suggest that cadmium has strong inhibitory potential on mixed rumen microorganisms and feedstuff pollution with this metal could seriously interfere with normal rumen microbial fermentation.

References

1. AOAC. 1990. Offcial Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

2. Babich H and Stotzky G 1977. Sensitivity of various bacteria, including actinomycetes, and fungi to cadmium and the influence of pH on sensitivity. Applied Environmental Microbiology 33, 681–695.
3. Chaney AL and Marbach EP 1962. Modified reagents for determination of urea and ammonia. Clinical Chemistry 8,130–132.
4. Cunningham DP and Lundie LL 1993. Precipitation of cadmium by Clostridium thermoaceticum. Applied Environmental Microbiology 59, 7–14.
5. Devant M, Ferret A, Calsamiglia S, Casals R and Gasa J 2000. Effects of protein concentration and degradability on performance, ruminal fermentation, and nitrogen metabolism in rapidly growing heifers fed high-concentrate diets from 100 to 230 kg body weight. Journal of Animal Science 78, 1667–1676.
6. Faixova Z and Faix S 2002. Influence of metal ions on ruminal enzyme activities. Acta Veterinari Brno 71, 451–455.
7. Forsberg CW 1978. Effects of heavy metals and other trace elements on the fermentative activity of the rumen microflora and growth of functionally important rumen bacteria. Canadian Journal of Microbiology 24, 298–306.
8. Gikas P and Romanos P 2006. Effects of tri-valent (Cr(III)) and hexa-valent (Cr(VI)) chromium on the growth of activated sludge. Journal of Hazardous Materials B133, 212–217.
9. Giller KE, Beare MH, Lavelle P, Izac AMN and Swift MJ 1997. Agricultural intensification, soil biodiversity and ecosystem function. Applied Soil Ecology 6, 3-16.
10. Harrison JJ, Ceri H and Turner RJ 2007. Multimetal resistance and tolerance in microbial biofilms. Nature Review Microbiology 5, 928–938.
11. Hoover WH, Crooker BA and Sniffen CJ 1976. Effects of differential solid-liquid removal rates on protozoa numbers in continuous culture of rumen contents. Journal of Animal Science 43,528–534.
12. Hughes MN and Poole RK 1989. Metals and Micro organisms. Chapman and Hall, London, UK.
13. Juliastuti SR, Baeyens J, Creemers C, Bixio D and Lodewyckx E 2003. The inhibitory effects of heavy metals and organic compounds on the net maximum specific growth rate of the autotrophic biomass in activated sludge. Journal of Hazardous Materials B100, 271–283.
14. Kurek E, Francis AJ and Bollag JM 1991. Immobilization of cadmium by microbial extracellular products. Archive Environmental Contamination and Toxicology 20, 106–111.
15. Laddaga RA, Bessen R and Silver S 1985. Cadmium-resistant mutant of Bacillus subtilis168 with reduced cadmium transport. Journal of Bacteriology 162, 1106–1110.
16. Lebrun M, Andurier A and Cossart P 1994. Plasmid-borne cadmium resistance genes in ¸*isteria monocytogenes* are present on Tn5422, a novel transposon closely related to Tn917. Journal of Bacteriology 176, 3049-3061.
17. Mihalikova K, Varadyova Z, Pristas P, Javorsky P and Kisidayova S 2009. Tolerance of a ruminant ciliate Entodinium caudatum against mercury, copper and chromium. Biologia 64, 1141–1145.
18. Mullen MD, Wolf DC, Ferris FG, Beveridge TJ, Flemming CA and Bailey GW 1989. Bacterial sorption of heavy metals. Applied Environmental Microbiology 55, 3143–3149.
19. Nies D H 1992. Resistance to cadmium, cobalt, zinc, and nickel in microbes. Plasmid 27, 17-28.

20. Nies DH 1999. Microbial heavy-metal resistance. Applied Microbiology and Biotechnology 51, 730–750.

21. Salem AZM, Ammar H, Lopez S and Gohar MY 2010. Sensitivity of ruminal bacteria isolates of sheep, cattle and buffalo to some heavy metals. Animal Feed Science and Technology 163, 143–149

22. Stoeppler M 1991. Cadmium. In: Merian, E. (Ed.), Metals and their Compounds in the Environment. VCH, Weinheim, New York, Basel, Cambridge, pp. 803–851.

23. Van Soest PJ, Robertson JB and Lewis BA 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583–3597.

24. Wilkinson JM, Hill J and Phillips CJC 2003. The accumulation of potentially toxic metals by grazing ruminants. Proceeding of Nutrition Society 62, 267–277.

25. Yue ZB, Yu HQ and Wang ZL 2007. Anaerobic digestion of cattail with rumen culture in the presence of heavy metals. Bioresource Technology 98, 781–786.

Original paper

COMBINES WORK QUALITY IN MAIZE SILAGE PRODUCTION

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Abstract

The paper presents testing results of three silage combines employed in maize silage preparation in Toplica region. It is focused on determination of technical working parameters of tested machines. Achieved results verified the superiority of silage combine John Deere 5820, which produced the chopped mass having particle lengths of the smallest deviation with respect to the preset cutting length. In this case, the average length of chopped mass was 9.9 mm, having 69 % mass in the range up to 8 mm. The other two silage combines produced lower mass percentage of this fraction and larger variations of particle lengths with respect to the preset length. Minimum mass flow rate was evidenced for the silage combine Fortschrit E-286: 7.3 kg s^{-1} (26.3 t h^{-1}) and the surface productivity of 0.83 ha h^{-1}, at the average speed of 4.0 km h^{-1}. Maximum production rate was achieved with silage combine John Deere 5820: 10.9 kg s^{-1} (39.1 t h^{-1}) at average working velocity of 4.7 km h^{-1} and surface efficiency of 1.21 ha h^{-1}.

Key words: *combine, mass flow rate, operational productivity, silage, work quality*

Introduction

Maize silage is popular forage, mainly used in ruminant nutrition to substitute expensive concentrated food. Many factors affect the quality of prepared fodder, including climatic conditions during the season, temperature during preparation, as well as the state of the crop used for silage production (Ball et al., 2001). Nowadays, a variety of different types of silage combines exists, Petrović et al., 2012. Depending on particular circumstances, like crop yield and species, terrain conditions, etc., specific harvester system can be used: a tractor-powered machine, self-propelled combine or a self-loading forage wagon system. To make an optimal choice of applied system, relevant and reliable information is necessary (Marsh, 2011).

Relying on the adequate value of preset cutting length, forage harvester John Deere 5830 can provide high-quality chopped material (Jonhson et al., 2002). Preparation of high-quality silage demands application of adequate machines that ensure proper mass distribution of specific fractions with respect to chopping length of ensiled plants (Ott, 2000; Lisowski, 2006; Van and Heinrichs, 2008). Srivastava et al. (2006) claimed that proper selection and definition of governing parameters and choice of silage combine type should result in reaching the upper limit of the possible machine mass flow rate.
Koprivica et al. (2007) reported that silage combine Fortschritt E-281C at working speed of 2.2 km h^{-1} has achieved a mass flow rate of 6.6 t h^{-1} and surface productivity of 0.76 ha

h^{-1}. Increasing the operational velocity to 2.8 km h^{-1} gave higher capacity (9.0 t h^{-1}) and surface productivity of 0.95 ha h^{-1}. Calvin (2007) reported that silage combines are designed for easy and efficient harvest of silage crop, which reduces share of human labor in time and allow optimal mass distribution of silage particles chopping lengths. Stanimirović et al. (2008), found that forage harvester Fortschrit E-286, with preset chopping length of 8 mm and operating speed of 4.1 km h^{-1}, achieved average mass flow rate of 7.2 kg s^{-1} (26.1 t h^{-1}) and surface productivity of 0.86 ha h^{-1}. According to Koprivica et al. (2009) harvester John Deere 5820 reached average daily performance of 7.25 ha. At average operating speed of 3.5 km h^{-1} combine achieved mass flow rate of 30.9 t h^{-1}. As it was expected, increasing the working speed to the maximum (4.5 km h^{-1}) resulted in higher productivity - 37.4 t h^{-1}.

Four different factors can limit machine capacity depending on field conditions and operation: power, mass flow capacity, speed and traction (Buckmaster, 2009). Potkonjak et al. (2010) reported that silage combine Krone Big X V8 achieves surface productivity of 2.79 ha h^{-1}, while the harvester John Deere 6810 reached only 1.4 ha h^{-1}. With preset cut length of 8 mm, combine Krone gave the average cut length of 9.8 mm, while the combine John Deere 6810 provided the cut length of 12.3 mm (preset value was 11 mm).

Stanimirović et al. (2009) analysed maize ensiling by silo-combine Zmaj 350. At average harvester speed of 5.1 km h^{-1}, they found surface productivity of 1.09 ha h^{-1} and mass flow rate of 9.1 kg s^{-1} or 32.9 h t^{-1}. However, at smaller velocity of 4.5 km h^{-1} the mass flow rate was only 7.2 kg s^{-1} (25.8 t h^{-1}). Mohammad et al. (2013) proposed the application of sensors to monitor the flow rate of harvested mass and reported that their accuracy was about 95%.

Materials and methods

Testing the work quality of silage combines Fortschritt E-286, Zmaj 350 and John Deere 5820 has been conducted in the Toplica region during 2013. All three self-propelled harvesters have been employed in maize silage preparation in the vicinity of village Zitoradja (43°11'14 "N, 21°42'28" E).

Primarily, test plots were planted by maize hybrid ZP-704. In all analysed cases, experimental data were obtained through 5 repetitions. Necessary adjustments of silage combines and presetting of the chopping length to 8 mm were carried out within the second phase of experiment. The length distributions of the chopped mass were determined by subsequent measurement and classification in the appropriate length fractions of the 5 kg of chopped mass taken from the transport trailer.

Mass flow rate was measured by capturing chopped mass after leaving combines within the specified time interval. The mean operating speed was determined by measuring the time of combine traveling between the two fixed points having mutual distance of 100 m. Final values were calculated by averaging five replicates. The aim of this study was to determine the work quality of tested silo-combines in the agro-ecological region of Toplica.

Results and discussion

Data presented in Table 1 show that silage combines were tested in similar conditions. The average yields were in the range between 23.91 t ha^{-1} and 24.49 t ha^{-1}, and the average heights of corn stalks were in the range from 2,560 to 2,732 mm. Numbers of plants per

hectare were in the range from 58,940 to 59,210. Moisture of the maize biomass during the ensiling process ranged between 69.9 % and 71.4 %.

Table 1. *Technical characteristics of silage harvesters and experimental conditions*

Parameters		Type of silage harvesters		
		Fortschritt E-286	Zmaj 350	John Deer 5820
Number of rows	/	/	3	4
Length	[m]	7.97	5.73	7.62
Width	[m]	3.14	2.51	3.30
Mass	[kg]	5,260	4,800	7,711
Chopping device type	/	Cylinder with knives	Cylinder with knives	Cilinder with knives
Drum rotation rate	[min^{-1}]	914	1,100	1,200
Maximal capacity	[t h^{-1}]	80	80	100
Cutting length	[mm]	3.3-15	4.8 -19	3-20
Operating speed	[km h^{-1}]	up to 8.7	up to 10	up to 10
Transport speed	[km h^{-1}]	20	20	20
Engine power	[kW]	110	125	206
The average plant height	[mm]	2,560	2,732	2,693
The average height set on ear	[mm]	726	730	717
Stem diameter	[mm]	22.6	23.2	23.4
Number of plants per hectare	[plant ha^{-1}]	58,940	59,210	59,143
Yield of maize	[t ha^{-1}]	24.49	23.91	24.14
Moisture	[%]	71.4	69.9	72.3
The average length of a clip	[mm]	233	240	215

Table 2 presents average values of chopped lengths of sillage mass, as well as mass participations of three particular fractions (< 8 mm, 8 – 19 mm and > 19 mm).

Based on these results, it can be noted that the silage combine Fortschritt E-286 achieved an average length of chopped mass of 9.4 mm with standard deviation of 0.8 mm and variation coefficient of 7.4 %. Most of chopped mass was in the shortest fraction having length of up to 8 mm (68.9%). The middle-length fraction having lengths between 8 mm and 19 mm comprehended mass participation of 29.4%, and the least mass percentage (1.7 %) was found for the largest fraction having lengths of chopped mass over 19 mm.

The average length ($\overline{X}$) of maize mass chopped by harvester Zmaj 350 was 11.7 mm, with a standard deviation (S_d) of 1.0 mm and variation coefficient (C_V) 4.1 %. Percentage of the fraction of chopped particles having lengths smaller than 8 mm took the value of 58.8 %, 39.4 % for the fraction between 8 mm and 19 mm, and 1.8 % for the largest fraction having particles over 19 mm in length.

In the latter case, the application of silage combine John Deere 5820 resulted in the average length of chopped mass of 9.9 mm, with a standard deviation from 0.5 mm and variation coefficient of 7.4 %.

Comparing the work quality of these three combines gave the following conclusion: the smallest deviation of actual average length of chopped mass in relation to the preset value was achieved by combine John Deere 5820. In that case, the average length of chopped mass was 9.86 mm, while 69.45 % of the chopped mass was shorter than 8 mm. These results are consistent with findings of other researchers: Ott, 2000; Jonhson et al., 2002;

Lisowski, 2006; Calvin, 2007; Stanimirović et al., 2008; Van and Heinrich, 2008; Potkonjak et al., 2010, among the thers.

Table 2. *Achieved chopped lengths of maize mass and presence of different fractions*

Type of silage combine	Average cut length and mass percentage of different chopped mass fractions	$\overline{X}$ (mm)	S_d (mm)	C_V (%)	Xmin (mm)	Xmax (mm)
Fortschritt E-286	Average cut length (mm)	9.4	0.8	7.4	4.9	21.0
	Fraction up to 8 mm	68.9	5.5	7.9	58.3	78.4
	Fraction 8 - 19 mm	29.4	4.6	16.0	21.1	39.0
	Fraction > 19 mm	1.7	1.7	107.2	1.0	5.0
Zmaj 350	Average cut length (mm)	11.7	1.0	4.1	5.0	22.1
	Fraction up to 8 mm	58.8	9.7	17.2	45.0	77.8
	Fraction 8 - 19 mm	39.4	11.0	26.4	18.3	57.0
	Fraction > 19 mm	1.8	1.5	86.4	1.0	5.0
John Deere 5820	Average cut length (mm)	9.9	0.5	7.4	5.4	21.6
	Fraction up to 8 mm	69.5	5.8	8.1	52.4	79.0
	Fraction 8 - 19 mm	29.1	4.9	17.2	20.6	39.0
	Fraction > 19 mm	1.4	2.0	108.1	1.0	6.8

Basic statistical parameters of working speed, mass flow rate and productivity of tested combines are listed in Table 3. Presented data show that traveling velocity of silage harvester Fortschritt E-286 varied between 3.0 km h^{-1} and 5.0 km h^{-1}, with average value of 4.0 km h^{-1}. Chopped mass flow rate was recorded in the range between 5.31 kg s^{-1} (19.33 t h^{-1}) and 9.46 kg s^{-1} (34.15 t h^{-1}), having the mean value of 7.30 kg s^{-1} (26.3 t h^{-1}). It follows that, under testing conditions, this machine reached mean capacity of only about 33 % with respect to declared value. Simultaneosly, the surface productivity varied between 0.61 ha h^{-1} and 1.13 ha h^{-1}, with average value of 0.83 ha h^{-1}.

Table 3. *Working speed, flow rate of chopped mass and productivity of tested combines*

Type of ensilage harvesters	Statistical property	Operating speed [km h^{-1}]	Mass flow rate		Surface productivity [ha h^{-1}]
			[kg s^{-1}]	[t h^{-1}]	
Fortschritt E-286	$\overline{X}$	4.0	7.30	26.3	0.83
	Sd	0.5	0.97	3.3	0.14
	Cv (%)	11.0	0.18	14.2	13.48
	min.	3.0	5.31	19.3	0.61
	max	5.0	9.46	34.2	1.13
Zmaj 350	$\overline{X}$	5.0	8.60	31.0	0.98
	Sd	0.4	0.98	4.1	0.14
	Cv (%)	7.8	10.38	11.4	10.36
	min.	4.4	6.93	25.0	0.87
	max	5.7	10.67	38.4	1.15
John Deere 5820	$\overline{X}$	4.7	10.87	39.1	1.21
	Sd	0.6	0.83	4.5	0.32
	Cv (%)	8.1	11.45	16.8	11.87
	min.	4.2	8.92	32.1	0.98
	max	5.7	11.76	42.3	1.39

During testing the silo-combine Zmaj 350, traveling velocity was in the range from 4.4 km h^{-1} to 5.7 km h^{-1}, with average value of 5.0 km h^{-1}. Mass flow rate took values between 6.93 kg s^{-1} (24.95 t h^{-1}) and 10.67 kg s^{-1} (38.41 t h^{-1}), while the average rate was 8.60 kg s^{-1} (30.96 t h^{-1}). Thus, measured value of mean flow rate of the combine was 38.75% of the value declared by manufacturer. Under specified testing conditions, silage harvester Zmaj 350 achieved surface productivity from 0.87 ha h^{-1} to 1.15 ha h^{-1}, while the average productivity was 0.98 ha h^{-1}.

Results of testing the combine John Deere 5820 also show its deficiency with respect to mass flow rate – real flow rate was only 39.13% of those declared by manufacturer. Testing speed varied in the range between 4.2 km h^{-1} and 5.7 km h^{-1}, while the mean velocity was 4.7 km h^{-1}. Average flow of chopped mass was 10.87 kg s^{-1} (39.13 t h^{-1}) and mass flow rate variations were from 8.92 kg s^{-1} (32.11 t h^{-1}) and 11.76 kg s^{-1} (42.34 t h^{-1}). Minimum, maximum and average registered surface efficiencies were 0.98 h ha^{-1}, 1.39 ha h^{-1} and 1.21 h ha^{-1}, respectively.

Experimental results show increase of the mass flow rate and surface productivity of all three combines with increasing the operating speeds. Analogue results have been also reported by: Harigan, 2003; Srivastava et al., 2006; Koprivica et al., 2007, 2009; Stanimirović et al., 2008; Potkonjak et al., 2010; Buckmaster, 2009; Barwicki et al., 2011.

Presented results of testing different combines show that forage harvester Fortschrit E-286 achieved much lower average speed in relation to other two silo-harvesters, Zmaj 350 and John Deere 5820, while the speeds of the two latter combines were similar. Silage combine John Deere 5820 achieved the highest mass flow rate, much over the combines Fortschrit E-286 and Zmaj 350, while the mass flow rates of these two combines can be rated as similar. Similar differences between the silo-combines were determined with respect to surface productivities.

Conclusion

Following the common practice, all three forage harvesters have been tested under fairly similar operational conditions. Among others, it can be noted that yields of maize crops collected and chopped by these harvesters at the experimental plot were close by values - between 23.91 t ha^{-1} and 24.49 t ha^{-1}.

However, the smallest deviation of actual cut length of chopped mass with respect to the preset cutting value was recorded when silo combine John Deere 5820 was used. In this case, the average length of chopped mass was 9.86 mm, and 69.45% chopped mass was in fraction having length of up to 8 mm. In opposite to this machine, the other two silo-combines expressed smaller participations of chopped particles of this fraction, as well as larger discrepancies of real (measured) chopped lengths from their preset values.

When compared to the other two harvesters, silo combine John Deere 5820 reached the highest average mass flow rate (10.87 kg s^{-1} or 39.13 t h^{-1}) and surface productivity (1.21 ha h^{-1}) at average working speed of 4.7 km h^{-1}.

Measured values of the average mass flow rate (7.30 kg s^{-1} or 26.28 t h^{-1}) and surface productivity (0.83 ha h^{-1}) of the combine Fortschritt E-286 were smallest. It should be noted that this harvester operated at smallest working velocity - only 4 km h^{-1}.

Finally, testing the silo combine Zmaj 350, which was performed at average operational velocity of 5.0 km h^{-1}, resulted in average mass flow rate of 8.60 kg s^{-1} (30.96 t h^{-1}) and surface productivity of 0.98 ha h^{-1}.

Results of present experiment clearly show that, under real agro-ecological conditions of Toplica region, all three forage harvesters were characterized by lower mass flow rate in comparison to values declared by manufacturers. Attempts of experimentators to increase the mass flow rates of tested combines failed at preset cutting lengths and testing conditions, because the speed increasing over the applied testing values resulted in congestion of combines during the work.

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References

1. Ball D, Colins M, Garry L, Neal M, David M, Ken O, Dan P, Dan U, Mike W 2001. Understanding forage quality. American Form Bureau Federation Publication, 1-01. Park Ridge IL, 1-18.
2. Barwicki J, Gach S, Ivanov S 2011. Input analyses of maize harvesting and ensilaging technologies. Agronomy Research Biosystem Engineering. Special Issue 1, p. 31-36.
3. Buckmaster DR 2009. Equipment matching for silage harvest. Applied Engineering in Agriculture. Vol. 25(1), 31-36.
4. Calvin HP 2007. An Updated, Automated Commercial Swather for Harvesting Forage Plots Agronomy Journal, Vol. 99, 1382- 1388.
5. Jonhson LM, Harrison JH, Davidson D, Robutti JL, Swift M, Mahanna WC, Shinners K 2002. Corn Silage Management I: Efects of Hybrid, Maturity and mechanical processing on chemical and physical characteristics. Journal of Dairy Science, Vol.85, No.4, 833-853.
6. Koprivica R, Stević V, Stanimirović N, Terzić D 2007. Use of Fortschritt E-281C Self-Propelled Harvester in Grass Ensiling. Acta Agriculturae Serbica, Vol. XII, 2, 69-75.
7. Koprivica R, Veljković B, Stanimirović N, Topisirović G 2009. Performance characteristics of John Deree 5820 harvester used for preparing maize silage for dairy cattle on family farms. Agricultural engineering. Vol. 34, No. 3, 23-30.
8. Lisowski A 2006. Maize harvesting - How to choose the harvester. Agrotechnika No. 8, 29-32.
9. Marsh B 2011. Forage Harvester Evaluation. Agriculture and Natural Resources, University of California, 1-7.
10. Mohammad M M, Saadat K, Mohammad L 2013. Field evaluation and comparison of two silage cornmass flowrate sensors developed for yield monitoring. International Journal of Agriculture: Res & Rev. Vol. 3 (4), 730-736.
11. Ott A 2000. Konkurrenzfähigkeit grosser Erntemaschinen. FAT-Berichte Nr 550, 8-12.
12. Petrović D, Radojević R, Radivojević D, Barać S 2012. Some operational parameters of silage harvesters. Proceedings of the first internationsl symposium on animal science (ANSISYM), pp. 562 – 568, Belgrade, Serbia.
13. Potkonjak V, Zoranović M, Turan J 2010. Exploitation parameters of silage combine during silage corn harvetsing. Tractors and power machines. Vol.15, No.4, 33-38.

14. Srivastava AK, Goering CE, Rohrbach RP & Buckmaster DR 2006. Engineering principles of agricultural machines, Second Edition, ASABE. Copyright American Society of Agricultural and Biological Engineers. Michigan, USA, pp. 491-524.

15. Stanimirović N, Koprivica R,Veljković B, Topisirović G 2008. Work quality of silage harvester Fortschrit E-286. Agricultural engineering. Vol.33, No.3, p.11-17. Vol.33, No.3, 11-17.

16. Stanimirović N, Koprivica R, Veljković B, Barać S 2009. Silage harvester ZMAJ 350 productivity in harvesting and chopping oh maize silage for dairy cows on a commercial farm. Agricultural Engineering Vol. 34, No.3, 17-22.

17. Van Saun RJ and Heinrichs AJ 2008. Troubleshooting Silage Problems: How to Identify Potential Problems. DAS-08-125. Factsheet, Pensilvania pp. 1-10.

Original paper

CUT LENGTH DISTRIBUTIONS OF HAYLAGE PARTICLES

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Abstract

Alfalfa is one of the most important crops for forage production. Traditional method of alfalfa conservation assumes hay preparation. However, nowadays it is also commonly processed in the form of silage and haylage. Physiological effects of forages that are included in diets depend on plant species, stage of maturity, method of preservation and diet composition. Physical characteristics of rations for ruminants are primarily influenced by dietary forage to concentrate ratio, type of forages and concentrates, and mean particle size of feeds. Length distribution of forage particles represents an important parameter for ruminant's diet formulation, especially for dairy cattle. During silage production, harvest considerations should be focused to obtaining the adequate particle size distribution of the ensiling crop particles.

This paper presents results of testing three contemporary types of self-propelled silage harvesters applied in the alfalfa haylage preparation: Claas Jaguar 950, Krone Big X 700 and Krone Big X 500. All machines were adapted with pick-up headers. In the study are analyzed length distributions of chopped alfalfa particles. Resulting frequency distributions of produced haylage are characterised by high mass percentage of the fraction comprehending the largest particles. It is also evident that harvester Class Jaguar 950 achieved the mean chopping length closest to preset value.

Key words*: alfalfa, chopper, cutterhead, length fraction, silage harvester*

Introduction

Alfalfa (*Medicago sativa* L.) plant, also called lucerne, is a relevant source for dairy cows feeding. Thanks to extensive adaptability and high nutritive value, alfalfa is the most widely cultivated forage legume worldwide. This crop is characterised by high level of proteins, vitamins and minerals content, but it does not contain the minimum sugar percentage needed for successful fermentation. However, producing the haylage by ensiling, compared to hay, is less sensitive toward the weather conditions, what results in smaller looses.

Haylage is preserved fodder of green plants that arise as a combination of wilting and ensiling. Under typical climate conditions in Serbia followed by dry farming, first moving of alfalfa crop provides the highest yield. Unfortunately, this period of the year is characterised by frequent rains, atmospheric and soil humidity. Consequently, harvested mass of wet alfalfa raw material is difficult to dry and leaves quickly fall, causing the loss of quality. In order to control the nutrient degradation during legumes ensiling, different

methods can be used: wilting, carbohydrate stimulation, inoculation and chemical preservation (Nadeau et al., 2000).

In opposite, haylage quality and production process are less dependent on weather conditions, than hay preparation, because hay production demands longer period for crops wilting in the field. During the haylage production process, alfalfa is left on the land for only few hours or maximum the whole day (depending on the temperature) in order to reduce the moisture to about 50 %. Finally, wilted alfalfa mass is collected, chopped and transported to the silo (Horrocks and Valentine, 1999).

In Serbia, alfalfa is the most important perennial forage crops, and is the second most important forage crop after maize. It is grown on 178,000 ha (STAT.YEARB.SERB. 2013). Contemporary agricultural production of alfalfa assumes machine crop harvesting. Modern self-propelled precision-cut forage harvesters, when combined with large transport units, reach a high harvest performance, over 150 tons of fresh material per hour (Shinners, 2003). However, besides harvesters of modern design (Marković et al., 2006; Potkonjak et al., 2010), the interest for non-contemporary models still exists in transition countries like Serbia (Stanimirović et al., 2008; Petrović et al., 2012).

Digestive effects of forages depend on plant species, stage of maturity, method of preservation and diet composition. Forage quality is first determined by content of useable energy, crude protein and its fractions - rumen degradable and undegradable protein, neutral detergent fiber-NDF and acid detergent fiber-ADF. The ensiling process represents storage and conserving forage system that includes mechanical and biological processes for preservation of quality and feeding characteristics of silage, maintaining the content of dry matter-DM, energy, protein and digestible fiber. Besides the chemical composition, physical effectiveness is basic parameter that influences nutritional value and quality of forages in ruminant nutrition (Stojanović et al., 2009).

Insufficient particle size of diet for dairy cows decreases ruminal acetate to propionate ratio, milk fat content and feed conversion efficiency, with possible causing ruminal acidosis, paraceratosis, lameness, dislocation of abomasus and fat cow syndrome (Stojanović et al., 2011).

Intake of diet with deficit of effective fiber cause disturbance of ruminal function, and ruminal fermentation. In opposite, excessive content of long and coarse forage particles in total mixed rations for dairy cows results in decreased feed intake and digestibility of consumed DM, and negatively affects cow's energy balance (Allen, 2000).

Fine chopping of roughage (corn silage, grass silage, alfalfa silage and barley silage) to a theoretical particle size of 4 to 6 mm adversely affect rumination activity and rumen fermentation in diets for lactating cows containing relatively large amounts of concentrate (50 to 60 % DM). Lowering the particle size of forages decreases ruminal pH and fiber degradation. Finally, moderate decreasing the theoretical particle size of forages to approximately 10 to 15 mm promotes ruminal digestion (Zebeli et al., 2012).

Materials and methods

The experiments were performed in the first decade of June 2014, at two locations planted with alfalfa in the second year of exploitation. The study is focused on preparation of alfalfa haylage in the second mowing.

Domestic variety of alfalfa "Banat" seeded at first location, was harvested with the self-propelled forage harvesters Krone Big X 700 and Krone Big X 500, equipped with pick-up

adapters. Average operational speeds were 6.1 and 6.9 km/h, respectively. The experiment was performed at flat terrain, parcel T-10 (25 ha) of "PKB" corporation, farm "Mladost - Jabučki Rit", Belgrade - GPS coordinates (44^o 55' 59" N, 20^o 33' 3" E). Achieved yield of wilted chopped mass was 3.2 t/ha.

The second domestic variety of alfalfa, "Morava", was seeded at the parcel "Directorate" (8 ha) of the "Almex" corporation, farm "Kačarevo" AD, Pančevo - GPS coordinates (44^o 54' 44" N, 20^o 38' 15" E). During the second experiment, this crop was harvested with the self-propelled forage harvester Claas Jaguar 950, equipped with pick-up adapter. Average operational speed was 9.8 km/h and yield of wilted chopped mass amounted to 8.3 t/ha.

Identical technollogy of preparation alfalfa haylage was applied at both locations. Before harvesting, plant was mowed by self-propelled mower, at the small-bud growth stage and average green mass humidity of about 70%. Partially wilted crop mass was gathered into swath tracks. Wilting process of the crop collected in swaths was finished when dry matter content was average 49 % ("PKB location ") and 38 % ("Almex" location). Under these conditions, mass was chopped with self-propelled forage harvesters, and ensiled. Technical characteristics of the applied machines are given in Table 1.

Table 1. *Technical characteristics of examined self-propelled forage harvesters*

Parameters	Unit	Type of silage harvesters		
		Claas JAGUAR 950	Krone BiG X 700	Krone BiG X 500
Engine		OM 502 LA V8 16 l	MAN D2868 V8 16 l	OM 460 LA R6 12.8 l
Exhaust gas standard		TIER 4i	TIER 2	TIER 3
Max. power (ECE R120)	kW	440	570	375
Feeder width / height	mm	730/180	760/170	760/170
Cutting cylinder width / diameter	mm	750/630	800/660	800/660
Number of knives on the drum		24	28	28
Corn cracker diameter	mm	250	250	250
Spout rotation angle		210°/225°	210°	210°
Base unit weight without header	kg	12,500	14,350	13,000
Particle length setting on plot	mm	44	10	8

Particle lengths of the forage samples were determined by hand separating and sieving of samples, acquired from the transport trailer, and classification in the appropriate length fractions.

Results and discussion

Elementary descriptive statistics parameters of mass distributions, related to length of chopped haylage particles are given in Table 2.

Table 2. *Descriptive statistics of alfalfa cut length distribution*

Krone Big X 700 - length setting 10 mm							
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Average
MEAN L (mm)	m_L	22.63	22.99	25.74	22.20	24.68	23.65
REL_ERR[MEAN L] (%)	ε_{mL}	126.32	129.87	157.39	122.00	146.83	136.48
ABS_ERR[MEAN L] (mm)	Δm_L	12.63	12.99	15.74	12.20	14.68	13.65
ST.DEV.[L] (mm)	σ_L	16.61	17.11	17.48	15.88	17.81	17.04
COEF. VAR. [L] (%)	C_{VL}	73.39	74.45	67.89	71.53	72.15	72.07
Claas Jaguar 950 - length setting 44 mm							
MEAN L (mm)	m_L	36.38	32.22	33.00	34.92	32.28	33.76
REL_ERR[MEAN L] (%)	ε_{mL}	-17.31	-26.78	-24.99	-20.65	-26.64	-23.27
ABS_ERR[MEAN L] (mm)	Δm_L	-7.62	-11.78	-11.00	-9.08	-11.72	-10.24
ST.DEV.[L] (mm)	σ_L	18.25	18.12	18.48	18.07	18.28	18.31
COEF. VAR. [L] (%)	C_{VL}	50.16	56.25	55.98	51.75	56.62	54.24
Krone Big X 500 - length setting 8 mm							
MEAN L (mm)	m_L	22.91	19.86	21.83	26.04	23.07	22.74
REL_ERR[MEAN L] (%)	ε_{mL}	186.39	148.20	172.90	225.45	188.35	184.26
ABS_ERR[MEAN L] (mm)	Δm_L	14.91	11.86	13.83	18.04	15.07	14.74
ST.DEV.[L] (mm)	σ_L	17.18	14.15	16.56	17.62	17.00	16.67
COEF. VAR. [L] (%)	C_{VL}	74.97	71.26	75.87	67.66	73.70	73.30

MEAN L - Mean length; REL_ERR[MEAN L] - Relative error of mean cut length with respect to preset value; ABS_ERR[MEAN L] - Absolute error of mean cut length with respect to preset value; ST.DEV.[L] - Standard deviation of cut length distribution; COEF. VAR. [L] - Variation coefficient of cut length distribution.

In addition, haylage mass frequency distributions $\mathbf{f_m}$ (%) of particles cut lengths $\mathbf{L_D}$ (mm), chopped by three harvesters are presented in figures 1, 2 and 3.

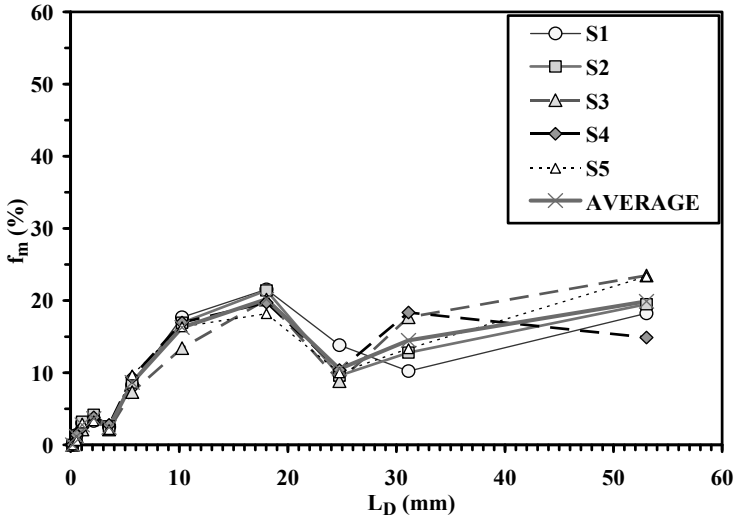

Fig. 1 *Mass % distribution of lucerne haylage cut lengths, chopped by Krone Big X 700*

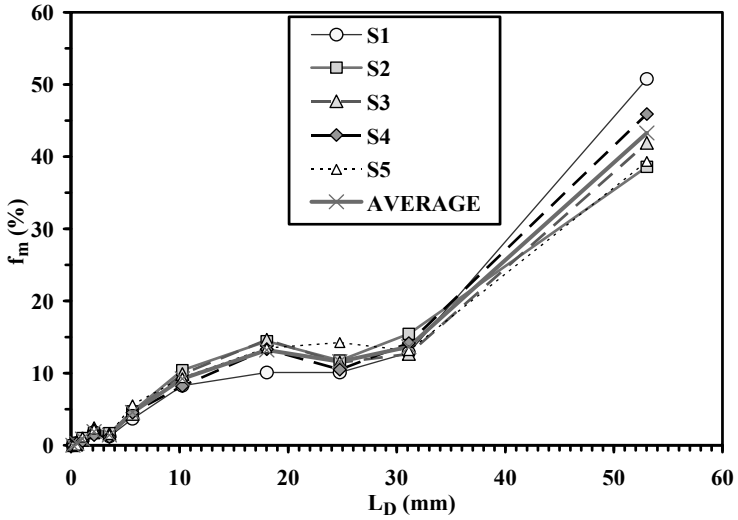

Fig. 2 *Mass % distribution of lucerne haylage cut lengths chopped by Claas Jaguar 950*

In general, it is evident from these figures that length frequency distributions are characterised by high mass percentage of the fraction comprehending the largest particles.

This evidence is in full agreement with reports of many other authors. As it was expected, general shapes of cut length distributions of haylage particles produced by combines Krone Big X 500 and Krone Big X 700 are similar, while Claas Jaguar 950 generates distributions of different shape. More precisely, frequency distributions related to Krone combines possess two extreme values, while the frequency of particles length distribution generally raise toward longest particles in the case of Class Jaguar 950 harvester.

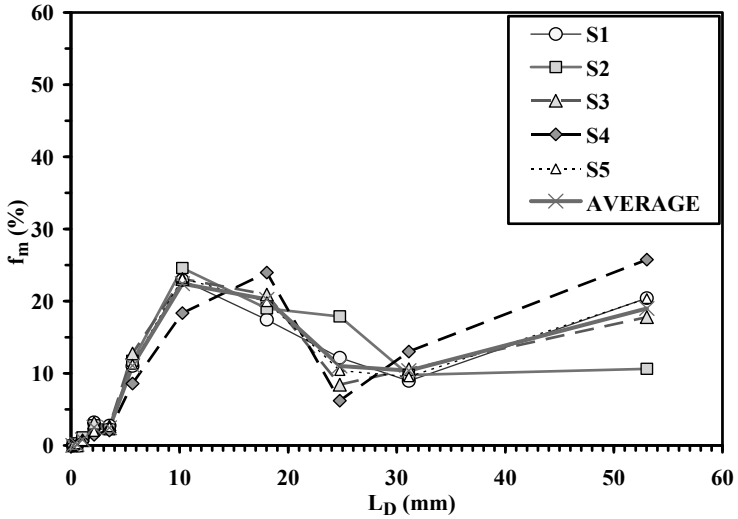

Fig. 3 *Mass % distribution of lucerne haylage cut lengths, chopped by Krone Big X 500*

Absolute errors of chopped particles mean lengths (mean value calculated on the basis of all five samples) were 13.65 mm, 10.24 mm and 14.74 mm, for combines Krone Big X 700, Claas Jaguar 950 and Krone Big X 500, respectively. Relative errors of particles cutting, defined with respect to the mean lengths, were 136.48 %, 23.27 % and 184.26 %, for combines Krone Big X 700, Claas Jaguar 950 and Krone Big X 500, respectively. For these three combines, standard deviations of cutting lengths were 17.04 mm, 18.31 mm, 16.67 mm, while the variation coefficients were 72.07 %, 54.24 % and 73.30 mm, respectively.

This means that combine Class Jaguar 950 achieved the mean chopping length closest to preset value. In addition, the variation coefficient of chopped particles length distribution was smallest, what means the highest concentration of length distribution around the mean value. Consequently, it follows that second harvester, produced by Class, provided the highest cutting precision with respect to preset value of chopping length.

Conclusion

The primary goal of farm management in this region is to facilitate further haylage compression in silos and haylage distribution within the livestock buildings after harvesting. Following this kind of approach, Serbian farming practice does not follow strictly the recommendations on the optimal length distribution of forage fractions, related to nutritional forage properties. During the presented experiment, real conditions that characterise current forage production of this kind have been followed.

Physical characteristics of rations for ruminants are influenced by dietary forage to concentrate ratio, type of forages and concentrates, and mean particle size of feeds. The particle length distribution of any forage is important parameter for ruminant's diet formulation, especially for dairy cattle. During silage production, harvest considerations should be focused to obtaining an appropriate particle size distribution of the ensiling crop.

Within the framework of the examined forage harvesters, Claas Jaguar 950 achieved a slightly shorter average mean length of particles from preset value, while harvesters Krone Big X 700 and Krone Big X 500 made several times longer average mean length of chopped particles with respect to the specified (theoretical) length.

Possible explanation of this fact, regarding the conditions at the parcels, can be found in the difference of wilted mass moisture on field; Krone's combines were used at lower moisture content of wilted mass in relation to combine Claas.

Other causes for the above results, which are not covered by this study, can be found in the constructional solutions and adjustment of the silage harvesters, as well as the reliability of individual machines. In further research it should be determined what constructional solutions and adjustment of the different silage harvesters could effect that considerable difference regarding the cut length of the haylage particles.

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References

1. Allen MS 2000. Effect of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83, 1598-1624.
2. Horrocks R and Valentine J 1999. Harvested Forages, Academic Press, 426 Pages, ISBN: 978-0-12-356255-5.
3. Marković D, Kekić M, Simčić A and Branković D 2006. Forage harvesters development. Cont. Agr. Engng. 32 (3-4), 132-260.
4. Nadeau EM, Russell JR and Buxton DR 2000. Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulose, inoculant, and formic acid fed to lambs. J. of Animal Science 78, 2980-2989.
5. Petrović D, Radojević R, Radivojević D and Barać S 2012. Some operational parameters of silage harvesters. Proceedings of the First International Symposium on Animal Science, November 8-10th 2012, Belgrade, Serbia, pp. 562-569.
6. Potkonjak V, Zoranović M and Anđelković S 2010. Technical-exploitation parameters of machines for haylage preparation. Tractors and power machines 15(4), 26-32.
7. Shinners KJ 2003. Engineering principles of silage harvesting equipment. In Silage Science and Technology (eds Buxton, DR, Muck RE and Harrison JH). pp. 361-403.
8. Stanimirović N, Koprivica R, Veljković B and Topisirović G 2008. On the Quality of Silage Harvester Fortschrit–286 (In Serbian). Agricultural Engineering 33(3), 11-17.
9. Statistical office of the Republic of Serbia 2013. Statistical yearbook of the Republic of Serbia 2013. ISSN 0351-4064 - Revised edition.
10. Stojanović B, Grubić G, Đorđević N, Božičković A and Ivetić A 2009. Chemical and physical quality of forages for dairy cows nutrition. 13. International Symposium Feed Technology, 29.09-1.10.2009., Novi Sad. Proceedings, pp. 217-228.
11. Stojanović B, Grubić G, Đorđević N, Božičković A and Ivetić A 2011. Effects of forages and total mixed rations particle size on physical effectiveness and chewing activity of lactating cows. Biotechnology in Animal Husbandry 27(3), 935-942.
12. Zebeli Q, Aschenbach JR, Tafaj M, Boguhn J, Ametaj BN and Drochner W 2012. Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. J. Dairy Sci. 95, 1041–1056.

Invited paper

DIFFERENT APPROACHES TO ASSESS THE WELFARE OF DAIRY COWS WITH SOME RESULTS IN SERBIA

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Abstract

In this paper, different methodologies for assessing the welfare of dairy cows, such as Animal Needs Index, system of welfare indicators, system of behaviour indicators and the Welfare Quality® assessment protocol for cattle were discussed. Also, the results of the usage of these methodologies in Serbia were analyzed. In the last several years in the country, numerous studies have been conducted about welfare of dairy cattle. State of welfare of dairy cows, on farms with tied and free system estimated by mentioned methodologies was generally acceptable. The major problems in the welfare of cows are insufficient amounts of floor litter, lack of cow access to outdoor runs or pasture, occurrence of lameness, dystocia, downer cow syndrome and mortality, the manifestation of aggression between the animals and improper relationship between stockmen and animals. On the basis of the results, it can be stated that in Serbia only recently enough attention has been paid to monitoring and understanding the current welfare state of dairy cows, which are the first important steps to achieve improvements in practical terms.

Key words: *dairy cows, methodology, welfare*

Introduction

From the very beginning of the scientific considerations of dairy cow welfare it was clear that assessment methodology would be an important and challenging issue. It was recognized that dairy cow welfare can be assessed in many different ways. The approaches to dairy cow welfare assessment include various individual indicators or their combinations. In the studies that have been conducted for many years, scientists have identified a number of dairy cow welfare indicators, adjusted for different purposes in practical terms. Nowadays as results from comprehensive studies there are many systematizations and categorizations of dairy cow welfare indicators. Profound scientific insight into features of the indicators categorizes them into those derived from the animals and those that reflect the state of the animals' environment (Bloom and Fraser, 2007; Hristov et al., 2012a; Hristov et al., 2012b).

For scientific and practical purposes, the easiness of application of the method for assessment of dairy cattle welfare and the quality of the obtained results in terms of their contribution to improve the state of dairy cow welfare are of crucial importance (Rousing et al., 2000; Whay et al., 2003; EFSA, 2009; EFSA, 2012; Hristov et al., 2012b). To improve dairy cow welfare at the population level it is essential to be able to identify farms

with impaired welfare in order to prioritize intervention plans. Among the different components of dairy cow welfare (health, feeding, housing and behaviour), the European Food Safety Authority (EFSA) reported that dairy cows are especially affected by poor health (EFSA, 2009; EFSA, 2012).

Based on this, the aim of the paper was to set out review of different methodologies to assess dairy cow welfare, such as Animal Needs Index (ANI) (Bartussek et al., 2000), system of welfare indicators (Anon., 2011), system of behaviour indicators (Hristov et al., 2010c) and the Welfare Quality® assessment protocol for cattle (Anon., 2009). In addition, the results obtained from the application of these methodologies in Serbia are briefly analyzed.

Dairy cattle welfare assessment methodology

The early days of considering the assessment of farm animal welfare and in that sense the assessment of dairy cow welfare date back to 1964 when the book "Animal Machines" was published by Ruth Harrison. The work of Harrison certainly initiated very important reforms, a better understanding of husbandry conditions for animals and increase of public awareness. In 1965, the UK government commissioned an investigation, led by Professor Roger Brambell, into the welfare of intensively farmed animals, partly in response to concerns raised in Harrison's previously mentioned book. The Brambell's report stated that animals should have the freedom to "stand up, lie down, turn around, groom themselves and stretch their limbs". This short recommendation later became known as Brambell's Five Freedoms. The Five Freedoms were used as the basis for the actions of professional group including veterinarians and have been adopted by representative groups internationally including, for example, the World Organization for Animal Health (OIE, 2013).

The Farm Animal Welfare Council (FAWC) was established by the British Government in July 1979. This body started to list the provisions that should be made for farm animals in five categories, which also became known as the Five Freedoms. The Five Freedoms that animals should have as currently expressed are: 1. freedom from hunger or thirst by ready access to fresh water and a diet to maintain full health and vigour; 2. freedom from discomfort by providing an appropriate environment including shelter and a comfortable resting area; 3. freedom from pain, injury or disease by prevention or rapid diagnosis and treatment; 4. freedom to express normal behaviour by providing sufficient space, proper facilities and company of the animal's own kind; and 5. freedom from fear and distress by ensuring conditions and treatment which avoid mental suffering.

Key concepts and the role of science in the welfare of dairy cattle are described by von Keyserlingk et al. (2009). Comprehensive description of dairy cows welfare indicators are given by Hristov et al. (2012b). In the latter paper only the most important principles, categories, indicators or behavioural systems of the methodologies that are used in our country are presented. The most important issues in dairy cattle welfare that impact the dairy industry today and tomorrow, especially dairy cattle welfare indicators and standards, and the most significant welfare problems, and to illustrate the role of science in addressing these challenges were discussed by Hristov et al. (2012a). Also, dairy cattle welfare standards were described by FAWC (2009) and Hristov et al. (2010a). An excellent review about associations between variables of routine herd data and dairy cattle welfare indicators was provided by de Vries et al. (2011). The influence of rearing conditions on the welfare of dairy cows and milk production is well documented in study by Zlatanović (2009).

The Five Freedoms were later partially or completely used in defining the methodology for the study of the welfare of dairy cows. The first approach was the ANI (Animal Needs Index). The ANI method includes the most important five animal welfare categories as: 1. possibility of movement; 2. possibility of social contacts with other cows; 3. type and quality of floor; 4. lighting and air quality in the accommodation facility; and 5. interaction of stockman with cattle (Bartussek et al., 2000). Basically, ANI almost exclusively uses resource- and management-based measures.

It took many years to define behavioural indicators for dairy cow welfare assessment that included nine complex behavioural systems. Each of these systems included assessment of a large number of behavioural strategies. These are the basic animal behavioural systems: reactivity, ingestion, exploratory behaviour, the kinetic system, behavioural system of associations (social behaviour, group behaviour), the body care system, territoriality, behavioural system of reproduction and behavioural system of rest and sleep (Vučinić, 2006; Broom and Fraser, 2007).

The Welfare Quality® assessment protocol for cattle (Anon., 2009) is a new multidimensional concept for evaluating dairy cow welfare. The four basic principles that are detailed in the protocol and observed through the expression of adequate criteria and indicators are: 1. the principle of provision of food and water to animal; 2. the principle of ensuring adequate housing conditions; 3. the principle of ensuring good health; and 4. the principle of ensuring the appropriate behaviour.

For dairy cattle welfare assessment, standards on farms in our country within the national project "TR 20110: Development and implementation of welfare and biosecurity standards to improve the technology of cattle and pigs production" have been developed. The assessment includes a written plan of welfare protection, management and leadership, competence of stockman, specialist competence, space, microclimate and hygienic conditions of rearing, veterinary and zootechnic practices, health status, satisfaction of animal behaviour needs, some physiological and behavioural indicators, and finally production indicators of welfare (Anon., 2011).

As already pointed out, all the analyzed indicators for assessment of animal welfare can be classified into two groups (Ostojić-Andrić et al., 2013) according to the impact on the status of animal welfare as: 1. indicators derived from the environment (non-animal-based measures); and 2. indicators of the body of animals (animal-based measures). In our country, researchers and stockmen have devoted more attention to non-animal-based measures (Hristov and Relić, 2009). This group of indicators point out the influence of the environment on animal welfare and include: type of animal housing, the degree of freedom of movement, the degree of contact with animals of the same species, the use of litter on floor, microclimate factors that act on an animal and the quantity and quality of offered food and water. In addition, the relationships of stockman to animals, as well as factors of those origins from the animals themselves are included. The relationship of stockman to animals involves the expertise of a certain form of exploitation of animals or some form of livestock production, feelings of stockmen to animals, the ability of stockmen to recognize the change in the status of the animal welfare, understanding and valuing of animal life, etc., (Waiblinger et al., 2006). Factors derived from the animals include genetic predisposition for a certain type of animal production, the use of appropriate animal breeds to certain type of production or other means of exploitation, the exploitation of animals in accordance with sex and age, etc.

The second group of the welfare indicators is measured by physiological, behavioural and production parameters. Physiological indicators include the physiological status of the

organism, the presence or absence of clinically manifest disease, the presence or absence of wounds and injuries, nutritional status, body condition, etc. Behavioural indicators include expression of physiological patterns of behaviour (ingestion of food and water, hygiene of the body, exploratory behaviour, space behaviour, social interactions, reproductive behaviour, rest and sleep, etc.), some changes in the behaviour and pathological form of behaviours. Production indicators include physiological level of production that corresponds to the standard of breed or age and production category, as well as changes in the level of productivity of the animals (Vučinić, 2006; Broom and Fraser, 2007).

Research in our country

In the last several years in our country numerous studies have been conducted regarding welfare of dairy cattle, namely using the methods of ANI (Hristov and Relić, 2009; Zlatanović, 2009; Hristov et al., 2010b; Relić et al., 2010), system of behaviour indicators (Hristov et al., 2010c), system of welfare indicators (Hristov et al., 2011a) and the Welfare Quality® assessment protocol for cattle (Hristov et al., 2011b; Ostojić-Andrić, 2013). Summary of conducted studies regarding welfare of dairy cattle in Serbia is given in Table 1.

In conducted studies (Table 1) different welfare assessment systems have been applied; in the course of the research activities, one national project (Anon. 2011) has been carried out and many papers (Hristov and Relić, 2009; Zlatanović, 2009; Hristov et al., 2010a; Hristov et al., 2010b; Hristov et al., 2010c; Relić et al., 2010; Hristov et al., 2011a; Hristov et al., 2011b), one magister thesis (Zlatanović, 2009) and one doctoral thesis (Ostojić-Andrić, 2013) have been published. Farms that we have so far tested in our country differ in capacity and system of rearing of the animals. Namely, research covered in total 47 farms, 32 farms with tied system and 15 farms with free housing system in the last several years. Investigated farms had different capacities from 12 to 1,250 dairy cows; there were 16 farms with capacity from 10 to 50 lactating cows, 12 farms from 51 to 100 cows, 14 farms from 101 to 500 cows, three farms from 501 to 1,000 cows and two farms with more than 1,000 lactating cows. When it comes to methodology, ANI was used on three farms, the Welfare Quality ® assessment protocol for cattle (2009) was applied to a total of 19 farms and the system of welfare indicators on 11 farms was applied. Simultaneously, ANI and the system of behaviour indicators were applied to a total of eight farms, ANI and system of welfare indicators on two farms, a system of welfare indicators and the Welfare Quality® assessment protocol for cattle to two farms and finally all four methods on two farms.

As mentioned previously, the measures could be categorized into animal-based measures and non-animal-based measures. Therefore, animal-based measures include: observations and measures from the animals made during the welfare assessment on farm, ante- or post-mortem. First of all, these are certain direct indicators (e.g. behaviour, clinical signs of injury or lameness). Some of these are veterinary procedures that can be obtained only by a veterinarian or other authorized person. Further animal-based measures comprise records of animal breeding, milk yield and milk quality, fertility, health, etc., (EFSA, 2012). These indicators may include records of animal-based measures obtained by the use of automated methods (e.g. progesterone in milk samples, locomotion scoring). Assessment of the welfare of dairy cattle using animal-based measurements by direct observations and investigation of farm records were conducted by Whay et al. (2003). Non-animal-based measures are designated as resource- and management-based (EFSA, 2012). These measures involve observations and measures of housing condition provided or of

management used (e.g. cubicle dimensions, quality of bedding and floor surfaces) and inspection of documentation (e.g. food provision strategies, foot care programme).

Table 1. *Summary of conducted study on dairy cattle welfare in Serbia*

Parameters	Number of farms	
Investigated farms	47	
Farms with tied system of rearing	32	
Farms with free system of rearing	15	
Farm capacity		
10 to 50 lactating cows	16	
51 to 100 cows lactating cows	12	
101 to 500 cows	14	
501 to 1000 cows	3	
More than 1001 cows	2	
Used assessment methods		
Welfare Quality ® assessment protocol for cattle	19	
ANI	3	
System of welfare indicators	11	
ANI and the system of behaviour indicators	8	
ANI and system of welfare indicators	2	
System of welfare indicators and the Welfare Quality ® assessment protocol for cattle	2	
All four methods	2	
The emergence of some welfare problems in farms	Number	Percentage
Malnutrition	16	34.04
Lameness	47	100
Mastitis	47	100
Metabolic diseases in cow	21	44.68
Diarrhoea in calves	29	61.70
Respiratory disease in calves	24	51.06
Dystocia	10	21.28
Downer cow syndrome	5	10.64
Mortality	15	31.91
Poor hygiene of floor surface in the stables	24	51.06
Inappropriate relationship between stockmen and animals	10	21.28

The aim of the monitoring of animal welfare indicators is to identify and determine the severity of the problem that endangers or impairs the welfare of the animals. Identification of welfare cow problems was achieved with general clinical examination of animals, special and specific examination of animals, examination of the housing system, handling of animals and the relationship of the dairy farmer to animals. Assessments of the seriousness of the problems that disturb cow welfare should take into account duration of causes and the number of animals for which the well-being is threatened or violated (FAWC, 2009; Hristov and Stanković, 2009; EFSA, 2012).

Applying the mentioned dairy cow welfare assessment methodologies in our country has produced a number of findings. First of all, it can be stated that knowledge relating to the definition and importance of introducing standards of animal welfare at cattle farms in our country do not apply enough or are applied selectively (Hristov et al., 2010a). In our country enough attention has only recently been paid to understanding the welfare problem

in scientific terms, which is the first important step to achieve improvements in practical terms (Hristov and Stanković, 2009; Hristov et al., 2011a). When we define the indicators of cow welfare it is very important to determine the monitoring system (Keeling and Veissiere 2005; Broom and Fraser, 2007; Hristov et al., 2010a).

Although experts and dairy farmers in our country have some knowledge regarding animal welfare, generally it is not sufficient for complete protection of cow welfare (Hristov and Stanković, 2009). They, in addition to scarce knowledge of indicators and standards of animal welfare, tend to have fragmented knowledge of the principles of animal welfare based on the five freedoms. One should always bear in mind that dairy farmers' attitudes and empathy towards animals are associated with animal welfare indicators (Hristov et al., 2010a; Kielland et al., 2010).

The average state of welfare of cows on farms in our country, in tied and loose housing systems, as estimated by ANI, system of behaviour indicators, the Welfare Quality® assessment protocol for cattle and system based on welfare indicators may be regarded as acceptable. The state of welfare of cows on the farm with free rearing system with the possibility of permanent use of outdoor runs is better than the state of welfare of cows in tied system. Loose housing provides significantly greater opportunities to meet the needs of all systems of behaviour of dairy cattle in relation to the tied system. Generally, knowledge that most stockmen possess in our country is inadequate for completely safeguarding the welfare of dairy cattle. Stockmen have many years of experience, but not the desire for additional training. There is a need to familiarize stockmen with ethological needs of the animals and to motivate them to meet those needs (Hristov et al., 2010a; Ostojić-Andrić, 2013).

Rearing conditions often do not correspond to dairy cattle welfare in terms of temperature and relative humidity (Hristov and Stanković, 2009). Maintenance of hygiene of all surfaces in the stables, and in particular the floor surface is not on an appropriate level as well. On the farms with tied system generally minimum standards of welfare are met and the biggest objections concern the inability of cow movement, thus demonstrating lack of other forms of behaviour in which the moving is part. Stockmen attitude towards cows is generally appropriate, but there were failures in usage of certain husbandry procedures due to non applied analgesia and anaesthesia. Due to lack of knowledge, stockmen often do not respond to the appearance of the initial symptoms of disease and veterinary assistance is requested only when the disease has already been manifested. Claw disorders and mastitis are the most important diseases that disrupt the welfare of cows, although metabolic diseases in cow and diarrhoea and respiratory disease in calves occur frequently. The major problems in the welfare of cows are the consequence of insufficient amounts of bedding material, lack of access of cows to outdoor outlets or pasture, occurrence of lameness, dystocia, downer cow syndrome and mortality, the manifestation of aggression between animals and inappropriate relationship between stockmen and animals (Hristov and Stanković, 2009; Hristov et al., 2010b; Relić et al., 2010; Hristov et al., 2011a; Ostojić-Andrić, 2013).

Although it is considered that the cow diet meets the needs at high level, malnutrition can be encountered in the different stages of lactation (Ostojić-Andrić, 2013). One should always bear in mind that as dairy farms become larger and diverge between grass-based and fully housed systems the interest in the welfare of the dairy cow and related environmental issues by consumers and legislators is increasing. These pressures mean that good nutrition and management, which underpins much of dairy cow welfare, are critical (Ostojić-Andrić, 2013; Logue and Sinclayr Mayne, 2014).

In our studies it was found that the protocol for assessment of dairy cattle welfare (2009) provides information about most consequences for cow welfare caused by the action of the major risk factors but which requires no longer observation time (behavioural disorders, thermal discomfort in cows). Also, there is a lack of specificity of some indicators (body condition, skin lesions which may be a consequence of several factors). Given the importance of both the inadequate body condition (malnutrition or too fat), the proportion of cows with normal body condition could be regarded as a more relevant indicator of the state of cow welfare. Age, productivity and genetic potential of animals significantly determine the potential risk and the impact on cow welfare and could also be examined as differential factors (Hristov et al., 2011b; Hristov et al., 2012b; Ostojić-Andrić, 2013).

Conclusion

On the basis of literature data about different approaches to assess the welfare of dairy cows and experience of authors the following can be concluded:

- Serbia has only recently paid enough attention to monitoring and understanding the current welfare state of dairy cows, which are the first important steps to achieve improvements in practical terms.
- The applied multidimensional approaches for assessment of dairy cow welfare are suitable for scientific consideration of the welfare level on farms, so that the methodology which is given therein may be introduced for practical assessment of the dairy cow welfare.
- The applied methodologies can be helpful to detect shortcomings in the protection of the welfare of the cows on the farm and to take action for resolving the problems of the welfare at the appropriate time.
- The results show that there are many opportunities for improving the quality of the welfare of dairy cows, which should be directed mostly to improving the housing conditions of dairy cows in terms of providing adequate space, comfort and hygiene.
- The most important problems of the welfare of cows in Serbia are the consequence of insufficient amounts of bedding material, lack of access of cow to outdoor outlets or pasture, occurrence of lameness, dystocia, downer cow syndrome and mortality, the manifestation of aggression between animals and inappropriate relationship between stockmen and animals.
- Stockmen and experts involved in the breeding and animal health protection should be familiar with cow welfare assessment methodologies, as well as with the parameters, indicators, criteria and principles used in the assessment of cow welfare.

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References

1. Anon. 2009. Welfare Quality ® assessment protocol for cattle. Welfare Quality ® Consortium, Lelystad, Netherlands.
2. Anon. 2011. Project TR 20110: Development and implementation of welfare and biosecurity standards to improve the technology of cattle and pigs production (2008-2011). Ministry of Education, Science and Technological Development, Republic of Serbia.
3. Bartussek H, Leeb CH and Held S 2000. Animal needs index for cattle. Federal Research Institute for Agriculture in Alpine Regions BAL Gumpenstein. Austria.
4. Brambell Report 1965. Report of the Technical Committee to enquire into the welfare of animals kept under intensive livestock husbandry systems. Her Majesty's Stationery Office, London, UK.
5. Broom DM and Fraser AF 2007. Domestic animal behaviour and welfare, 4th Ed. CAB International, Oxfordshire, UK.
6. Capdeville J and Veissier I 2001. A method for assessing dairy cows welfare in a loose housing herd focussing on animal observations. Acta Agriculturae Scandinavia, Suppl 30:62-68.
7. de Vries M, Bokkers E, Dijkstra T, van Schaik G and de Boer I 2011. Invited review: Associations between variables of routine herd data and dairy cattle welfare indicators. J. Dairy Sci. 94: 3213–3228.
8. EFSA 2009. Scientific report on the effects of farming systems on dairy cow welfare and disease. Annex to the EFSA Journal 2009:1143.
9. EFSA 2012. Scientific Opinion on the use of animal-based measures to assess welfare of dairy cows. EFSA Journal EFSA Journal 2012; 10(1):2554, 81 pp.
10. FAWC 1979. Farm Animal Welfare Council press statement. Available at http://www.fawc.org.uk/pdf/fivefreedoms1979.pdf (Accessed 11 July 2014).
11. FAWC 2009. Opinion on the welfare of the dairy cow. Farm Animal Welfare Council, London, SW1P 3JR., 16 pp.
12. Harrison R 1964. Animal Machines: The new factory farming industry. Vincent Stuart Pblishers, London.
13. Hristov S and Relić R 2009. Ocena uslova smeštaja sa osvrtom na dobrobit krava. Zbornik naučnih radova, Agroekonomik 3-4, 79-87.
14. Hristov S and Stanković B 2009. Najznačajniji propusti u obezbeđenju dobrobiti životinja na farmama goveda i svinja. Zbornik naučnih radova, Agroekonomik 3-4, 95-102.
15. Hristov S, Stanković B and Dokmanović M 2010a. Standardi dobrobiti goveda i svinja. Zbornik radova: Prvi naučni simpozijum agronoma sa međunarodnim učešćem, Jahorina 9-11. decembar 2010., 143-150.
16. Hristov S, Stanković B and Maksimović N 2012a. Welfare of dairy cattle – today and tomorrow. Third International Scientific Symposium "Agrosym Jahorina 2012", 55-62.
17. Hristov S, Stanković B and Zlatanović Z 2012b. The most important indicators of dairy cows welfare evaluation. Proceedings of the first international symposium on animal science. Book I., 8-10 November 2012, Belgrade, Serbia, 313-327.
18. Hristov S, Stanković B, Todorović-Joksimović M, Mekić C, Zlatanović Z, Ostojić-Andrić D and Maksimović N 2011a. Welfare problems in dairy calves. Biotechnology in Animal Husbandry 27 (4), 1417-1424.

19. Hristov S, Zlatanović Z, Skalicki Z and Stanković B 2010b. Procena dobrobiti krava primenom indeksa potreba životinja. Zbornik radova XV Savetovanje o biotehnologiji, Čačak, 15 (17), 473-478.

20. Hristov S, Zlatanović Z, Skalicki Z, Stanković B and Maksimović N 2010c. Procena dobrobiti krava na osnovu sistema ponašanja. Zbornik radova XXV Savetovanja agronoma, veterinara i tehnologa, Vol. 16(3-4): 79-86, 24-25. Februar 2010, Padinska Skela, Beograd.

21. Hristov S, Zlatanović Z, Stanković B, Ostojić-Andrić D, Davidović V, Joksimović Todorović M, Plavšić B and Dokmanović M 2011b. Procena dobrobiti krava u slobodnom sistemu držanja. Veterinarski glasnik 65, 5-6, 399-408.

22. Keeling L and Veissier I 2005. Developing a monitoring system to assess welfare quality in cattle, pigs and chickens. In Science and society improving animal welfare. Welfare Quality conference proceedings 17/18 November 2005. Edited by: Butterworth A. Brussels, Belgium, 46-50.

23. Kielland C, Skjerve E, Østerås O, Zanella AJ 2010. Dairy farmer attitudes and empathy toward animals are associated with animal welfare indicators. Journal of Dairy Science 93, 2998-3006.

24. Logue DN and Sinclayr Mayne C 2014. Welfare-positive management and nutrition for the dairy herd: A European perspective. The Veterinary Journal 199, 1, 31–38.

25. OIE (Office International Epizooties) 2013. Terrestrial Animal Health Code. From http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.7.1.htm.

26. Ostojić-Andrić D 2013. Ocena kvaliteta dobrobiti na farmama mlečnih krava. Doktorska disertacija, Univerzitet u Beogradu - Poljoprivredni fakultet, Beograd.

27. Ostojić-Andrić D, Hristov S, Petrović MM, Pantelić V, Aleksić S, Nikšić D and Dokmanović M 2013. Welfare indicators of dairy cows – selection and implementation in assessment. Proceedings of the 10th International Symposium Modern Trends in Livestock Production, October 2-4, 2013, 66-79.

28. Relić R, Hristov S and Bojkovski J 2010. Application of Methods to Assess the Welfare of Dairy Cows on Farms in Serbia. 9th International Symposium "Prospects for the 3rd Millennium Agriculture", 30th September-2nd October 2010, UASVM Cluj-Napoca, Romania. Bulletin UASVM, Veterinary Medicine 67(1)/2010, 256-262.

29. Rousing T, Bonde M and Sorensen TJ 2000. Indicators for the assessment of animal welfare in a dairy cattle herd with a cubicle housing system. In: Blokhuis, Ekkel and Wechsler, Editors, Improving health and welfare in animal production, EAAP Publ. vol. 102, Wageningen Pers Publ., Wageningen, The Netherlands, pp. 37–44.

30. von Keyserlingk GAM, Rushen J, de Passillé MA and Weary M D 2009. Invited review: the welfare of dairy cattle—Key concepts and the role of science. J. Dairy Sci. 92, 4101–4111.

31. Waiblinger S, Boivin X, Pedersen V, Tosi MV, Janczak AM, Visser EK and Jones RB 2006. Assessing the human – animal relationship in farmed species: A critical review. Applied Animal Behavior Science 101, 185-242.

32. Whay HR, Main DCJ, Green LE and Webster AJF 2003. Assessment of the welfare of dairy cattle using animal-based measurements: direct observations and investigation of farm records. Veterinary Record 153, 197-202.

33. Zlatanović Z 2009. Uticaj uslova gajenja na dobrobit krava i proizvodnju mleka. Magistarski rad, Univerzitet u Beogradu-Poljoprivredni fakultet, Beograd.

Original paper

REPRODUCTIVE AND RELATED DISORDERS ON DAIRY FARMS WITH DIFFERENT LEVELS OF WELFARE QUALITY

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Abstract

In this paper reproductive results of six dairy cows farms with total of 766 (farm 1 – 107; farm 2 –175; farm 3 – 49; farm 4 – 400; farm 5 –20 and farm 6 – 11 milking cows) with different system of rearing and welfare level were analyzed. A dairy cow reproductive efficiency is a key factor for milk production - impaired reproductive performance is a major cause of reduced production in dairy industry.

Welfare and reproductive disorders data were collected by questionnaire regarding criteria of Animal Need Index (ANI – Bartussek et al., 2000) and compared by multidimensional criteria of total discriminating effect. Possibility of movement, lighting and air quality in the accommodation facility, type and quality of floor, possibility of social contacts with other cows and interaction of stockman with cattle were compared and analysed in respect of farm welfare. In respect to the welfare level of lowest ranked farm (farm 6), farms 1, 2, 3, 4, 5 and 6 were ranked as 4th, 2nd, 1st, 5th, 3rd, and 6th, respectively, but in respect to the reproductive and related disorders occurrence rate lowest ranked farm (farm 4), farms 1, 2, 3, 4, 5 and 6 were ranked as 1st, 4th, 2nd, 6th, 5th and 3rd. Discrepancy derives from the fact that reproduction data were collected for a year, while welfare assessment describes reached level in on particular moment of time, not covering all potential causes of reproductive disorders.

Reduced reproductive success would seem promising as information about poor farm welfare, although good results often are not related to good welfare. Nevertheless, assessed welfare protection level provides important information about herd health and potential reproduction problems, pointing out that there are many opportunities for improving the quality of the welfare of dairy cows, mostly through improving the housing conditions of dairy cows.

Key words*: dairy cow, farm, reproductive disorder, welfare*

Introduction

There are three types of concern about animal welfare that prevalence: those involving the biological functioning of the animal, those involving the animals "feeling", and those that

involve the ability of the animal to live a "natural" life (Fraser, 2003). People concerned with the biological functioning of the animal (most often veterinarians and farmers) generally focus on disease, injury, poor growth rates, and reproductive problems. There is little disagreement about whether such problems are of welfare concern, and much research in animal welfare has focused on these issues (Rushen et al., 2008).

Reproductive efficiency in dairy cows is essential in dairy milk production, and many studies have identified impaired reproductive performance as a major cause of diminished production efficiency in the dairy industry. Reduced reproductive success would seem promising in providing information about poor welfare. There is much evidence that animals in poor condition (e.g. those that are ill or suffering from chronic stress) are less likely to reproduce successfully. Critics of animal agriculture often argue that the low reproductive rate of dairy cattle is an indicator of poor welfare. However, as we argue later, while poor welfare may indeed lead to lower reproductive success, it does not follow that high reproductive success indicates a lack of welfare problems (Rushen et al., 2008).

According to EFSA (2009), reproductive disorders reflect prolonged or short-term poor welfare, such as lack of oestrus, embryonic loss or early abortion due to stress experienced for longer or shorter time periods around parturition and in early lactation, or related to the poor welfare directly, particularly dystocia and genital infections associated with pain or inflammatory reactions. The aim of this paper was to establish the relationship between the most significant reproductive disorders and welfare level on the farm.

Materials and methods

Incidence of the most significant reproductive disorders in six dairy farms with total of 766 cows (farm 1 – 107; farm 2 –175; farm 3 – 49; farm 4 – 400; farm 5 –20 and farm 6 – 11 dairy cows) with different system of rearing and different welfare level were analyzed.

Welfare quality level and information regarding reproductive disorders in the last 12 months were collected regarding criteria of Animal Need Index (ANI – Bartussek et al., 2000). The ANI method includes the most important five animal welfare categories: 1. possibility of movement, 2. lighting and air quality in the accommodation facility, 3. type and quality of floor, 4. possibility of social contacts with other cows and 5. interaction of stockman with cattle.

The obtained data were analyzed and compared by method of multidimensional criteria of total discriminating effect.

Results and discussion

As it could be seen in Table 1, in respect of possibility of movement, the best marked was farm 3 (9 points), then farms 1 and 2 (5 points), farms 4 and 5 (4.5 points), and finally, farm 6 (2.5 points). In respect of type and quality of floor, the best assessed farms were farm 3 (6 points), and farms 1, 2 and 4 (4.5 points). Farm 5 gained 3 and farm 6 2.5 points. Considering lighting and air quality in the accommodation facility, the best marked farm was farm 1 again (7 points), then farm 2 (5.5 points), farms 4 and 5 followed with 5 points, farm 1 had 4.5 points and farm 6 had 2.5 points. Chances for possibility of social contacts with other cows

were the best on farm 3 (5 points), than on farm 5 (3.5 points), 3 points on farm 2, 2.5 points on farm 1, and finally, 1.5 points on farm 6. Interaction of stockman with cattle was the best on farm 5 (8 points), then farms 2 and 6 (7.5 points), and on farms 3, 1 and 4 marks were 7, 6 and 5.5, respectively. Welfare rank of these farms was assessed and farms were ranked, as it is given in Table 2.

Table 1. *Score ANI points (minimum -9.5, maximum 45 points)*

Parameter	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6
System of rearing	*loose*	*loose*	*loose*	*loose*	*loose*	*tied*
Possibility of movement	5	5	9	4.5	4.5	2.5
Type and quality of floor	4.5	4.5	6	4.5	3	2.5
Lighting and air quality in the accommodation facility	4.5	5.5	7	5	5	2.5
Possibility of social contacts with other cows	2.5	3	5	2	3.5	1.5
Interaction of stockman with cattle	6	7.5	7	5.5	8	7.5
Score ANI points	**22.5**	**25.5**	**34**	**21.5**	**24**	**16.5**
total discriminating effect	***7.116***	***10.568***	***17.871***	***6.039***	***9.345***	***2.257***
rank	***IV***	***II***	***I***	***V***	***III***	***VI***

Table 2. *Farm ranking according to ANI-welfare categories (Bartussek, 2000)*

Sum of ANI points	Names of categories of housing conditions with respect to welfare	Verbal school grades	Farm	Farm score ANI points	Rank
< 11	Not suitable	Insufficient	-	-	-
11-16	Scarcely suitable	Sufficient	-	-	-
16.5-21	Little suitable (mediocre)	Satisfactory	**Farm 6**	**16.5**	**VI**
21.5 -24	Fairly suitable	Good	**Farm 5,** **Farm 1** **Farm 4**	**24** **22.5** **21.5**	**III** **IV** **V**
24.5-28	Suitable	Very good	**Farm 2**	**25.5**	**II**
> 28	Very suitable	Excellent	**Farm 3**	**34**	**I**

Occurrence of reproductive disorders is presented in Table 3. According to total discriminating effect in respect to the reproductive disorders occurrence rate, lowest ranked farm (farm 4), farms 1, 2, 3, 4, 5 and 6 were ranked as 1st, 4th, 2nd, 6th, 5th and 3rd, respectively. Rather high rates of certain reproductive disorders were noticed on farm 5, especially placenta retention, metritis and finally mastitis (17.39%, 13.70% and 13.04%, respectively), which was moderate high on farm 6 (8.33%).

Comparison of the results given in all three tables points out that, according total discriminating effect in respect to the welfare protection level of lowest ranked farm (farm 6), farms 1, 2, 3, 4, 5 and 6 were ranked as 4th, 2nd, 1st, 5th, 3rd, and 6th, respectively, but in respect to the true reproductive and related disorders occurrence rate lowest ranked farm (farm 4),

farms 1, 2, 3, 4, 5 and 6 were ranked as 1st, 4th, 2nd, 6th, 5th and 3rd, respectively, with rather low coefficient of correlation (r = 0.218).

Table 3. *Reproductive indicators*

Observation in the last 12 months	**Farm 1**	**Farm 2**	**Farm 3**	**Farm 4**	**Farm 5**	**Farm 6**
System of rearing	*loose*	*loose*	*loose*	*loose*	*loose*	*tied*
	n					
1. Total herd size	280	400	102	750	23	12
2. Milking cows	107	175	49	400	20	11
	%					
3. Calves lost	0.5	2	2	5	3	2
4. Cows lost	0	0.25	0.98	2	8.70	0
5. Mastitis	2.89	4.25	1.96	4	13.04	8.33
6. Puerperal paresis	1.76	1.75	0	0	8.70	0
7. Retention of placenta	3.57	4.25	0	2	17.39	0
8. Metritis	0	4.25	0	6.93	13.70	0
9. Heath detection and insemination	100	90	85	95	90	80
10. Conception rate	50	50	70	50	30	30
11. Dystokia	0.71	2.25	0	1.33	0	0
total discriminating effect	***22.4513***	***16.58309***	***22.42150***	***16.16071***	***5.68021***	***17.38789***
rank	***I***	***IV***	***II***	***VI***	***V***	***III***

The dairy cows fertility is influenced by many factors. These factors include management regime, environment, genetics, nutrition, and biological and health status. The heat detection rate or heat detection efficiency (HDE) is crucial when wanting to impregnate cows. If few cows in heat are detected, few cows will subsequently be inseminated and few cows will become pregnant. Herds with good HDE can achieve better results according to many reproductive performance indicators (Mayne et al., 2002).

Similar to the situation with mastitis, reports of the relationships between health status, expressed through condition scoring (CS) and calving-related problems are equivocal. Markusfeld et al. (1997) reported that poor body condition is associated with a risk of retained placenta and uterine infection after calving while Berry et al. (2007) could find no relationship between body condition and dystocia or still births.

Cows in low body condition have poorer reproductive performance even when data was adjusted to account for differences in yield (Pryce et al., 2001). Failure to get in calf, especially where the farming system has a high level of reliance on seasonal pasture growth, is a major cause of culling in New Zealand dairy systems (Xu and Burton, 2000) hence survival characteristics and longevity are negative correlated to CS. However, the full extent to which this attribute of longevity is a valid indicator of welfare, particularly where shortened life is based upon a management decision to cull, is subject for debate. Moderate body condition at calving for mature cows and some over it for first and second calved cows is advised because cows calving at less than moderate will produce less milk and are more likely to have reproductive problems (Macdonald and Roche, 2004).

Although there was rather low coefficient of correlation and discrepancy between estimated ranks of reached welfare level and reproductive parameters of the observed farms obvious,

there is much evidence that animals in poor condition (e.g. those that are ill or suffering from chronic stress) are less likely to reproduce successfully. Established discrepancy derived from the fact that reproduction data were collected for entire year, while reached welfare protection level, although consequently from the previous efforts and work done, describes obtained level of welfare in on particular moment of time and do not cover all potential causes of reproductive disorders. Critics of animal agriculture often argue that the low reproductive rate of dairy cattle is an indicator of poor welfare. However, while poor welfare may indeed lead to lower reproductive success, it does not follow that high reproductive success indicates a lack of welfare problems (Rushen et al., 2008).

Reproductive disorders risk identification and reproductive disorders risk management, as primary preventive issues are pivotal in modern animal health care in all size types dairy farms. Welfare protection and quality risk management can both be integrated into current operational veterinary herd health and production management programs (Noordhuizen and Da Silva, 2009), particularly in monitoring and protocols of reproduction, such as insemination, calving and postpartal regimes on farms. Cows with reproductive-related diseases have been associated with impaired reproductive performance (Dubuc et al., 2011). Oltenacu et al. (1990) found that cystic ovarian disease and silent heat syndrome each increased the day open interval by 40 days. They also found that metritis prolonged the interval by 20 days and retained placenta by seven days. Cows calving twins are at greater risk of reproductive disorders, including retained placenta, dystocia, and metritis, which increase average days open and services per conception following the subsequent lactation (Nielen et al., 1989). Peake et al. (2011) found prolongation of the interval from calving to onset of the first luteal phase for cows with one or more of three production stressors: lameness, subclinical mastitis, and body condition score loss. However, no significant associations were found between disease events and overall reproductive performance.

The most common reproductive disease in dairy cattle is metritis, an inflammation of the uterine wall caused by bacterial infection and usually diagnosed by elevated body temperature, vaginal discharge, and a large, flaccid uterus. The latter is usually determined by rectal palpation, although it appears to be a poorer diagnostic tool than examining vaginal discharge directly (Le Blanc et al., 2002).

Herd managing staff that performs inseminations themselves instead of using specialized technicians risk reduced herd reproductive performance (Buckley et al., 2003), possibly due to lack of training.

Free-stall herds have displayed better reproductive efficiency (Valde et al., 1997), and studies have demonstrated that the interval between calving and first ovulation and oestrus is shorter in free-stall than tie-stall herds, enabling earlier insemination in free-stall herds (Petersson et al., 2006). When examining the effects of automatic milking on fertility, Kruip et al. (2002) found that automatic milking increases the number of days to first service. Fahey et al. (2002) reported lower calving rates in larger herds, whereas Simensen et al. (2010) found that larger herds had better fertility.

Calving difficulty (dystocia) is another common disorder with clear implications for animal welfare. Calving difficulties can be a leading cause of calf death. Among dairy cattle, dystocia is a major cause of stillbirths (Meyer et al., 2001). The welfare of surviving calves is also affected: calves that needed assistance during delivery developed enteritis at an earlier age

than calves that did not need such assistance (Sivula et al., 1996). Dairy herds with a high incidence of dystocia also tend to have a higher incidence of health problems in calves (Sanderson and Dargatz, 2000). Calving difficulties appear to have less of an impact on the cow than on the calf; although dystocia can cause reproductive problems in the cows, this appears to have only moderate effects on milk production or feed intake (Bareille et al., 2003). However, dystocia is associated with increased incidence of metritis and retained placenta (Gröhn et al., 2003), as it increases trauma to the uterine wall and increases the susceptibility to disease by increasing the risk of harmful bacteria entering the reproductive tract (Bruun et al., 2002) and it increases the likelihood that the cow will be culled (Rogers et al., 2004). One barrier to research in this area is of measuring calving difficulty. However, farms likely vary greatly in when they feel that some assistance is needed and measures of calving assistance may reflect the farmer's attitudes rather than anything else (Nix et al., 1998).

Lame cows have been reported to have poorer reproductive performance. Sprecher et al. (1997) found that cows with high lameness scores had longer intervals from calving to first service and to conception and also required more services per pregnancy. In addition, Hultgren et al. (2004) found that the first-service conception risk was lower for cows with sole ulcer. Garbarino et al. (2004) found that cows classified as lame had 3.5 times greater odds of delayed cyclicity than did cows classified as non-lame.

Many forms of illness, such as calving difficulties, lameness, and metritis can lead to reproductive failure, and so measures of reproductive success may be indirect measures of these health problems. Furthermore, considerable research on a number of mammalian species has also shown the depressive effects of stress on reproductive capacity of both males and females and has described the physiological mechanisms underlying these effects. Such suppressive effects of stress have been found in cattle (Dobson and Smith, 2000) and a number of studies show that stressors reduce reproductive efficiency in cows.

Risk conditions can be identified through monitoring plans, their impact assessed by adaptive conjoint analysis procedures involving experts (Horst et al., 1996) or quantified by epidemiological studies yielding odds ratios or relative risks (Noordhuizen et al., 2001). Risk conditions can be found at the animal/herd level (parity; milk yield; breed; lactation stage), the level of cows environment and management (barn climate; housing conditions; feed quality), and collected data (milk recording; feedstuff analysis).

Welfare protection plans refer to health management strategies and comprise key components like disease or injury risk identification. Good hygiene is crucial at calving to reduce risk of genital infections (EFSA, 2009). Proper use of the issues goes through a set of so-called working instructions or protocols (Noordhuizen and da Silva, 2009). Therefore, dairy cows welfare builds on further on a general good farming practice attitude.

Conclusions

Taking into account all presented data concerning assessed biosecurity level of six observed dairy farms the following can be concluded:

- according to total discriminating effect in respect to the welfare protection level of lowest ranked farm (farm 6), farms 1, 2, 3, 4, 5 and 6 were ranked as 4^{th}, 2^{nd}, 1^{st}, 5^{th}, 3^{rd}, and 6^{th}, respectively, but in respect to the true reproductive and related disorders

occurrence rate lowest ranked farm (farm 4), farms 1, 2, 3, 4, 5 and 6 were ranked as 1st, 4th, 2nd, 6th, 5th and 3rd, respectively;

- although there was rather low coefficient of correlation and discrepancy between estimated ranks of reached welfare level and reproductive parameters of the observed farms obvious, there is much evidence that animals in poor condition are less likely to reproduce successfully. However, it should be bear in mind that reduced reproductive success would seem promising in providing information about poor welfare, although good reproductive results often do not stand related to high welfare of the farm, making this relationship more indirect;
- nevertheless, assessed welfare protection level provides important information about herd health, and potential reproduction problems;
- there are many opportunities for improving the quality of the welfare of dairy cows, mostly through improving the housing conditions of dairy cows in terms of providing adequate space, comfort and hygiene.

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References

1. Bareille N, Beaudeau F, Billon S, Robert A and Faverdin P 2003. Effects of health disorders on feed intake and milk production in dairy cows. Livestock Production Science 83, 53–62.
2. Bartussek H, Leeb CH and Held S 2000. Animal needs index for cattle ANI 35 L/2000 – cattle. Federal Research Institute for Agriculture in Alpine Regions BAL Gumpenstein, A 8952 Irdning, of the Federal Ministry of Agriculture and Forestry, Environment and Watermanagement, A 1010 Vienna, Austria.
3. Berry DP, Lee JM, Macdonald KA and Roche JR 2007. Body condition score and body weight effects on dystocia and still births and consequent effects on postcalving performance. Journal of Dairy Science 90: 4201–4211.
4. Bruun J, Ersbøll AK and Alban L 2002. Risk factors for metritis in Danish dairy cows. Preventive Veterinary Medicine 54, 179–190.
5. Buckley F, Mee J, O'Sullivan K, Evans R, Berry D and Dillon P 2003. Insemination factors affecting the conception rate in seasonal calving Holstein-Friesian cows. Reproduction, Nutrition, Development 43(6), 543-555.
6. Dobson H and Smith R. F. (2000). What is stress, and how does it affect reproduction? Animal Reproduction Science 61, 743–753.
7. Dubuc J, Duffield TF, Leslie KE, Walton JS and LeBlanc SJ 2011. Effects of postpartum uterine diseases on milk production and culling in dairy cows. Journal of Dairy Science 94(3), 1339-1346.

8. EFSA 2009. Scientific Opinion on the overall effects of farming systems on dairy cow welfare and disease. The EFSA Journal 2009. 1143, 1-38.
9. Fahey J, O'Sullivan K, Crilly J and Mee JF 2002. The effect of feeding and management practices on calving rate in dairy herds. Animal Reproduction Science 74(3-4), 133-150.
10. Fraser D 2003. Assessing animal welfare at the farm and group level: The interplay of science and values. Animal Welfare 12, 433–443.
11. Garbarino EJ, Hernandez JA, Shearer JK, Risco CA and Thatcher WW 2004. Effect of Lameness on Ovarian Activity in Postpartum Holstein Cows. Journal of Dairy Science 87(12), 4123-4131.
12. Gröhn YT, Rajala-Schultz PJ, Allore HG, DeLorenzo MA, Hertl JA and Galligan DT 2003. Optimizing replacement of dairy cows: Modeling the effects of diseases. Preventive Veterinary Medicine 61, 27–43.
13. Horst HS, Huirne RBM and Dijkhuizen AA 1996. Eliciting the relative importance of risk factors concerning contagious animal diseases using conjoint analysis: a preliminary report. Prev.Vet.Medicine. 27: 183—195.
14. Hultgren J, Manske T and Bergsten C 2004. Associations of sole ulcer at claw trimming with reproductive performance, udder health, milk yield, and culling in Swedish dairy cattle. Preventive Veterinary Medicine 62(4), 233-251.
15. Kruip, TAM, Morice H, Robert M and Ouweltjes W 2002. Robotic milking and its effect on fertility and cell counts. Journal of Dairy Science 85: 2576-2581.
16. LeBlanc SJ, Duffield TF, Leslie KE, Bateman KG, Keefe GP, Walton JS, and Johnson WH. 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J. Dairy Sci. 85:2223-2236.
17. Macdonald K and Roche J 2004. Condition Scoring Made Easy. Dexcel Limited.
18. Markusfeld O, Galon N and Ezra E 1997. Body condition score, health, yield and fertility in dairy cows. The Veterinary Record 141: 67–72.
19. Mayne C, McCoy M, Lennox S, Mackey D, Verner M, Catney D, McCaughey W, Wylie A, Kennedy B and Gordon F 2002. Fertility of dairy cows in Northern Ireland. Veterinary Record 150(23), 707-713.
20. Meyer CL, Berger PJ, Koehler KJ, Thompson JR and Sattler CG 2001. Phenotypic trends in incidence of stillbirth for Holsteins in the United States. Jouranl of Dairy Science, 84, 515–523.
21. Nielen M, Schukken YH Scholl DT, Wilbrink HJ and Brand A 1989. Twinning in dairy cattle: A study of risk factors and effects. Theriogenology 32(5), 845-862.
22. Nix JM, Spitzer JC, Grimes LW, Burns GL and Plyler BB 1998. A retrospective analysis of factors contributing to calf mortality and dystocia in beef cattle. Theriogenology 49, 1515–1523.
23. Noordhuizen JP, Frankena K, Thrusfield MV, Graat EAM 2001. Application of quantitative methods in veterinary epidemiology. 2nd edition. Wageningen Academic Publ., Wageningen, The Netherlands.
24. Noordhuizen JP and Da Silva JC 2009. Animal hygiene and animal health in dairy cattle operations. The Open Veterinary Science Journal. 3:17-21.

25. Oltenacu PA, Frick A and Lindhe B 1990. Epidemiological study of several clinical diseases, reproductive performance and culling in primiparous Swedish cattle. Preventive Veterinary Medicine 9(1), 59-74.

26. Peake KA, Biggs AM, Argo CM, Smith RF, Christley RM, Routly JE and Dobson H 2011. Effects of lameness, subclinical mastitis and loss of body condition on the reproductive performance of dairy cows. Veterinary Record 168(11), 301.

27. Petersson KJ Strandberg E, Gustafsson H and Berglund B 2006. Environmental effects on progesterone profile measures of dairy cow fertility. Animal Reproduction Science 91(3-4), 201-214.

28. Pryce JE, Coffey MP and Simm G 2001. The relationship between body condition score and reproductive performance. Journal of Dairy Science 84: 1508–1515.

29. Rogers PL, Gaskins CT, Johnson KA and MacNeil, MD 2004. Evaluating longevity of composite beef females using survival analysis techniques. Journal of Animal Science 82, 860–866.

30. Rushen J, de Passillé AM, von Keyserlingk M.AG and Weary DM 2008. The Welfare of Cattle. In Chapter 2 Health, Disease, and Productivity, 15-43, Springer, Dordrecht, The Netherlands.

31. Sanderson MW and Dargatz DA (2000). Risk factors for high herd level calf morbidity risk from birth to weaning in beef herds in the USA. Preventive Veterinary Medicine 44, 97–106.

32. Simensen E, Osteras O, Boe K, Kielland C, Ruud L and Naess 2010. G Housing system and herd size interactions in Norwegian dairy herds; associations with performance and disease incidence. Acta Vet. Scand. 52: 14.

33. Sivula NJ, Ames TR and Marsh WE 1996. Management practices and risk factors for morbidity and mortality in Minnesota dairy heifer calves. Preventive Veterinary Medicine 27, 173–182.

34. Sprecher DJ, Hostetler DE and Kaneene JB 1997. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology 47(6), 1179-1187.

35. Valde JP, Hird DW, Thurmond MC and Osteras O 1997. Comparison of ketosis, clinical mastitis, somatic cell count, and reproductive performance between free stall and tie stall barns in Norwegian dairy herds with automatic feeding. Acta Veterinaria Scandinavica 38(2), 181-192.

36. Xu ZZ and Burton LJ 2000. Reproductive performance of dairy cows in New Zealand. Proceedings of the Australian and New Zealand Combined Dairy Cattle Veterinarians Conference - incorporating the 17th Annual Seminar of the Society of Dairy Cattle Veterinarians of the New Zealand Veterinary Association 17: 23–41.

Original paper

INFLUENCE OF MICROCLIMATE IN A BARN ON DAIRY COWS' WELFARE AND PRODUCTION

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Abstract

Microclimate in a barn has a major influence on cows' health and welfare, as well as on milk production of dairy cows. Extreme values of air temperature and humidity can negatively influence barn conditions, having thus negative effect on cows kept in barns. The research aimed to investigate influence of basic microclimate parameters on a modern dairy farm located in Eastern Croatia. Research period referred to summer season, from 01 June – 31 August 2013. Measurements were carried out in three time intervals (00:00-08:00; 08:00-16:00; 16:00-00:00) for the following parameters: temperature (T), humidity (H) and temperature-humidity index (THI). The results showed that the values of T and THI were higher than optimal values recommended by the scientific literature. Differences between the daily interval for T, H and THI were statistically highly significant ($p<0.0001$). Furthermore, significant difference ($p<0.0001$; $p<0.05$) was determined for all investigated parameters between June and July, and June and August. However, between July and August there was no significant difference. Although measurements were performed on a modern dairy farm equipped with additional cooling by fans, it was not sufficient to create an optimal microclimate environment for dairy cows. Because of that, additional measures need to be undertaken during summer months (for example, installation of water sprinklers) to reduce the air temperature and temperature-humidity index, all with the purpose to increase comfort of dairy cows and to prevent decrease in milk production.

Key words: *ambiance of cows, dairy farm, microclimate parameters*

Introduction

Specific microclimatic parameters occurring in a barn, as caused by high air temperatures and air humidity, can negatively influence animals' health (Kadzere et al., 2002; Mijić et al., 2007) and violate welfare norms (Mačuhová et al., 2008; Brouček et al., 2009). In such conditions, dairy cows are exposed to heat stress, which results in lowered feed consumption and milk production (Young, 1993). In comparison to ordinary cows, high producing dairy cows should be provided with better housing environment, since negative microclimate influences are more exhibited in such cows (Bobić et al., 2011). Heidenreich et al. (2004) stated that microclimate in a barn should be considered as a very important factor in animal welfare measures, while Brunsch et al. (1996) published data on optimal temperature (4 - 16^0C) and optimal air humidity (60 - 80%) for dairy cows. However, unfavorable combination of air temperature and air humidity occurs often. Nauheimer and

Weniger (1986) concluded that both conditions of 30^0C and relative humidity of 50%, and 26^0C and relative humidity of 70% had equally negative influence on dairy cows' welfare. Cows tend to avoid heat stress differently, which depends on environment they are in. Thus, grazing cows will seek for shades, while cows in barns will look for a coolest place (Brouček, 1997). Unfavorable climate and microclimate parameters negatively influence not only animals in barns, but also workers who spend their work time in production facilities (Matković et al., 2006).

The aim of this research was to investigate influence of some microclimate parameters on ambience of dairy cows in a modern dairy farm during summer period.

Materials and methods

The research was carried out on a dairy farm in the Osijek-Baranja County (Eastern Croatia). Experimental animals were primiparous Holstein cows in full lactation stage. The farm was of a modern construction: free keeping of cows, semi-open type, parlor (Figure 1). Research was carried out from 01 June to 31 August, 2013. Measurements were performed by a digital device Data Logger PCE-HT71", which was placed in the middle of a barn at a 2 m height. Every two hours the device recorded air temperature (T) and air humidity (H) in the facility, based on which temperature-humidity index (THI) in a facility was calculated. Daily measurements were divided into three intervals and one interval referred to eight hours, as follows: Interval 1 from 00:00-08:00 hours, Interval 2 from 08:00-16:00 hours, and Interval 3 from 16:00-00:00 hours. Data were processed in the StatSoft Statistica 8 (2008). Differences between mean values were performed by One - Way ANOVA, and significance of differences was tested by Post Hoc and Fisher's LSD tests ($p<0.0001$; $p<0.01$; $p<0.05$).

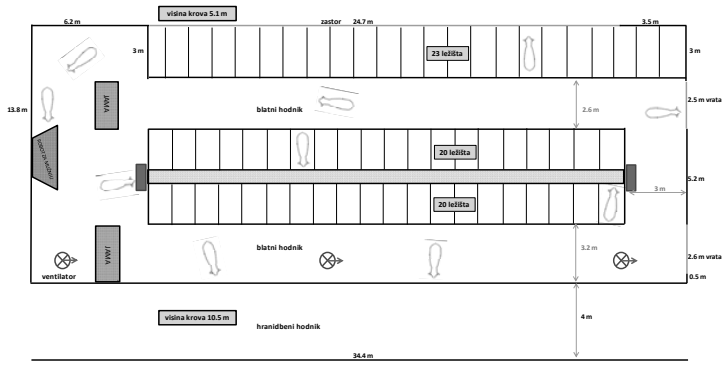

Figure 1. *Scheme of investigated farm*

Table 1 presents average values for investigated parameters. Air temperature was on average 23.54^0C, fluctuating from 13.10 to 38^0C. Average value of air humidity was 67.74%, fluctuating from 28.70 to 90.20%, while THI was on average 70.81, fluctuating between 55.81 and 84.62.

Table 1. *Basic statistical results for investigated microclimate parameters*

Parameters	N	Average	Min.	Max.	S. D.	S. E.
T (^{0}C)	1.104	23.54	13.10	38.00	5.00	0.15
H (%)	1.104	67.74	28.70	90.20	14.23	0.43
THI	1.104	70.81	55.81	84.62	6.12	0.18

T= air temperature; H= air humidity; THI= temperature-humidity index; N = number of measurements; Min. = minimum; Max. = maximum; S.D. = standard deviation; S.E. = standard error

Results and discussion

Referring to the investigated time intervals, research results, as presented in Table 2, indicated that there were statistically highly significant differences ($p<0.0001$) determined between all time intervals for all investigated parameters (air temperature and air humidity, as well as for temperature-humidity index).

Table 2. *Values and significance level of microclimate parameters referring to time interval*

Parameter	Interval (N = 368)			p
	I	II	III	
T(^{0}C)	20.40^a	27.51^b	22.70^c	***
H (%)	77.11^a	54.98^b	71.13^c	
THI	67.14^a	75.10^b	70.17^c	

***$p<0.0001$ = values marked by different letter are statistically significant; I = interval from 00.00 – 08.00 hours; II = interval from 08.00 – 16.00 hours; III = interval from 16.00 to 00.00 hours; T = temperature; H = air humidity; THI = temperature-humidity index

According to Berman et al. (1985), 25^{0}C is an optimal temperature for Holstein cows to maintain stable body temperature. Higher air temperature requires undertaking of measures for air conditioning. Our research showed that the time interval 2 (from 8.00 – 16.00 hours) with an average temperature of 27.51^{0}C was the most unfavorable for cows. Although in that interval the air humidity was the lowest (54.98%), high air temperatures affected also THI (75.10). According to the results of Ravagnolo et al. (2000), critical THI values were from 64 to 77, while West et al. (2003) concluded that production of milk per one THI increase unit lowered for 0.20-0.88 kg/cow. Results of this research showed that THI values were unfavorable for dairy cows and that they exceeded the allowable limits. Obtained THI in this research was higher than the average THI value published by Gantner et al. (2011). Such unfavorable combination of microclimate parameters was surely affecting reduce in milk production of observed primiparous cows, if referring to statement of Bianca (1965), which claimed that barn temperature of 29^{0}C and air humidity of 40% influenced milk yield reduce for about 3.7%.

Table 3. *Values and significance level of microclimate parameters referring to research month*

Parameter	Month			p
	June	July	August	
T(^{0}C)	21.97^a	24.36^b	24.25^{bc}	***
H (%)	72.78^a	66.03^b	64.56^{bc}	***
THI	68.96^a	71.91^b	71.49^{bc}	*

***$p<0.0001$; *$p<0.05$

Referring to research results in Table 3, high air temperature (24.25^0C) and high temperature-humidity index (71.91) were also determined for specific months. Differences in air temperature and air humidity between June and July, and between June and August were highly significant ($p<0.0001$), while differences for THI between June and July and between June and August were less significant ($p<0.05$).

Conclusion

Based on the performed research, it was concluded that values for T and THI were higher than optimal ones. This was especially exhibited during the time interval 2 (from 8.00 – 16.00 hours) and during July. Statistical analysis determined highly significant differences ($p<0.0001$) for T, H and THI between particular time intervals. Referring to month of measurement, there were also highly significant differences ($p<0.0001$) determined for T and H between June and July, and between June and August. Less significant differences ($p<0.05$) were determined for THI between June and July, and June and August. Although measurements were performed on a modern dairy farm, this was not enough to assure favorable microclimate ambience for dairy cows. Therefore, additional measures shall be undertaken (e.g. installation of automatic water mist air conditioning system) to reduce T and THI, in order to improve cow comfort and to prevent reduce in milk production.

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References

1. Berman A, Folman Y, Kaim M, Mamen M, Herz Z, Wolfenson D, Arieli A and Graber Y 1985. Upper critical temperatures and forced ventilation effects for high yielding dairy cows in a subtropical climate. Journal of Dairy Science 68, 1488-1495.
2. Bianca W 1965. Reviews of the progress of dairy science. Section A. Physiology. Cattle in a hot environment. Journal of Dairy Research 32, 291-345.
3. Bobić T, Mijić P, Knežević I, Šperanda M, Antunović B, Baban M, Sakač M., Frižon E and Koturić T 2011. The impact of environmental factors on the milk ejection and stress of dairy cows. Biotechnolgy in Animal Husbandry 27, 919-927.
4. Brouček J 1997. The effect of high temperatures on cattle. Agriculture 43, 522-542.
5. Brouček J, Kisac P and Uhrincat M 2009. Effect of hot temperatures on the hematological parameters, health and performance of calves. Journal of Biometeorology 53, 201-208.
6. Brunsch R, Kaufmann O and Lüpfert T 1996. Milchkühe. In: Rinderhaltung in Lauf-ställen. (ed. A. Krugmann), pp. 19. Eugen Ulmer GmbH and Co. Stuttgart, D.
7. Gantner V, Mijić P, Kuterovac K, Solić D and Gantner R 2011. Temperature-humidity indeks values and their significance on the daily production of dairy cattle. Mljekarstvo 61, 56-63.
8. Heidenreich T, Büscher W and Cielejewski H 2004. Vermeidung von Wärmebelastungen bei Milchkühen. Deutsche Landwirtschafts Gesellschaft, Merkblatt 336.
9. Kadzere CT, Murphy MR, Silanikove N and Maltz E 2002. Heat stress in lactating dairy cows: a review. Livestock of Production Science 77, 59-91.

10. Mačuhová J, Enders S, Preis R, Gutermann S, Freiberger M and Haidn B 2008. Untersuchungen zur Optimierung des Stallklimas in Außenklimaställen für Milchvieh. Bayerische Landesanstalt für Landwirtschaft 8, 1-97.
11. Matković K, Vučemilo M, Vinković B, Seol B, Pavičić Z, Tofant A and Matković S 2006. Effect of microclimate on bacterial count and airborne emission from dairy barns on the environment. Annals of Agricultural and Environmental Medicine 13, 349-354.
12. Mijić P, Knežević I, Matković M, Baban M and Ivkić Z 2007. The milk ability and somatic cell counts of milkcow sinvarious systems of keeping and milking. Biotehnology in Animal Husbandry 23, 209-216.
13. Nauheimer H and Weniger JH 1986. Auswirkungen einer Temperaturbelastung von Milchkühen. Züchtungskunde 60, 388- 397.
14. Young BA 1993: Implication of excessive heat load to the welfare of cattle in feedlots. In: Recent Advances in Animal Nutrition in Australia (ed. Farell DJ), pp. 45-50. University of New England, Armidale, Australia.
15. Ravagnolo O, Misztal I and Hoogenboom G 2000. Genetic component of heat stress in dairy cattle, development of heat index function. Journal of Dairy Science 83, 2120-2125.
16. West JW, Mullinix BG and Bernard JK 2003. Effects of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. Journal of Dairy Science 86, 232-242.

Original paper

THE POSSIBILITY OF CONTAMINATION OF DEEP FROZEN BULL SEMEN DURING LONG PERIODS OF STORAGE IN CONTAINERS WITH LIQUID NITROGEN

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Abstract

Preservation of bull sperm by deep freeze is a technological process that allows you to store semen in theory for an unlimited period of time, national and international transport, without significant loss of quality and safety of semen. According to the literature, some microorganisms successfully survive the low temperatures during storage of semen in liquid nitrogen (-196 ° C), in deep-frozen semen, seeds, and in liquid nitrogen and ice sediment in the storage container for deep-frozen bull semen. The aim of this study was to do microbiological analysis of samples semen frozen bull and liquid nitrogen in containers for bull semen storage. 414 samples of frozen bull semen, and 53 samples of liquid nitrogen ice sediment were examined. From the deeply frozen semen were isolated *Candida albicans, Citrobacter freundii and Pseudomonas stutzeri*. Microorganisms isolated from the storage container of frozen semen are mostly members of the family *Enterobacteriacae*, and *Citrobacter freundii* was isolated from the largest number of samples. Considering the findings of microorganisms in the semen, and liquid nitrogen, there is the possibility of connection of contamination of the semen with microorganisms of the liquid nitrogen, and reversely, as would be the goal of our future investigations.

Key words: *Citrobacter freundi, Candida albicans, deep frozen bull semen, liquid nitrogen*

Introduction

For the production and distribution of quality bull semen in artificial insemination the bulls that have passed the relevant tests are used. The bulls must be disease free, and their semen is collected and processed according to standard protocols. However, the microorganisms are present in most of the semen samples, in a smaller or larger number. These organisms are opportunistic pathogens of low importance for causing infection. Nevertheless, even such microflora can lead to infection (OIE Appendix 3.2.1.), under the conditions where the immune system of the body is compromised. According to literature data, the individual microorganisms can successfully survive the low-temperature storage of semen in liquid nitrogen (-196 ° C), both in semen straws, as well as in liquid nitrogen and ice sediment in the containers for storage of bull frozen semen. According to the "Regulations on the manner of sperm marking, keeping records of the production of sperm, and the conditions that must be

met in terms of semen quality of the Republic of Serbia" Article 5, the number of microorganisms after thawing of frozen semen should not exceed 500 cfu / ml. Vakanjac et al. (2013) isolated samples of *Candida albicans* in 9 and *Citrobacter Freundi* in 5 of 351 samples of deep frozen bull semen after thawing. Abro et al (2009) examined 100 samples of frozen bull semen and isolated seven different bacterial species (*Acinetobacter, Actinobacillus lignieisis, Citrobacter, Micrоccus luteus, Pseudomonas auruginosa, Staphylococcus epidermidis* and *Staphylococcus intermedius*). Bielanski et al. (2003) in their work indicated that they isolated 13 different bacterial species from deep-frozen bull semen which were stored in liquid nitrogen from 6 to 35 years. The same author also states that in 69% of samples of the liquid nitrogen, 62% of samples of the deep frozen semen and 32% of the samples of embryos the microorganisms were isolated, and in 14 (35%) of the samples is isolated from any microorganism of the sample. Bielanski (2012) states that the viruses are isolated from the liquid nitrogen, but a larger number of ubiquitous microorganisms were isolated from the liquid nitrogen and the ice sediment. Morris (1999) in his paper states that he isolated 100 cfu anaerobic microorganisms and aerobic microorganisms 10 cfu per 10 kg of liquid nitrogen. Radnoti and Farkas (1966) isolated 1 cfu of microorganisms on 5-10 ml of liquid nitrogen. Piasecka-Seraline (1972) describes in his work that there were 94% samples of frozen semen showing bacterial contamination after freezing and storage in liquid nitrogen, which was experimentally contaminated with *Escherichia coli* and *Staphylococcus aureus*. Nedić et al. (2013) in total of 35 samples of liquid nitrogen confirmed the presence of microorganisms in 21 samples, and identified *Citrobacter freundi, Klebsiella oxitoca, Acinetobacter braumannii, Acinetobacter Iwoffi, Pseudomonas stutzeri, Citrobacter diversus, Citrobacter koseri, Proteus mirabillis* and *Aspergillus sp*.

Very extensive testing possibilities of cross-contamination of samples and liquid nitrogen are described by Bielanski (2005). In his work he states three experiments, the first experiment, 15 ml of bacterial culture and 10 ml of viral culture poured into liquid nitrogen in which bovine embryos are stored, in the second experiment, samples of bovine semen and embryos exposed to viral and bacterial culture prior to freezing, and in the third experiment 1 ml of the bacterial and viral culture is frozen and stored, and wherein the same container and the sample of bovine embryos and semen. The results of this study show that there is no cross-contamination of samples in one of these three experiments if they were frozen 7 days in the tested container.

Materials and methods

A total of 414 samples of frozen bull semen and 53 samples of ice sediment of liquid nitrogen were checked. After dissolution in the water bath at 37 ° C, with a deletion of straws 70% alcohol, and careful trimming of the top, straws were seeded in medium (blood agar supplemented with 10% sheep blood, Mac Conkey agar, and Sabouraud dextrose agar). Samples of liquid nitrogen were taken with sterile swabs, dip in bowl filled with liquid nitrogen, in which frozen bull semen straws are stored. After enrichment in nutrient broth (24 hours at 37 ° C) samples were streaked onto solid medium (blood agar supplemented with 10% sheep blood, Mac Conkey agar, and Sabouraud dextrose agar). All plates were incubated for 24-48h at 37°C. Bacterial strains obtained in pure cultures were examined on the basis of morphological, cultural and biochemical characteristics. As a test to confirm, in the process of identification Microgen ID (Camberley, UK) was applied.

Results and discussion

Results of 414 samples of frozen bull semen show that *Candida albicans* was isolated in pure culture in nine samples, and *Citrobacter freundi* in 5 samples. We isolated two types of microorganisms in samples of deep frozen bull semen, in contrast to Bielanski et al. (2003) who in his work isolated as many as 13 different bacterial species. Abro et al. (2009) who examined 100 samples of frozen bull semen isolated seven different bacterial species. Although the temperature of liquid nitrogen is -196 °C it is considered that contamination of the samples stored in liquid nitrogen is possible when the container is not properly sealed.

Piasecka-Serati in 1972 was first to describe contamination of deep frozen bull semen in liquid nitrogen, which is experimentally contaminated with *Escherichia coli* and *Staphylococcus aureus.* From a total of 53 samples of ice sediment of liquid nitrogen that we examined in our study, organisms were isolated in 28 samples. *Citrobacter freundi* were isolated in 9 samples, *Pseudomonas aeruginosa* and *Klebsiella oxitoca* in 2 samples, *Acinetobacter iwoffi* in 3 samples ,*Pseudomonas stutzeri* in 4 samples, *Staphylococcus saprophyticus, Citrobacter diversus, Klepsiella ozaenae, Proteus mirabillis* and *Aspergillus sp*. in one sample, Candida albicans in 3 samples. High levels of liquid nitrogen sediment contamination, of the container for storage of human embryos are described by Morris (2005). The author states that the bacteria are isolated from all of the samples, the mold in 9 of the 10 samples, wherein *Acinetobacter baumannii, Micrococcus sp. Chrysenomonas luteola, Klepsiella oxytoca, Sphinogobacterium spiritivorum, Weaksella virosa* and nonhemolytic streptococci were identified. A large number of different microorganisms isolated from liquid nitrogen (*Staphylococcus auricularis, Bacillus sp, Alcaligensis faecalis, Stenotrophomonas maltophilia,* etc.). Bielanski got in his work (2003). Fountain et al. (1997) reported the isolation of *Aspergillus sp, Penicillium sp, Paecilomyces*, α hemolytic *Staphylococcus, Bacillus sp, Corynobacterium* and coagulase-negative *staphylococci* in liquid nitrogen for storage of hematopoietic stem cells.

Conclusion

Although there is no conclusive evidence about the possibility of transmission of microorganisms from a container of liquid nitrogen in the samples of stored deep frozen bull semen, it is still necessary to implement measures to ensure that contamination of liquid nitrogen and samples is reduced to a minimum. Containers in which deep-frozen bull semen are kept, should be new or previously thoroughly cleaned and disinfected, filled with new liquid nitrogen and sealed under veterinary supervision. Containers must be annually emptied, cleaned and disinfected to reduce possible contamination of liquid nitrogen and stored samples.

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References

1. Abro Sh, Wagan R, Tunio MT, Kamboh AA and Munir M 2009. Biochemical activities of bacterial species isolated from the frozen semen of cattle. J Agric Soc Sci, 109-113.
2. Bielanski A, Bergeron H, Lau P C and Devenish J 2003. Microbial contamination of embryons and semen during long term bankng in liquid nitrogen. Cryobiol, 46, 146-152.
3. Bielanski A 2005. Non-transmission of bacterial and viral microbes to embrions and semen stored in vapour phase of liquid nitrogens in dry shippers. Criobiology 50, 206-210.
4. Bielanski A 2012. A review of the risk of contamination of semen and embryons during cryoprezervation and measures to limit cross-contamination during banking to prevent diease transmission in Et practices. Theriogenology 77, 467-482.
5. Fountain D, Ralston M, Higgins N, Gorlin JB, Uhl L and Wheeler C 1997. Liquid nitrogen freezers: a potential source of microbial contamination of hematopoetic stem cell components. Transfusion 37, 6, 585-91.
6. Nedić Svetlana, Vakanjac Slobodanka, Đurić M and Maletić M 2013. Mikrobiološka analiza tečnog azota iz kontejnera za skladištenje dubokog zamrznutog semena. Reprodukcija domaćih životinja, Divčibare, Srbija, 181 pp.
7. Morris J 1999. Contamination issues. In: safe Cryopreservation of Gametes and Embrions. Mc Laughlin E, Pacey A, Elliot T, (Eds), Ladybrook Publishing, Australia pp. 26.
8. Piasecka-Seralin M 1972. The effects of the sediment accumulated in containers under experimental conditions on the infection of semen stored directly in liquid nitrogen (-196°C). Bull Acad Pol Sci Biol 20, 263-267.
9. Radnot D and Farkas E 1966. Sterility and cryosurgery. Arch Ophrhalmol 75, 302-3.
10. Vakanjac Slobodanka, Pavlović M, Nedić Svetlana, Pavlović V, Masgaš V, Maletić M, Đurić M, Isolation of *Candida albicans* and *Citrobacter freundi* from deep frozen semen of bulls, XIII Middle Europian Buiatrics Congress, Belgrade, Serbia, 2013, 182 pp.

Original paper

EFFECT OF HIGH LEVELS OF AMMONIA IN AIR ON ADRENAL RESPONSE TO ADRENOCORTICOTROPIN AND FORCED RUNNING IN RABBITS

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Abstract

The aim of the present study was to assess adrenal response to high air ammonia levels in rabbits. Twenty male rabbits of the New Zealand White breed at the age of 4 months were randomly allocated into two groups: control - reared under low air ammonia levels (1.4-14.6 ppm) and experimental – reared under higher ammonia levels (28-57 ppm). The rabbits of both groups were subjected to forced running for 15 min on day 37 of the trial and two weeks later they received i.m. injection of 0.1 mg synthetic adrenocorticotropin ($ACTH_{1-24}$) per rabbit. The animals were sacrificed two days after termination of the trial and some internal organs and glands were excised and weighed. Plasma cortisol levels in both groups were not significantly altered at 20 and 60 min following the end of forced running relative to basal levels. Plasma corticosterone level in the control rabbits declined at 20 min ($P<0.01$) and 60 min ($P<0.05$) following exposure to forced running and remained unchanged in the experimental group. Plasma cortisol and corticosterone concentrations in both groups declined significantly at 60 and 120 min following ACTH injection. The rabbits under high ammonia levels had heavier adrenal glands than control rabbits ($P<0.01$). The results are interpreted to suggest that ammonia-induced higher adrenal weight was due to hypertrophy of adrenal zona glomerulosa.

Key words: *adrenal gland, corticosterone, cortisol, rabbit, tetracosactide*

Introduction

Most of the ammonia related investigations are focused on the deleterious effect of ammonia on defense mechanisms of the respiratory tract against pathogens (Drummond et al., 1981; Al-Mashhadani, 1984; Gustin et al., 1994).

It is worth to note that exposure of weaned pigs to 35 and 50 ppm of ammonia for 19 days was found to increase serum cortisol level on day 19, but the magnitude of the increase was of moderate value (von Borell et al., 2007). On the contrary, exposure of pigs to 0, 25, 50 and 100 ppm of ammonia for 6 days did not modify plasma cortisol level (Gustin et al., 1994). Slight but significant increase of plasma cortisol levels have been reported in nursery pigs following chronic (20 days) or acute (96 h) exposure to 30 and 50 ppm of ammonia (Mitloehner, 2004). Olanrewaju et al. (2008) did not find significant

change of plasma cortisol level in broiler chickens exposed to 0, 25 and 50 ppm of ammonia for 14 days. Thus, the aim of the present study was to investigate the effect of enhanced level of air ammonia on adrenal response to stress.

Materials and methods

Twenty New Zealand male rabbits (*Oryctolagus cuniculus*) at the age of 4 months and average weight of 3 kg were randomly allocated into 2 groups (control and experimental) - 10 rabbits in each. Rabbits were reared individually in wire-floor cages, provided with feeders and automated drinkers - feed and drinking water were supplied ad libitum. Air temperature, relative humidity and CO_2 levels were within the following limits - 16-24 °C; 40-70% and 480-1260 ppm. The rabbits of both groups were reared together from birth until the start of the experiment in a room with natural ventilation and relatively high air ammonia levels (15-21 ppm). During the 51 days long experiment the rabbits of the control group were reared under low air ammonia levels (1.4 – 14.6 ppm), whereas the experimental rabbits were kept under higher levels of naturally occurring ammonia (28-57 ppm) via window closing. Thirty seven days from the start of the experiment all rabbits were exposed to 15 min forced running. Blood samples were collected by ear venepuncture before (basal level) and after the forced running session (at 20 and 60 min). Two weeks later the rabbits of both groups were injected with 0.1 mg tetracosactide (Adrenocorticotropin, 1-24). Blood was taken before and following adrenocorticotropin (ACTH) injection (at 1 and 2 h). The acute effect of air ammonia on adrenal function was assessed by spreading liquid ammonia on the floor, immediately after the end of forced running session and ACTH injection, which led to increased air ammonia level as high as 158 ppm. All rabbits were sacrificed two days after termination of the trial and the following internal organs and glands were excised and weighed – liver, lung, spleen, testes and adrenal glands. Plasma cortisol and corticosterone were measured by Rabbit cortisol and corticosterone ELISA kits, manufactured by Cusabio Biotech. CO., LTD. Air ammonia was recorded via AeroQual S200 Monitor, equipped with ammonia sensor head (0-100±0.1 ppm). The results of one factor statistical analysis are expressed as means ± S.E.M. and were analyzed by ANOVA.

Results and discussion

Chronic exposure to ammonia had no effect on basal plasma levels of cortisol (Figure 1). Ammonia prevented cortisol decline, observed in the control rabbits at 20 min following the end of forced running session (Figure 1). Our data, concerning rabbit exposure to forced running, are not consistent with those reported in rats subjected to acute exercise session (Fortunato et al., 2008). The authors found an increase of corticosterone level at 30 min after the termination of 20 min long treadmill exercise followed by sharp decline. The observed discrepancy of cortisol dynamics between our and their experiment could be due to species specific difference in the rate of cortisol clearance.

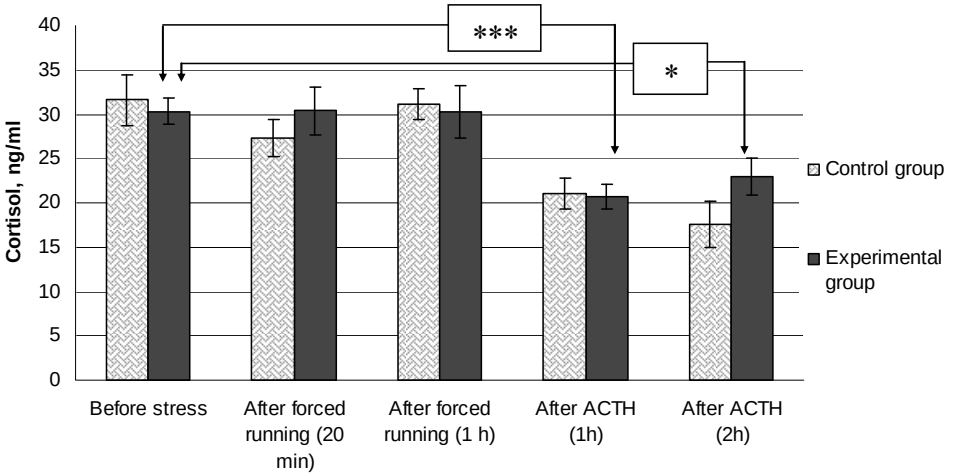

Figure 1. *Effect of forced running and ACTH injection on plasma levels of cortisol in rabbits, reared under low and high ammonia levels*
* - P<0.05 *** - P<0.001

Table 1. *Relative weight of liver, lung, spleen, testes and adrenal glands in rabbits, reared under low and high ammonia levels*

Organs and glands	n	Control group		Experimental group	
		x	Sx	x	Sx
Liver	9	0.0272	0.0012	0.0249	0.0017
Lung	9	0.0039	0.00014	0.0042	0.00026
Spleen	9	0.00049	0.00006	0.00037	0.00004
Testes	9	0.00126	0.00020	0.00182	0.00036
Adrenal gland	9	0.000073	0.0000036	0.000101**	0.0000081

** - P<0.01 significantly different versus control group

Surprisingly, plasma cortisol levels declined significantly in both groups at 1 and 2 h following adrenocorticotropin ($ACTH_{1-24}$) injection. Our data are not consistent with the reported 7-fold increase of corticosterone within 30 min after ACTH injection in neonatal mother-deprived rabbits (Brecchia et al., 2009). Earlier studies involving wild rabbits have shown completely different stress response to psychological stress which did not involve the adrenal cortex (Kracht and Kracht, 1952; Kracht, 1954.) The authors found symptoms of thyrotoxicosis and suggested that the observed stress response was completely mediated by the pituitary-thyroid system. On the contrary, Brown-Grant et al. (1954) reported that rabbits subjected to restraint or electrical current had reduced rate of release of Iodine-131 from the thyroid gland, which was not due to stress-induced renal clearance of iodine, since the inhibitory effect of stress on thyroid function was also observed in adrenalectomized rabbits. It seems that the lack of adrenal response to forced

running and ACTH injection in our study was influenced by NH_3 despite the similar response in the control and experimental rabbits. This view stems from the fact that many generations of rabbits in the rabbit farm have been raised under high ammonia levels, especially in winter when all doors and windows were kept closed because of the low ambient temperature. Furthermore, the rabbits of the control group have been reared under high ammonia level for 4 months before being moved to a clean, well ventilated room at the start of the experiment. Numerous experiments on animal and man indicated that exposure to stress during pregnancy and neonatal period alters hypothalamic-pituitary adrenal activity of the offspring (Sternberg and Ridgway, 2003; Davis et al., 2004; Huang, 2011). Consequently, we assume that adrenal response to stress in the control rabbits might have been modified by the elevated air ammonia level during pregnancy, neonatal or early postnatal period.

Alleviated adrenal response to ACTH in hyperammonemic rats was reported by Llansola et al. (2013**)**. Ammonia is known to increase NO synthesis (Swamy et al., 2005) and NO was found to modulate cortisol and corticosterone secretion (Adams et al., 1992).

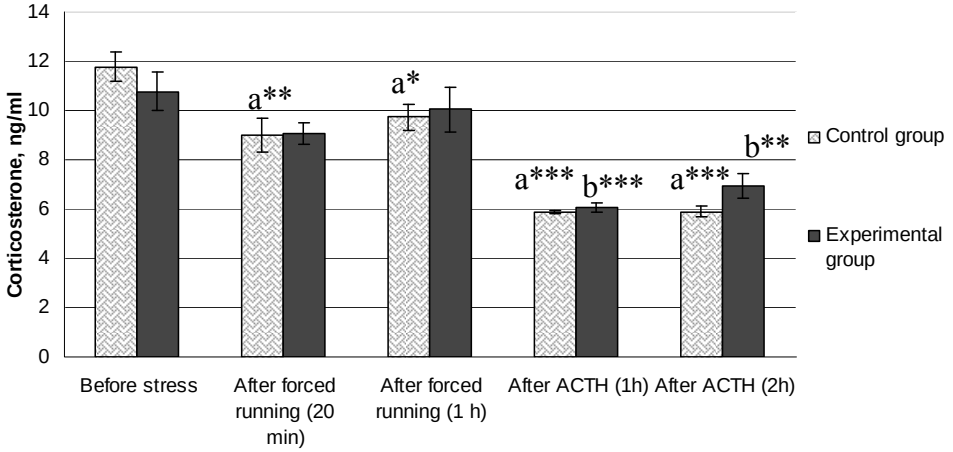

Figure 2. *Effect of forced running and ACTH injection on plasma levels of corticosterone in rabbits, reared under low and high ammonia levels*

* - P<0.05 ** - P<0.01 *** - P<0.001

a – significantly different versus basal level in the control group

b – significantly different versus basal level in the experimental group

Plasma corticosterone levels declined in the control group (P<0.01) at 20 and 60 min following exposure to 15 min forced running (Figure 2). It is worth to note that plasma corticosterone level in the experimental group did not decline significantly at 20 and 60 min. following the termination of forced running session. The observed difference in plasma corticosterone levels between the two groups could be due to ammonia-induced increase of plasma aldosterone and the use of corticosterone as a precursor of aldosterone (Müller, 1965, Perez et al., 1977; Yamauchi et al., 1997). The experimental rabbits had heavier adrenal glands (P<0.01) relative to the control rabbits (Table 1). Adrenal

hypertrophy in the hyperammonemic rabbits was obviously not due to increased secretion of glucocorticoids as seen in Figure 1 and 2. Ammonium chloride has been used in many animal studies to induce metabolic acidosis since around 98% of endogenous ammonia exists as NH_4^+ (Wright, 2010**)**. Ammonium chloride ingestion was reported to decrease serum cortisol level (Müller, 1965; Yamauchi et al., 1997; Llansola et al., 2013). Also, hyperammonemia-induced metabolic changes are only partially mediated by adrenaline (Wiechetek et al., 1989). Consequently, the higher adrenal weight in the experimental rabbits was most probably due to hypertrophy of adrenal zona glomerulosa. This view is in agreement with the reported zona glomerulosa hypertrophy in ammonium chloride treated rats (Lina and Kuijpers, 2004). Furthermore, hepatic encephalopathy is accompanied by hyponatremia (Bhatia et al., 2006) and it is well known that hyponatremia stimulates aldosterone secretion.

Adrenocorticotropin administration caused significant decline of plasma corticosterone levels in both groups of rabbits at 1 and 2 h following the injection (Figure 2). However, the rate of corticosterone decline was more pronounced than that of cortisol level and constituted almost half of the basal corticosterone level.

Conclusion

Rabbits did not respond to forced running and ACTH administration by increase of cortisol and corticosterone levels. Exposure to high air ammonia caused adrenal hypertrophy.

References

1. Adams ML, Nock B, Truong R and Cicero TJ 1992. Nitric oxide control of steroidogenesis: endocrine effects of NG-nitro-L-arginine and comparison to alcohol. Life Science, 50(6), 35-40.
2. Al-Mashhadani EH 1984. Respiratory tract damage, heat loss patterns and performance of poultry exposed to atmospheric ammonia. Dissertation/Thesis, University of Nebraska – Lincoln, United States.
3. Bhatia V, Singh R and Acharya SK 2006. Predictive value of arterial ammonia for complications and outcome in acute liver failure. Gut, 55(1), 98-104.
4. Brecchia G, Bonanno A, Dall'Aglio C, Mercati F, Zerani M, DiGrigoli A and Boiti C 2009. Neuroendocrine responses in neonatal mother-deprived rabbits. Brain Research, 1304, 105-112.
5. Brown-Grant K, Harris GW and Reichlin S 1954. The effect of emotional and physical stress on thyroid activity in the rabbit. The Journal of physiology, 126(1), 29-40.
6. Davis EP, Townsend EL, Gunnar MR, Georgieff MK, Guiang SF, Ciffuentes RF and Lussky RC 2004. Effects of prenatal betamethasone exposure on regulation of stress physiology in healthy premature infants. Psychoneuroendocrinology, 29(8), 1028-1036.
7. Drummond JG, Curtis SE, Simon J and Norton HW 1981. Effects of atmospheric ammonia on young pigs experimentally infected with Ascaris suum. American journal of veterinary research, 42(6), 969-974.

8. Fortunato RS, Ignácio DL, Padron AS, Peçanha R, Marassi MP, Rosenthal D, Werneck-de-Castro JP and Carvalho DP 2008. The effect of acute exercise session on thyroid hormone economy in rats. Journal of Endocrinology, 198(2), 347-353.
9. Gustin P, Urban B, Prouvost JF and Ansay M 1994. Effects of atmospheric ammonia on pulmonary hemodynamics and vascular permeability in pigs: interaction with endotoxins. Toxicology and applied pharmacology, 125(1), 17-26.
10. Huang LT 2011. The link between perinatal glucocorticoids exposure and psychiatric disorders. Pediatric research, 69, 19R-25R.
11. Kracht J 1954. Fright–thyrotoxicosis in the wild rabbit, a model of thyrotropic alarm-reaction. Acta endocrinologica, 15(4), 355-367.
12. Kracht J and Kracht U 1952. Zur Histopathologie und therapie der Schreckthyreotoxicose des wildkaninchens. Virchouts. Arch., 321, 238-274.
13. Lina BAR and Kuijpers MHM 2004. Toxicity and carcinogenicity of acidogenic or alkalogenic diets in rats; effects of feeding NH4CL, KHCO3 or KCL. Food and Chemical Toxicology, 42(1), 135-153.
14. Llansola M, Ahabrach H, Errami M, Cabrera-Pastor A, Addaoudi K and Felipo V 2013. Impaired release of corticosterone from adrenals contributes to impairment of circadian rhythms of activity in hyperammonemic rats. Archives of biochemistry and biophysics, 536(2), 164-170.
15. Mitloehner FM 2004. Acute and chronic effects of ammonia on inflammation, immunology, endocrine function, performance and behavior of nursery pigs. Research Report. Animal welfare, NPB #03-159, 1-22. http://www.pork.org/FileLibrary/ResearchDocuments/03-159-MITLOEHNER.8-27-04.pdf
16. Müller J 1965. Aldosterone stimulation in vitro. Acta endocrinologica, 48(2), 283-296.
17. Olanrewaju HA, Thaxton JP, Dozier WA, Purswell J, Collier SD and Branton SL 2008. Interactive effects of ammonia and light intensity on hematochemical variables in broiler chickens. Poultry science, 87(7), 1407-1414.
18. Perez GO, Oster JR, Vaamonde CA and Katz FH 1977. Effect of NH4Cl on plasma aldosterone, cortisol and rennin activity in supine man. The Journal of Clinical Endocrinology & Metabolism, 45(4), 762-767.
19. Sternberg WF and Ridgway CG 2003. Effects of gestational stress and neonatal handling on pain, analgesia, and stress behavior of adult mice. Physiology & behavior, 78(3), 375-83.
20. Swamy M, Zakaria AZ, Govindasamy C, Sirajudeen KN and Nadiger HA 2005. Effect of acute ammonia toxicity on nitric oxide (NO), citrulline-NO cycle enzymes, arginase and related metabolites in different regions of rat brain. Neuroscience research, 53(2), 116-122.
21. Von Borell E, Özpina RA, Eslinger KM, Schnitz AL, Zhao Y and Mitloehner FM 2007. Acute and prolonged effects of ammonia on hematological variables, stress responses, performance, and behavior of nursery pigs. Journal of swine health and production, 15(3), 137-145.

22. Wiechetek M, Podgurniak P, Zabielski R and Podgurniak M 1989. The effect of adrenal denervation on the metabolic effects of hyperammonemia in sheep. Canadian journal of physiology and pharmacology, 67(9) 1062-1066.
23. Wright GAK 2010. Hepatic encephalopathy: the role of inflammation, ammonia and aquaporin expression in the pathogenesis of cerebral oedema. PhD, University College London.
24. Yamauchi T, Harada T, Matsumura Y, Sueda K and Matsui N 1997. Effect of acid-base disturbances on aldosterone secretion in man. Journal of endocrinological investigation, 20(10), 576-579.

Original paper

ESOPHAGOGASTRIC ULCER IN PIGS ON COMMERCIAL FARMS

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Abstract

Intensive swine production in modern conditions of breeding and use of technological processes has influenced the occurrence of wide range of breeding and technopathy diseases. One of the most abundant breeding diseases declared in literature as an independent disease is esophagogastric ulcer characterized by erosions and ulcers in mostly esophagogastric part and rarely in mucous part of stomach. Esophagogastric ulcer is a disease of multifactorial etiology caused by genetic predisposition, diet, and presence of certain pathogenic microorganisms (for example *Helicobacter pylori*). The goal of our research was to examine the frequency of esophagogastric ulcer in pigs on commercial farms. One commercial farm has its own slaughterhouse for producing meat of fattened pigs. In our experiment we used 103 pigs in fattening with body weight between 100 and 107 kg and aged from 6 to 7 months. In the process line of slaughterhouse we established thickening of esophageal surface, hyperkeratosis, nonstructural yellow surface in 37 of total of 103 animals, while erosion of esophageal part of stomach, surface damage which does not include damage of muscular layer of mucous membrane, was present in 29 of 103 animals. Ulcers of esophageal part of stomach which affect total thickness of mucosal membrane were present in 4 of 103 examined animals.

Key words: *commercial farms, esophagogastric ulcer, pigs*

Introduction

Esophagogastric ulcer occurs most commonly as an independent disease in pigs. The largest number of cases was recorded in sows and finishing pigs. It can be said that the incidence of esophageal ulcers is the most significant in young males aged about 8 weeks. A great number of articles suggest that the most frequent occurrence of ulcers is in swine body weight from 40 to 90kg (Lončarević et.al., 1997; Šamanc, 2009). According to some sources, frequency of occurrence of the ulcer in pig suggests the most common body weight of 60kg, then, from 70 to 100kg the incidence is falling and rising again in pigs heavier than 100kg. Primitive breeds of pigs are far more resistant than the noble ones in which the disease is far more common. There was a significantly higher incidence of these lesions in pigs of Duroc breed than in the Yorkshire breed (Guise et al., 1997). The goal of our research was to examine the frequency of esophagogastric ulcer in pigs on commercial farms.

Material and methods

Commercial farm has a slaughterhouse and in this particular case there is no transport and handling of animals in depot prior to slaughter. On the slaughter line 103 animals were examined chosen by method of accidental choice. The average age of animals was between 6 and 7 months and the body weight varied between 100 and 110 kg. The stomach was opened, freed of content and washed with water in order to see the inner part of mucus. The photo was taken of all the samples and they were processed for histological analysis.

Results and discussion

The stomach of pig has the shape of bag and it is located in transversal way in abdomen. Two parts can be differed - left or cranial and right or pyloric. There are also two surfaces (cranial and caudal), two edges (dorsal and ventral) and a central part *corpus ventriculi.*There are two openings on the stomach, one connecting with oesophagus –*ostium cardiacum* and one leading to small intestine-*ostium pyloricum*. Dorsal edge is a concave one and it is known as *curvatura ventriculi minor*, located between oesophagus and duodenum. Ventral edge is convex and bigger known as large curvature-*curvatura ventriculi major.* In order to make the inner part of stomach visible the cut was made across *curvatura ventriculi major.* In the inner part there are cutaneus and glandular part of mucosa. This type of stomach is known as composite stomach – *ventriculus compositus.* The part of stomach containing only cutaneous mucosa located arround the cardia is named *pars oesophagica* or *proventricularis*. Other part of stomach containing glandular mucosa is the glandular stomach –*pars glandularis*. The border line between one type of mucosa and the other has the obvious difference in the color.

The results of occurence of esophagogastric ulcer in investigated swine on the slaughter line are given in Table 1.

Table 1. *The occurence of esophagogastric ulcer in swine on the slaughter line*

Fattening pigs	Number of examined pigs	Age	Body weight (kg)	Positive	Negative
Total	100	6-8 months	100-110	24	76

In Table 2 we present the number of positive results of ulceration presence in relation to the age category.

Table 2. *Number of positive animals in relation to age*

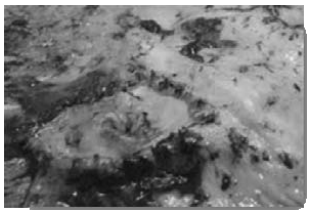
	Number of animals	Positive results of ulceration presence
Animals from 6-7 months	51	10
Animals older than 7 months	49	14

The results of histological examination of esophageal part of stomach on the salughter line are given in the Table 3.

Table 3. *Histological examination of esophageal part of stomach on the slaughter line*

The findings	Number of positive animals	Number of tested animals
Thickness of esophageal surface of mucosa-Hyperkeratosis, non-structural yellow surface	37	103
The erosions of esophageal surface of mucosa-surface erosion which do not get to *Muscularismucose*	29	103
The ulcerations of esophageal surface of mucosa-damage of whole depth of mucosa	4	103

In pictures 1, 2, 3 and 4 we presented the founded changes in mucosa.



Picture 1. *Ulcer which is obviously separated from healthy mucosa*

Picture 2. *Hyperkeratosis of the cutaneous mucosa surface*

Picture 3. *Erosions of mucosa/ different sizes and shapes*

Picture 4. - *Mucosa of esophageal area without changes*

On esophagogastric stomach area the established changes mostly consist of different numbers and range with erosions or without erosions. The lining of the affected had changed color (tawny, yellow-green or gray). Its area is roughly thickened and wrinkled, like the oak bark and therefore surpasses the mucous membrane environment. Along with this, there were erosions which were different**.** This shape and size changes usually began with that of the periphery of the *margo plicatus*, spreading into the center and affecting the entire lining. When there was independent erosion, as for the major destructive changes- ulcers of deeper nature, we occasionally found them alone, and many times combined with other pathological alterations. The most frequently we found chronic ulcers, which were round, oval or irregular in shape, with extensive lesions. They looked like many small ulcers merged into one large that was often spread to the whole esophageal region. The edges of the ulcer were prominent and sharp. The crater was covered with a layer of necrotic mass. Ulcer color is gray to brown chocolate. Sometimes the blood clot was glued to the base of the ulcer. Bottom of the chronic necrotic ulcer crater was covered with purulent-fibrous exudate. In several samples we found edema in the submucosa around the edges of ulcers in the form of cut watermelon slices. In most chronic ulcers the areas in the process of rehabilitation can be observed, as well as fields that are pathologically active. Surface repair patches are characterized by the presence of connective tissue in the bottom of the crater and *cicatrisatio*-regeneration of epithelium at the edges of the ulcer. Ulcers were found in alterations whose localization was mostly in the folds of mucous membrane or their foot, and rarely were scattered throughout mucosa, often accompanying mucosal fold along its length. Rarely have we found round oval ulcers, as well as incorrectly rectangular ones. Size varied. Shallow ulcers lining resembled cracked bark and deep ulcerations had the look like a sultry of mucosa with some sharp object. Walls were irregular, jagged, while they were inflamed and edematous**.** In the bottom of the crater there is the necrotic mass of dirty yellow color or small blood clot of darker color.

Etiological factors that contribute to the emergence of diseases can be classified into several groups, some of which stand out as the most important like food and polyvalent stress (Kopinski et al., 2007; Bojkovski et al., 2010, 2011). Food can significantly contribute to the occurrence of ulcers, particularly for hogs. Infestation is common, because of the commercial reasons and the concentrated feed for pigs with increased proportion of corn at the expense of barley and oats. Meals with finely minced and powdered food with a large number of small particles favor the occurrence of cutaneous mucous membrane processes on proventriculus (esophageal) and erosions in the other part of the stomach. This means that the meal does not contain a greater percentage of coarse particles, lacking natural wear and restoring cutaneous epithelial lining of proventricular part of the stomach (Šamanc, 2009). Disorder in the renewal of the epithelium could be the result of long-term acidosis related to the chemical processes of digestion, and less influenced by the gastric juice. During degradation of starch under the influence of microorganisms, large amounts of lactic acid which has a corrosive effect are made, and they are likely to cause initial appear-acute cutaneous erosions on the mucosal surface. It was observed that the state of stress, caused by overcrowding the facility, and the conditions, drastically changed environmental conditions (especially temperature) as a result of changes in the appearance of gastric mucosa in a larger number of animals. Many authors point out that stomach ulcers in pigs, like ulcers in people, develop under the influence of various stresses. Under modern conditions of production, pigs are exposed to many nonspecific stimuli, which cause tension among them, fear, pain, etc. This acts as a stressor and psychosomatic physical nature, requiring intermittent or continuous adaptation of animals

given environmental conditions and its frequent changes. Esophagogastric ulcer would therefore have to be included in the group of diseases whose causes are the result of the general adaptation syndrome. Observations concerning the state of stress caused by cramped living space, sudden temperature changes, transportation, room in the depot before slaughter, mixing of animals who do not know a new unfamiliar environment. In pigs with esophagogastric ulcer it violated the neuroendocrine regulation in terms of reduced secretion of pituitary hormones and increased thyroid secretion of cortisol. Given that the stress states increased corticotropin secretion of endocrine changes described correlations can be interpreted as a contribution to the neuro stress is very important in the pathogenesis of peptic ulcer disease **(**Krakowka et al., 1998 **)**. Microbiological examination of material from the ulcer showed that there are often fungus *Candida* species. Experimental work has shown that *Candida albicans* is a normal resident flora of pig stomach and turns off its role in ulcer development (Krakowka et al., 2006). Today it is considered to belong to the group of *Helicobacter heilmanii* very important enteropathogenic microorganisms in pigs that have an important role in the formation of gastric ulcers in these animals. Although these microorganisms invade the pars oesophagica and do not use toxic destruction of cellular elements, it attributes their role in the pathogenesis of the lesion. One way ulceration action of bacteria could be their ability to produce lower fatty acids. Lower fatty acids quickly pass through the outer barrier of gastric mucosa and increase the acidity of gastric contents. The primary cause of infectious diseases has not been proven nor is it typical for pigs. Several different causes are associated with the occurrence of disease in pigs, but the exact cause has never been confirmed. In a few cases the gastric ulcers were found in pig circovirus lesions (Ivetić et al., 2002). Gastric ulcers are associated with the emergence of swine influenza and swine respiratory disease complex especially during the summer months (Gagričin and Došen, 2004). Regardless of the fact that the cause or risk factors will reduce the effects of normal gastric function. Abnormal fluid content of the stomach is the result of changes in pH that normally exists between terminal part of the esophagus and pylorus. The result is an intense secretion of gastric acid. The increased acidity of gastric juices irritates the esophageal lining part and leads to initial changes squamous epithelium (hyperkeratosis), which can further lead to ulceration. Clinical picture accompanying esophagogastric ulcer is usually correlated with the stage of development and intensity of pathomorphological changes in the pars esophagica. In per acute cases, without the appearance of clinical symptoms, the animals die suddenly. In acute cases skin and mucous membranes are very pale. Some animals grind their teeth because of pain in the stomach, taking less food. They retreat into the corners of rooms, reluctant to move and usually lie. In some cases, it may be noted that they vomit. An influence of acid in the blood is black vomit. Faeces are dark. In sub-acute and chronic cases the disease is longer and symptoms are milder. The animals are anemic, taking less food and gradually become thin. Occasionally dark faeces can be noticed. Often the only indication of the existence of ulcers is occasional occurrence of constipation due to hard stools. In fact most of the pigs are sub clinically ill and the incidence of disease can be determined only at the slaughter line. Clinical effects of ulcers are usually associated with blood loss. Similar clinical symptoms that accompany esophagogastric ulcer happen also in cases of dysentery, salmonellosis, TGE, intestinal adenomatosis, coccidiosis and others. Bacterial and viral infections are usually manifested with fever and sickness in a large number of animals at the same time, in contrast to the esophagogastric ulcer, in which the temperature is within the normal range and which occurs sporadically (Milić, 1968). If esophagogastric ulcer is not timely diagnosed and appropriate treatment is not taken, the disease, after a shorter or longer period, ends lethally.

Pigs suspected to have ulcers should be separated into separate boxes. Treatment of anemia may include application of vitamin K although hemostatic effectiveness of the therapy is questionable. In some cases, antacid agents (aluminum hydroxide or magnesium silicate) can be successfully applied. As in all cases of symptomatic treatment, it is recommended to provide infusion solutions or preparations based on iron and vitamin B complex. Change of diet and a higher percentage of fiber in the diet contribute to a faster recovery from illness. In addition, the food needs to be kept in order to prevent fungal growth and thus prevent the creation of unsaturated fatty acids. Prevention is based on the exclusion of risk factors, minimizing stress and improving nutrition in terms of changes in the composition and the adjustment of time of a meal is beneficial as well. Quality of food ingredients must be acknowledged, especially if food is long standing or of poor quality. Successful control of swine respiratory disease complex will reduce losses in ulcerative gastroenteritis on some industrial pig farms (Filipović, 2009).

Conclusion

Pathology of pigs is a very dynamic discipline and control and health protection must be an integral part of each program. It is very important to access technological and production diseases whose detection and suppression is not a legal obligation, but an economic necessity. In the process line of slaughterhouse we have established thickening of esophageal surface, hyperkeratosis, nonstructural yellow surface in 37 animals out of total of 103 animals, while erosion of esophageal part of stomach, surface damage, which does not include damage of muscular layer of mucous membrane, was present in 29 of 103 animals. Ulcers of esophageal part of stomach which affect total thickness of mucosal membrane were present in 4 of 103 examined animals.

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References

1. Bojkovski J, Relić R, Hristov S, Stanković B, Savić B and Petrujkić T 2010. Contribution to knowledge of health, reproduction,biosecurity and ecological problems in intensive pig production. The 9th International Symposium "Prospects for the 3rd millennium agriculture" Cluj-Napoca Symposiums 1-5 pp.
2. Bojkovski J, Radojičić B, Savić B, Petrujkić T,Pavlović I and Relić R 2011. Bolesti stada i ekološki problem u intenzivnoj proizvodnji svinja. 46th Croatian and 6th International Symposium on Agriculture, Opatija Croatia, 828-832 pp.
3. Gagrčin M and Došen R 2004. Kompleks respiratornih oboljenja svinja,Veterinarski glasnik, 58(3-4), 409-418.
4. Guise HJ, Carlyle WW, Penny RH, Abbot TA, Riches HL and Hunter EJ 1997. Gastric ulcers in finishing pigs: their prevalence and failure to influence growth rate. Vet. Rec. 141, 563-566.
5. Ivetić V, Savić B,Valter D and Milošević B 2002. Cirkovisrune infekcije svinja,Veterinarski glasnik, 56(1-2) ,33-40.

6. Krakowka S, Eaton KA, Rings DM and Argenzio RA 1998. Production of gastroesophageal erosions ans ulcers (GEU) in gnotobiotic swine monoinfected with fermentative commensal bacteria and fed high-carbohydrate diet. Vet. Pathol. 35, 274-282.
7. Krakowka S and Ellis J 2006. Reproduction of severe gastroesophageal ulcers (GEU) in gnotobiotic swine infected with porcine Helicobacter pylori-like bacteria. Vet.Pathol. 43, 956-962.
8. Kopinski JS and McKenzie RA 2007. Oesophagogastric ulceration in pigs: a visual morphological scoring guide. Aust. Vet. J., 85, 356-361.
9. Lončarević A, Maričić Z, Toševski J and Pavlović I 1997. Osnove sistematskog zdravstvenog nadzora i programiranje zdravstvene zaštite svinja u intenzivnom odgoju. U monografiji: A. Lončarević: Zdravstvena zaštita svinja u intenzivnom odgoju, pp. 517-523, Naučni institut za veterinarstvo Srbije, Beograd.
10. Milić D 1968. Problem ezofagogastricnog ulkusa kod svinja na farmama. PhD, Fakultet veterinarske medicine, Univerzitet u Beogradu, Beograd.
11. Filipović V 2009. Iskustva primene preventivnih mera na farmama intenzivne proizvodnje, Zbornik radova sedmog simpozijuma zdravstvena zaštita, selekcija i repodukcija svinja, Srebrno jezero, pp. 21-23.
12. Šamanc H 2009. Bolesti svinja, Naučna knjiga, Beograd.

Original paper

CONTROL OF THE RESPIRATORY DISEASES IN A PIG HERD USING DATA OF THE RESPIRATORY ORGANS EXAMINATION OF FATTENING PIGS AT A SLAUGHTERHOUSE

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Abstract

In everyday farming practice, assessment of economical impact of respiratory diseases at herd level relies on the following information: data records on the diseases within the herd, productivity and slaughterhouse records. The data available from the slaughterhouse encompass the number and percentage of low weight pigs, number of diseased animals, the severity of lesions observed at slaughter as well as the amount of discarded organs and carcasses. The aim of this investigation is to improve the assessment of health status of pigs at herd level and design programs for the control of respiratory syndrome in swine based on the analysis of the data collected at slaughter line. In the slaughterhouse, the thoracic cavity organs from 105 fatlings that have reached the slaughter body mass, 20 underweight fatlings and 20 underdeveloped piglets were examined. The weight of both half-carcasses was measured. Tissue samples of altered organs (lungs, mediastinal lympho nodes, tonsils) were collected and subjected to bacteriological analysis. The average weight of both half-carcasses was 81.54kg in fatteners with full slaughter body mass, and 58.29kg and 14.95kg in low-weight fatlings and piglets, respectively. Changes affecting 10% of lung tissue were established in 9 animals, 11-20% in 10 and 21-32% in 10 fatlings. Pathological process is characterized by hepatization of lung tissue, inflammation of the pleura or, even more frequently, adhesions between the visceral and parietal pleura and pericardium. In underweight fatlings, the rates of lung changes were <20% in 5, 11-30% in 3, <40% in 4, and >40% in 6 animals. Applying bacteriological testing, the following microorganisms were isolated: *Haemophilus parasuis*, *Arcanobacterium pyogenes*, and *Pasteurella multocida*. The examination performed at the slaughter line strongly suggested the necessity of designing a new, updated vaccination program taking into consideration the causative agents and vaccination schedule.

Key words: *control, fatteners respiratory organs, slaughterhouse*

Introduction

A number of researches demonstrated that respiratory diseases of swine result in substantial decrease in production performance and increase of production costs in pig industry (Van Alstine, 2012). Economical losses are mainly associated with increased consumption of drugs

and labor, veterinary services, decreased feed conversion, increased mortality rate, body weight loss and prolonged fattening period (Dosen et al., 2008; Hurnik et al, 1994). The analysis of economical effects of an outbreak of *Actinobacillus pleuropneumoniae* infection diagnosed on pig farms in the United States (US) in 1995 revealed a total economical loss of 32 million US dollars (Willard, 2005). Some estimations reported in the US indicated that reproductive problems, increased mortality rate and decreased growth and performance rate induced by Porcine reproductive and respiratory syndrome virus (PRRSV) infection are responsible for economic losses of some 66.75 million US dollars in breeding herds and some 493.57 million US dollars on fattening pig farms in the U.S. (Neumann et al., 2005). Although the specific economic effect of mycoplasmal pneumonia can be difficult to ascertain, a review of a number of studies of herds with enzootic pneumonia demonstrated 17% decrease in daily weight gain and a 14% decrease in feed efficiency. In addition, it has been estimated that for every 10% of the lung with pneumonia, the mean daily gain is reduced by 37 grams (Leneveu et al., 2005).

In everyday farming practice, assessment of economical impact of respiratory diseases at herd level relies on the following information: data records on the diseases within the herd (occurrence of clinical form of respiratory disease and/or other diseases, mortality rate, autopsy results, material costs of applied therapy and prophylactic strategies), productivity data (current weight gain, number of days until slaughter, conversion) and slaughterhouse records (the percentage of low-weight pigs, number of diseased animals, severity of lesions observed at slaughter and amount of discarded organs and carcasses) (Dailidavičienė et al., 2008; Došen et al., 2008; Došen et al., 2013). The inspection at slaughter might be a useful tool for monitoring the health status of animals and data source for further epidemiological studies (Došen et al., 2011). In many countries, health recording protocols for collecting relevant data at the slaughterhouses were developed (Christensen et al., 1999; Sorensen et al., 2006). Comprehensive post-mortem examination in the slaughterhouse enables identification of certain pathological changes in clinically healthy pigs. Such pathologies are associated with decreased production performances and indicate potential health problems on these farms (Sorensen et al., 2006; Van Alstine, 2012).

The aim of this research was to improve the assessment of health status of pigs at herd level and to design programs for the control of respiratory syndrome in swine based on the analysis of the data collected at slaughter line.

Materials and methods

The material for this research derived from swine farm, where certain disorders and health respiratory problems in fatteners were detected. The applied research methods included gross pathological examination and standard bacteriological examination for detection of the presence of aerobic and anaerobic bacteria in the organs and tissue samples derived from slaugtered pigs.

All examined animals at the slaughterhouse originated from the same farm with the capacity of 14,000-15,000 fatlings. It should be noted that during the previous period, samples from dead fatlings were examined revealing presence of the viral genome Porcine circovirus (PCV) type 2 and PRRSV (Real Time RT-PCR). At the slaughterhouse, thoracic cavity organs from 105 fatteners that have reached the slaughter body mass, 20 underweight fatlings (the same

age as the previous category) and 20 underdeveloped piglets (age 70-80 days) were examined. The results of the examination and severity of the changes were recorded in forms prepared according to Christensen et al. (1999). The weight of both half-carcasses was measured. Moreover, the tissue samples of altered respiratory organs (lungs, mediastinal lympho node, tonsils) were collected and subjected to bacteriological examination.

Results and discussion

The half-carcass weight of healthy fatlings, underweight fatlings and underdeveloped piglets

The substantial variability in the carcass weight and leanness (share of muscle tissue) was established. The carcass weight of clinically healthy fatling ranged within a wide range of 67.95 kg to 101.4 kg. The carcass weight below 80 kg was established in 45 (44.55%) fatteners. The highest and the lowest leanness values being 64.9 kg and 49.2 kg, respectively, were established in the fatling group with carcass weight ranging between 80.1 and 90 kg.

Table 1. *Half-carcass weight of clinically healthy fatteners at slaughter line*

less than 70kg (67.95kg)	71-75 (72.13)	76-80 (77.81)	81-85 (82.45)	86-90 (87.43)	91-95 (92.62)	96-100 (96.2)	over 100 (101.4)
4 pcs*	12 pcs	29 pcs	26 pcs	18 pcs	10 pcs	1 pcs	1 pcs
The average half-carcass mass = 81.54kg							

* pieces

Table 2. *Half-carcass weight / leanness ratio of clinically healthy fatteners at slaughter line*

Less than 70 kg - 5 carcasses	Average leanness 54.8%		
	max. 56.9%	min. 50.5%	over 55% -3 carcasses
70.1 -75kg - 12 carcasses	Average leanness 54.53%		
	max. 61.9 %	min. 49.5%	over 55% - 3 carcasses
75.1- 80kg - 28 carcasses	Average leanness 52.36%		
	max. 61.6 %	min. 49.3%	over 55% - 7 carcasses
80.1-85kg - 26 carcasses	Average leanness 53.99%		
	max. 64.9 %	min. 49.2%	over 55% - 5 carcasses
85.1-90kg - 18 carcasses	Average leanness 54.92%		
	max. 60.9 %	min. 51.2%	over 55% - 7 carcasses
over 90kg - 12 carcasses	Average leanness 53.37%		
	max. 57.8 %	min. 51.2%	over 55% - 2 carcasses

The average carcass weight of underdeveloped fatlings was 58.2 kg, ranging from 43.6 kg to 69.6 kg. Although the fatteners were of the same age and originated from the same farm (facilities), their carcass weight was lower for 23.34 kg. The average carcass weight of piglets

aged 70-80 days was 13.23 kg. The piglets of the same age and body mass of 7.5 kg (weaned at age of 28 days) would weigh around 26 kg, provided that the daily weight gain was 400 g.

Table 3. *Half-carcass weight of underweight fatteners and underdeveloped piglets*

Category	Body mass (groups)	Number of fatlings	Individual carcass weight (kg)	Average carcass weight (kg)
Emergency slaughter	<50kg	6	43.4; 44.0; 46.6; 47.0; 49.2; 49.8	X=58.29
	50-60kg	3	56.4; 58.0; 58.0	
	61-70kg	11	61.0; 62.2; 62.8; 63.0; 63.8; 64.0; 64.8; 66.4; 67.0; 68.8; 69.6	
Piglets	13-13.9kg	9	13.2; 13.2; 13.2; 13.2; 13.2; 13.2; 13.3; 13.3. 13.3	X=13.23

Type of lesions in lung tissue, pleura and heart of clinically healthy fatlings

Low-grade lobular hepatization localized predominantly in the left and right apical lung was observed in 6 fatlings. In the two animals from the same population, localized pleuritis was observed. The changes were of very low grade, thus not quantified. In another 6 fatlings, lobular collapse of lung tissue was observed in the left and right diaphragm lobe. In 22 fatteners, localized pleuritis of both diaphragm lobes was noticed, whereby in 2 animals it was associated with adhesion complications and in 9 with abscesses. In 20 (19.05%) fatlings, the changes affected 11-40% of lung tissue. Most commonly, the entire cardiac lobes of the lung was affected.

Table 4. *Type of lesions in clinically healthy fatteners examined at slaughter line*

	Normal lung			Lung inflammation			Severe lung inflammation	
	Affected			Affected			Affected	
	0	Not measurable	1-10%	11-20%	21-30%	31-40%	41-50%	>50%
Pcs*	33	43	9	10	8	2	-	-
%	31.43	40.95	8.57	9.52	7.62	1.9	-	-
Total	80.95%			19.05%			-	

* pieces; - no detected

The majority of gross pathological changes were associated with localised chronic pleuritis (35.58%), *Pneumonia catarrhalis*, mycoplasmal infection (18.27%), *Pneumonia catarrhalis disseminata* (13.46%) as well as *Pleuropneumonia chronica / adhesiva* (7.69%). Interestingly, pericarditis (*Synechia pericardi)* was diagnosed in only one case (0.96%).

In the period from October 2003 to March 2004, number of 110, 865 pigs originating from 1,196 pig farms were examined at slaughterhouses in France. Vaccination against *Mycoplasma hyopneumoniae* has been performed at the majority of farms (899 herds) throughout a period of more than one year. The findings revealed the average rates of pneumonia, pleuritis and abscess being 27.6%, 14.4% and 2.3%, respectively. In almost 10% of the herds, pneumonia was diagnosed in more than 30% of animals. In vaccinated herds, the

rate of observed lesions was lower than that in non-vaccinated ones (Leneveu et al., 2005). In Lithuania, the examination of respiratory organs at slaughter revealed "enzootic pneumonia" in 46.14% of examined animals. Pleuritis, alone or associated with lung inflammation, was established in 29.55% of examined cases (Dailidavičienė et al., 2008). In our research, the bacteriological analysis of lung tissue, mediastinal lympho nodes and heart revealed the presence of *Bacillus sp*., *Streptococcus alfa hem*., *E. coli*, *Haemophilus parasuis.*

Primary causative agents of respiratory diseases are PRRSV, PCV-2, Swine influenca virus and infections caused by *M. hyopneumoniae*, *Actynobacillus pleuropneumoniae*, *Bordetella bronchiseptica*, *Haemophilus parasuis* and sometimes *Aujeszky's disease virus* (Došen et al., 2013; Prodanov-Radulović et al., 2011; Van Alstine, 2012). The secondary important infectious agents include *Pasteurella multocida*, *Streptococcus suis* and *Actinobacillus suis* (Došen et al., 2008; Neumann et al., 2005). Regrettably, the majority of infections implicate more than one causative agent. If the bacterial infection is associated with the viral one, the disease takes an acute course spreading rapidly between the housing facilities and is associated with higher morbidity and mortality rates. Subsequently, the epidemic takes a chronic course characterized by sporadic manifestations such as coughing, sneezing, anorexia and fever. The frequency and severity of the epidemic outbreak is strongly dependent on the location of the farm, bio-safety measures, farm management, vaccination program, as well as on the type and species of the agent (Hurnik et al., 1994).

Swine production management is of great influence on the occurrence of respiratory diseases on the farm. The most common infection routes include purchasing of infected pigs or semen for artificial insemination originating from another swine farm (Christensen, 1999; Došen et al., 2011; Prodanov-Radulović et al., 2011). Purchasing fattening pigs from different sources (farms), farms with floor-system production and vicinity of other pig-farms is a potential risk factors for the occurrence of respiratory diseases (Sorensen et al., 2006; Prodanov-Radulović et al., 2011). Large number of pigs on the farm and high-density of the animals also play a role in the outbreak of the disease (Stark, 2000). Besides, air quality, temperature, nutrition and hygiene regimen are important factors in the development and course of the disease (Hurnik et al., 1994; Sorensen et al., 2006).

Type of lesions in lung tissue, pleura and heart in underdeveloped fatlings and piglets

Lung changes affecting 13-78% of lung tissue were observed in all fatteners. All lung lobes were affected, and pleuritis was established in 50% of cases. In eight fatlings, the changes affected over 40% of lung tissue.

Table 5. *Type of lesions in underdeveloped fatteners and piglets*

Group	Normal lung		Lung inflammation			Severe lung inflammation	
	0 affected	1-10% affected	11-20% affected	21-30% affected	31-40% affected	41-50% affected	>50% affected
			5/25%	3/15%	4/20%	2/10%	6/30%
Total	20 (40%) fatlings						

In the examined samples, the following microorganisms were isolated: *H. parasuis* (4 samples), *Arcanobacterium pyogenes* and *P. multocida* (2 samples), *A. pyogenes* and *H. parasuis* (2 samples), *Pasteurella multocida* and *H. parasuis* (2 samples), and *A. pyogenes*, *H. parasuis* and *P. multocida* (1 sample). In the examined piglets, lobular collapse of lung tissue (4), emphysema (2), interstitial edema (2), fibrinous pneumonia and hepatization (2) and lung tissue hepatization were diagnosed.

Conclusion

High rate of changes in respiratory organs of clinically healthy fatteners was evident. It substantially affects the fluctuation in body weight, leanness and carcass quality. In spite of prophylactic measures and persistent therapy, a number of fatteners are underdeveloped. The apparent high-grade changes in thoracic cavity organs of examined pigs raise the issue of economical feasibility of their raising. Moreover, such fatlings are potential carriers of infectious agents. The examination performed at the slaughter line strongly suggested the necessity of designing a new, updated vaccination program taking into consideration the causative agents and vaccination schedule.

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References

1. Christensen LS, Sorensen V, Mousing J 1999. Disease of the respiratory system. In Disease of Swine (eds B Strawe, SD Allaire, W Mengeling, D Taylor), pp. 927-928 Iowa State University Press, USA.
2. Dailidavičienė J, Januškevičienė G, Jukna V, Pockevičius A, Kerzienė S 2008. Typically definable respiratory lesions and their influence on meat characteristics in pigs. Veterinatrija ir zootechnika T. 43,65.
3. Došen R, Prodanov J, Pušić I, Stojanov I, Maljković M 2008. Uticaj oboljenja respiratornog trakta na zaostajanje svinja u porastu. Arhiv veterinarske medicine 1, 35-40.
4. Došen R, Prodanov-Radulović J, Pušić I, Stojanov I, Stojanović D, Ratajac R 2011. Resistance *Escherichia coli* isolates to antibiotics from the organ samples originating from swine farms. Biotechnology in Animal Husbandry 27, 3, 861-866.
5. Došen R, Prodanov-Radulović J, Stojanović D, Petrović T, Pušić I, Ratajac R 2013. Virusne bolesti respiratornog trakta svinja. II International Symposium and XVIII Scientific Conference of Agronomists of Republic of Srpska, Trebinje, Bosnia and Herzegovina, 371 pp.
6. Hurnik D, Dohoo IR, Bate LA 1994. Types of farm management as risk factors for swine respiratory disease. Preventive Veterinary Medicine 20, 147-157.
7. Leneveu P, Robert N, Keïta A, Pagot E, Pommier P, Tessier P 2005. Lung Lesions in Pigs at Slaughter: A 2-Year Epidemiological Study in France, Intern J Appl Res Vet Med. 3, 259-265.

8. Neumann EJ, Kliebenstein JB, Johnson CD, Mabry JW, Bush EJ, Seitzinger AH, Green AL, Zimmerman JJ 2005. Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. Journal of the American Veterinary Medical Association 227, 3, 385-392.
9. Prodanov-Radulović J, Došen R, Pušić I, Stojanov I, Lupulović D, Ratajac R 2011. The transmission and spreading routes of Aujeszky's disease in swine population. Biotechnology in Animal Husbandry 27, 3, 867-874.
10. Sorensen V, Jorsal SE, Mousin J 2006. Diseases of Respiratory System. In: Diseases of Swine (eds Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ), pp. 149-177. Blackwell Publishing, UK.
11. Stärk K 2000. Epidemiological Investigation of the Influence of Environmental Risk Factors on Respiratory Diseases in Swine- A Literature Review. The Veterinary Journal 159,37–56.
12. Willard C 2005. Economic impacts of reduced pork production associated with the diagnosis of *Actinobacillus pleuropneumoniae* on grower/finisher swine operations in the United States. Preventive Veterinary Medicine 68,181–193.
13. Van Alstine WG 2012. Respiratory System. In Diseases of Swine (eds Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW), pp. 348-362. Wiley-Blackwell Publishing, USA.

Original paper

UNDERSTANDING INTENTION TOWARD ANIMAL WELFARE BETWEEN IRANIAN STOCKPERSON

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Abstract

Although conventional livestock farming has been successful at production and cost-benefits rate however, besides these successes, modern livestock system, also, created ethical, social and environmental challenge. In other words, it is really unsuccessful at the issues of environmentally friendly production, animal health and animal welfare. In this situation, organic livestock has been introduced as an alternative for conventional livestock. Organic livestock is guided by a set of fundamental goals and ideas. Providing animal welfare can be related to some overall goals for organic. As such understanding how different actors perceive it is a precondition for the successful improvement of animal welfare. To our knowledge, no studies undertake to investigate stockmen's intention toward animal welfare in Iran and MENA region. As such, the aim of this study is to investigate the intention of stockmen regarding importance and necessity of animal welfare at Sirjan County in Kerman province in the middle of Iran through a psychological perspective. Therefore, 100 industrial stockmen through simple random sampling were selected who, as we assume, will be the key stakeholders, and will contribute to the animal welfare in Iran. Data were analyzed using the SPSS. The results through a multiple regression revealed that attitude toward animal welfare, moral norm, control perceived behaviour and outcome expectations were predictor of stockmen' intention toward animal welfare. The findings of this study provided preliminary data toward improvement of animal welfare between Iranian stockmen.

Key words: a*nimal welfare, industrial stockperson, intention, Iran*

Introduction

Although conventional livestock farming has been successful at production and cost-benefits rate however, besides these successes, modern livestock system, also, created ethical, social and environmental challenge. In other words, it is really unsuccessful at the issues of environmentally friendly production, animal health and animal welfare. In this situation, organic livestock has been introduced as an alternative for conventional livestock. Organic livestock is guided by a set of fundamental goals and ideas. Providing animal welfare can be related to some overall goals for organic. Organic livestock farming has an explicit goal of improved animal health and welfare compared with non-organic

farming (Vaarst and Alroe, 2012). The welfare of animals can be defined in many ways (Kauppinen et al., 2010) and there are many different conceptions of what welfare is, and animal welfare is both an evaluative concept, as well as a normative concept, which involves both value judgments and ethical concerns (Vaarst and Alroe, 2012). In organic agriculture, a number of animal welfare issues differ clearly when compared to non-organic farming. This means that not only is there an explicit goal of improved livestock welfare, but—more important—an underlying philosophical and ethical idea and definition of what constitutes good animal welfare. As such, understanding how different actors perceive it is a precondition for the successful improvement of animal welfare (Kauppinen et al., 2010). The role of the stockperson in the welfare and productivity of farm animals has received increasing attention over recent years (Coleman et al., 2000). For example, stockperson behavior has been shown to be strongly related to fear and reproductive performance in pigs (Coleman et al., 2000). Therefore, the most relevant attitudes are those of the farmers and the farmer who as a care giver has a vital influence on animal welfare (Kauppinen et al., 2010).

In this paper we deal with the question of how a group of stakeholders, such as farmers, perceive animal welfare and potentials for their behavior in Iran. This is especially interesting in Iran, which is marked by Islamic country, because Islam frequently advocated for respect to animal. We believe that farmers, who will be key stakeholders, will play an important role in deployment animal welfare in Iran. As such the aim of this research is investigated intention toward animal welfare in industrial dairy sector in Iran.

Materials and methods

The study was designed as a cross-sectional survey. The population of interest consists of industrial dairy farmers in Sirjan city, eastern Iran. The study sample consisted of 100 farmers selected through a random sampling. The participants aged from 25 to 71 and had a mean age of 49/17 years (SD = 11/05). All farmers were males.

The variables were quantitatively tested using the survey methodology to understand individual intention. An in-depth literature review was used to develop the questionnaire to collect data for this study. Data were collected through personal interviews based on a structured questionnaire. The questionnaire was used for a face-to-face survey with farmers, conducted in the period from 2013. Researchers received all completed questionnaires directly after the survey; no intermediaries were involved into the analysis or interpretation of results. All farmers received the right to refuse to participate, to refuse to answer any question they deemed to be too sensitive or that they felt uncomfortable about. The questions were scored on a 1-5 point scale (very low, low, moderate, high, very high) to reduce the statistical problem of extreme skewness.

Results and discussion

A multiple regression analysis, with intention regarding animal welfare as the dependent variable, and with attitude, moral norm, self-efficacy, outcome expectations and moral norm as independent variables, revealed that attitude, self-efficacy and outcome expectations are significant predictors (Table 1).

These three variables predicted 47% of the variance in intention regarding animal welfare, with outcome expectations appearing to contribute most to the model, (β=0.61, $p<0.000$),

followed by self-efficacy (β=0.19, p<0.000) and then attitude (β =0.15, p<0.009). Analysis revealed that relationships between moral norm and intention were not significant.

Table1. *Enter regression of variables on intention regarding animal welfare*

Independent variables	b	Std.er	Beta	Signif T
Outcome expectations	0.79	0.10	0.61	0.000
Self-efficacy	0.14	0.03	0.19	0.000
Attitude	0.06	0.02	0.15	0.009
Constant= -0/17, F= 55/40, Sig= 0/000				
Variable	Multi. R	R^2	R^2 Adjust	
Intention	0.68	0.47	0.46	

Intention is a plan or motivation which influences human behavior (Ajzen, 1991). It refers to the individual attempts to doing special behavior and clearly, probably the occurrence of behavior is directly dependent on his/her intention (Ajzen, 1991). The past research revealed that attitude is the most important factor which can influence intention (Kielland et al., 2010; Breuer et al., 2000; Coleman et al., 1998). Attitude refers to "the degree of a person's favorable or unfavorable evaluation or appraisal of the behavior in question" (Fishbein and Ajzen, 1975). As such we expected that farmers attitude toward animal welfare directly influence their intention toward animal welfare. The second factor which can influence farmers' intention is self-efficacy. It refers to the "people's perception of ease or difficulty in performing the behavior of interest" (Liao et al., 2007), or "the extent to which individuals perceive the behavior to be under their volitional control" (Fielding et al., 2008). We added to other factor to framework, moral norm and farmers' expectation regarding animal welfare. Expectations refers, individual's beliefs about costs and benefits of the behavior or judgments about the results of behavior have an important effect on intention (Bundura, 2004). Outcome expectations are the anticipated consequences of a behavior. It is the relative value that an individual places on each outcome expectation (Winters et al., 2003). When animal welfare can contribute to more production in animal (Kauppinen et al., 2012) farmers' expectation regarding their benefits can influence their intention toward animal welfare. Moral norms are internal moral rules or values, motivated by anticipated self-administered rewards or punishments (Arvola et al., 2008). Moral norms originate from the psychologist Schwartz's (1977) norm-activation theory of altruism. Schwartz's theory claims that pro-environmental actions occur in response to personal moral norms about them and that these are activated in individuals who believe that environmental conditions pose threats to other people, other species, or the biosphere and that actions they initiate could avert those consequences (Stern et al., 1995). Since many researchers (Kauppinen et al., 2010; Vaarst and Alroe, 2012) acknowledged that animal welfare behavior is a moral behavior we added it to our framework.

Conclusion

Our analysis revealed that expectation about outcome of animal welfare, attitude and self-efficacy can determine farmers' intention toward animal welfare. And moral norm was not significant predictor of intention. Based on this finding we can conclude that farmers see animal welfare more instrumental than humanity and morality. Furthermore, for policy implication we suggest that at first stage, farmers be aware about the effects of animal

welfare and their animal productivity. This clarity makes them more motivated to doing behavior regarding animal welfare.

Our results, also, revealed that self-efficacy of animal welfare will be expected to have an impact on the possibility of doing this behavior. This finding suggests that animal welfare programs should seek to gain widespread support from the community parts to animal welfare and provide strategies that ensure us people find it easy to engage in this behavior. Researchers aiming to increase animal welfare behavior may need to focus on the strategies that strengthen farmers' plans and objectives to animal welfare. Promoting farmers' confidence through educational programs in order to enable them to overcome any perceived barriers and difficulties in actions resulting in welfare behavior, will improve adherence to animal welfare behavior among the population. Furthermore, while attitude was another significant variable, in order to increase animal welfare behavior, we should learn about and understand farmers' attitudes toward animal welfare. We believe that in Iran, the success of many policy instruments will be limited unless we succeed in installing a more positive attitude toward this behavior practices in the minds of stakeholders.

References

1. Ajzen I 1991. The theory of planned behavior. Organizational behavior and human decision processes, 50(2), 179-211.
2. Arvola A, Vassallo M, Dean M, Lampila P, Saba A, Lähteenmäki L and Shepherd R 2008. Predicting intentions to purchase organic food: The role of affective and moral attitudes in the Theory of Planned Behaviour. Appetite, 50(2), 443-454.
3. Bandura A. 2004. Health promotion by social cognitive means. Health education & behavior, 31(2), 143-164.
4. Breuer K, Hemsworth PH, Barnett JL, Matthews LRand Coleman GJ. 2000. Behavioural response to humans and the productivity of commercial dairy cows. Applied Animal Behaviour Science, 66(4), 273-288.
5. Coleman GJ, Hemsworth PH and Hay M 1998. Predicting stockperson behaviour towards pigs from attitudinal and job-related variables and empathy. Applied Animal Behaviour Science, 58(1), 63-75.
6. Coleman GJ, Hemsworth PH, Hay M and Cox M 2000. Modifying stockperson attitudes and behaviour towards pigs at a large commercial farm. Applied Animal Behaviour Science, 66(1), 11-20.
7. Fielding K, McDonald SR and LouisWR 2008. Theory of planned behaviour, identity and intentions to engage in environmental activism. Journal of Environmental Psychology, 28(4): 318–326.
8. Fishbein M and Ajzen I 1975. Belief, Attitude, Intention and Behavior: An Introduction to Theory and Research. Addison-Wesley Publishing Company Inc: Reading, Massachusetts, USA.
9. Kauppinen T, Vainio A, Valros A, Rita H and Vesala KM2010. Improving animal welfare: qualitative and quantitative methodology in the study of farmers' attitudes. Animal Welfare, 19(4), 523.
10. Kauppinen T, Vesala, KM and Valros A 2012. Farmer attitude toward improvement of animal welfare is correlated with piglet production parameters. Livestock Science, 143(2), 142-150.

11. Kielland C, Skjerve E, Østerås Oand Zanella AJ 2010. Dairy farmer attitudes and empathy toward animals are associated with animal welfare indicators. Journal of dairy science, 93(7), 2998-3006.
12. Liao Chechen, Jain-Liang Chen, and David CYen 2007. "Theory of planning behavior (TPB) and customer satisfaction in the continued use of e-service: An integrated model." Computers in Human Behavior 23.6 2007: 2804-2822.
13. Stern PC, DietzT and Guagnano GA 1995. The new ecological paradigm in social psychological context. Environment and Behaviour, 27: 723-743.
14. Vaarst Mand Alroe HF 2012. Concepts of animal health and welfare in organic livestock systems. Journal of agricultural and environmental ethics, 25(3), 333-347.
15. Winters ER, Petosa RL and Charlton TE 2003. Using social cognitive theory to explain discretionary "leisure-time" physical exercise among high school students. Journal of adolescent health, 32(6), 436-442.

Original paper

INFLUENCE OF BLOOD AND MILK SELENIUM CONCENTRATION ON SOMATIC CELL COUNT IN EARLY AND MID LACTATION

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Abstract

The aim of this study was to determine the effect of selenium concentration in blood and milk in early lactation on somatic cell count. The average selenium concentration in the blood serum was 0.62 ± 0.11 μmol/l and in the milk serum 0.61 ± 0.07 μmol/l. Optimal blood selenium concentrations were found in 19 cows and suboptimal concentrations in 11 cows. Optimal milk selenium concentration was found in 14 cows and suboptimal in 16 cows. The average milk production per cow was 23.12 ± 3.1 liters / day and the average somatic cell count in the first and sixth months of lactation was 5.34 ± 5 (log transformed) and 5.12 ± 4.9, respectively. Blood selenium concentration correlated negatively with milk somatic cell count in early and mid lactation. The classification of cows based on blood selenium concentration gave results which suggested that selenium-deficient cows had a significantly higher somatic cell count in early lactation (the first month) and mid lactation (the sixth month). Blood selenium is an important predictor of milk somatic cell count. Somatic cell count in milk is not dependent upon selenium concentration in milk or interaction blood×milk selenium. Selenium concentration is not in connection with milk production.

Key words: *cows, selenium, somatic cell count*

Introduction

Selenium acts as a cellular antioxidant in the cell cytoplasm by preventing cell damage due to peroxidase and plays a major role in the function of the immune response (Miller et al., 1993). Parturition and early lactation lead to a weakened immune system and a subsequent increase in the risk of infection in dairy cows (Mallard et al., 1998). Miller et al. (1995) found that blood selenium concentrations decrease at parturition. Uncontrolled peroxide is highly damaging to healthy cells and healthy tissue of the mammary gland (Kommisrud et al., 2005). Selenium is essential in helping leukocytes: it reduces the formation of peroxides, translates them into safe substances and, then, destroys phagocytized pathogens (Larsen, 1993; Finch and Turner, 1996; Smith et al., 1997; McKenzie et al., 1998). We hypothesized that selenium deficiency in early lactation can affect mammary gland health in cows. The aim of this study was to determine the effect of selenium concentration in blood and milk in early lactation to somatic cell count.

Materials and methods

Animals: The experiment included 30 high-producing dairy Holstein-Friesian cows raised under farm conditions. The cows had similar body condition scores. They were in their third (14 cows) or fourth (16 cows) lactation and gave approximately the same amount of milk in the previous lactation (7000 liters).

Blood analysis: Blood was taken during the first month of lactation (25-30 days) by v.coccygea to determine selenium concentration. Blood sera were further analyzed by atomic absorption spectrometry (AAS) on a Perkin Elmer Elan 6100 ICPMS, Massachusetts, USA. Selenium concentration was determined using the method described by Maas et al. (1992).

Milk analysis: Milk samples were taken at the time of blood sampling. Somatic cell count (SCC) was determined in bulk milk samples from every quarter using MILKOSCAN appliances. Milk serum was separated for the purpose of measuring selenium concentration and further analyzed by atomic absorption spectrometry. The methodology was the same as for the blood serum.

Model and statistics: In the first step results are presented as mean ±SD. In second step we examined correlation (Pearsons) between Se concentration in blood and milk and SCC in early and mid lactation. Finally, a statistical model to assess the impact of selenium in the blood and milk of the number of somatic cells in milk of cows is formed. On the basis of the value of selenium in the milk and blood the cows were denoted as cows with optimal or suboptimal value of selenium in blood and milk. Lower reference range of selenium is 0.6 μmol/l. Data were analyzed by ANOVA as a manual 2x2 factorial experiment, and the influence of the concentration of selenium in blood serum selenium in milk serum and their interaction, according to the model: $yi = \mu + Bi + LJ + FC + fjk + l \times \varepsilon ijkl$, where y - the dependent variable – SCC or milk production, Bi - effect block, Ij - effect of selenium concentration in blood serum (j - optimal concentrations, suboptimal concentration), fk - effect of selenium concentration in the milk serum (k - optimal concentrations, suboptimal concentration), $L \times fjk$ - interaction between the two variables and $\varepsilon ijkl$ - residual error.

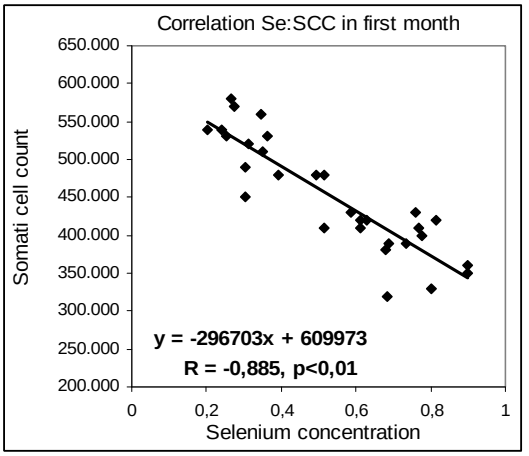
Results and discussion

The average selenium concentration in the blood serum was 0.62 ± 0.11 μmol/l and in the milk serum 0.61 ± 0.07 μmol/l. Optimal blood selenium concentrations were found in 19 cows and suboptimal concentrations in 11 cows. Optimal milk selenium concentrations were found in 14 cows and suboptimal in 16 cows. The average milk production per cow was 23.12 ± 3.1 liters / day and the average somatic cell count in the first and sixth months of lactation was 5.34±5 (log transformed) and 5.12±4.9, respectively. The above data are shown in Table 1.

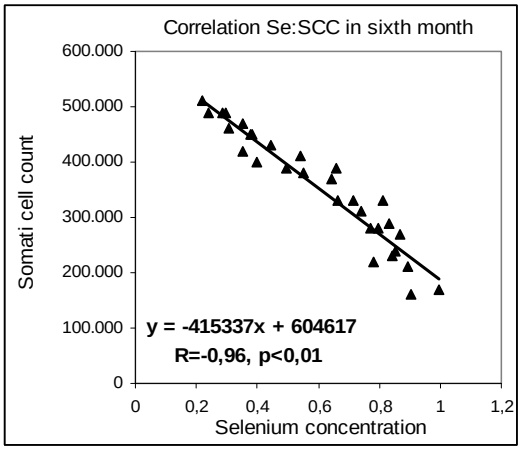
Table 1. *Description data from experimental cows*

Parameter	Mean±SD
Blood selenium (μmol/l)	0.62±0.11
No. of cows with optimal / suboptimal blood selenium concentration	19 / 11
Milk selenium (μmol/l)	0.61±0.07
No. of cows with optimal / suboptimal milk selenium concentration	14 / 16
Milk yield (liters/day/cow)	23.12±3.1
SCC in the first month of lactation (log/ml)	5.34±5
SCC in the sixth month of lactation (log/ml)	5.12±4.9

Blood selenium concentration correlated negatively with milk somatic cell count in early and mid lactation. The above results are given in Graphs 1 and 2. Correlations between other variables were not determined.



Graph 1. *Correlation between blood selenium concentration and milk somatic cell count at first lactating month – early lactation*



Graph 2. *Correlation between selenium blood concentration and milk somatic cell count at sixth lactating month – mid lactation*

The classification of cows based on blood selenium concentration gave results which suggested that selenium-deficient cows had a significantly higher somatic cell count in early (the first month) and mid lactation (the sixth month). Blood selenium is an important predictor of milk somatic cell count. Somatic cell count in milk is not dependent upon selenium concentration in milk or interaction blood×milk selenium. Selenium concentration is not in connection with milk production.The above results are given in Table 2.

Table 2. *Influence of selenium concentration on milk production and SCC in early and mid lactation*

	Treatment					p value		
	OBS*	OMS	SBS	SMS	SEM	BS**	MS	BS×MS
Milk production	25.4	23.5	24.5	24.7	1.4	NS	NS	NS
SCC early (log)	5.12	4.93	5.58	5.45	0.9	<0.01	NS	NS
SCC mid (log)	5.05	4.82	5.33	5.06	0.8	<0.01	NS	NS

*OBS – optimal Se value in blood, OMS – optimal Se value in milk, SBS – suboptimal Se value in blood, SMS – suboptimal Se value in milk.

**BS – influence of blood Se to SCC, MS – influence of milk Se to SCC, BS×MS – influence of interaction of blood and milk Se to SCC.

The range of physiological values for blood selenium in dairy cows is 0.6 to 0.9 mmol/l (Erdeljan et al., 2011, Juniper et al., 2006, Gunter et al., 2003). Our results are in accordance with the above. Pechová et al. (2008) reported that there is no significant correlation between blood selenium concentration and milk selenium concentration, which is consistent with our results. However, Grace et al. (2001) found a statistically significant linear correlation between blood and milk selenium concentrations. The concentration of selenium in the blood and breast milk depends on selenium supplements used on farms, since the use of selenium leads to a significant increase in its concentration in the blood and breast milk (Ran et al., 2010).

Atroshi et al. (1986) and Hogan et al. (1993) found that the occurrence of mastitis in cows is associated with low glutathione peroxidase and vitamin E in the blood plasma. Kruze et al. (2007) observed that cows infected with *Staphylococcus aureus* receiving selenium in their diet showed a significantly higher glutathione peroxidase activity and a significantly lower milk somatic cell count. Low levels of glutathione peroxidase were found to reduce the antioxidant capacity of the defense system of the mammary gland, leading to an increase in mastitis incidence and somatic cell count in milk (Mukherjee, 2008). Selenium concentrations and glutathione peroxidase activity are positively correlated (Pilarczyk et al., 2012). Selenium is an integral part of the enzyme, and this can explain why selenium-deficient cows exhibit higher infiltration of inflammatory cells undergoing excessive inflammation. Selenium deficiency provokes an inflammatory process due to reduced antioxidant activity in tissues when there is an accumulation of immune cells in response to prolonged inflammation; therefore, the concentration of selenium is negatively correlated with the degree of cellular infiltration in the parenchyma of the udder. A reduction in mastitis after dietary selenium and vitamin E intakes occurs as the result of enhanced activities of glutathione peroxidase (Hemmingway, 1999; Weiss et al., 1997). Selenium supplementation leads to a reduction in subclinical mastitis and somatic cell count in dairy cows (Barbano et al., 2006; Cope et al., 2009; Rabiee et al., 2010; Weiss et al., 2002; Davidov et al., 2012).

Conclusion

Blood selenium concentration plays an important role in maintaining mammary gland health. Selenium-deficient cows were found to have a large milk somatic cell count. Blood selenium is an important predictor of milk somatic cell count. Somatic cell count in milk is not dependent upon selenium concentration in milk or interaction blood×milk selenium. Selenium concentration is not in connection with milk production.

Acknowledgments

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References

1. Atroshi F, Parantainen J, Sankari S and Osterman T 1986. Prostaglandin and glutathione peroxidase in bovine mastitis. Res Vet Sci 40, 361-366.
2. Barbano DM, Ma Y and Santos MV 2006. Influence of raw milk quality on fluid milk shelf life. J Dairy Sci 89, 15-19.
3. Cope CM, Mackenzie AM, Wilde D and Sinclair LA 2009. Effects of level and form of dietary zinc on dairy cow performance and health. J Dairy Sci 92, 2128-2135.
4. Davidov I, Radinović M, Erdeljan M, Belić B, Cincović MR and Boboš S 2012. Blood selenium concentration, somatic cell count and their correlation at first and sixth month of lactation in dairy cows. Contemporary Agriculture 61, 95-103.
5. Erdeljan M, Davidov I, Boboš S, Radinović M and Stančić I 2011. Nalaz nivoa selena u krvnom serumu kod krava u laktaciji. Letopis naučnih radova Poljoprivrednog fakulteta u Novom Sadu 35, 104-109.
6. Finch JM and Turner RJ 1996. Effects of selenium and vitamin E on the immune response of domestic animals. Res Vet Sci 60, 97-106.
7. Grace ND, Ankenbauer-Perkins KL, Alexander AM and Marchant RM 2001. Relationship between blood selenium concentration or glutathione peroxidase activity, and milk selenium concentrations in New Zealand dairy cows. NZ Vet J 49, 24-28.
8. Gunter SA, Beck PA and Phillips JM 2003. Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves. J Anim Sci 81, 856-864.
9. Hemmingway RG 1999. The influences of dietary selenium and vitamin E intakes on milk defense responses to mastitis. J Dairy Sci, 76, 2795-2803.
10. Hogan JS, Weiss WP and Smith KL 1993. Role of vitamin E and selenium in host defense against mastitis. J Dairy Sci 76, 2795-2803.
11. Juniper DT, Phipps RH, Jones AK and Betrin G 2006. Selenium supplementation of lactating dairy cows: effect on selenium concentration in blood, milk, urine and feces. J Dairy Sci 89, 3544-3551.
12. Kommisrud E, Osteras O and Vatn T 2005. Blood selenium associated with health and fertility in Norwegian dairy herds. Acta Vet Scand 46, 229-240.
13. Kruze J, Ceballos A, Stryhn H, Mella A, Matamoros R, Contreras PA, Leyan V and Wittwer F 2007. Somatic cell count in milk of selenium-supplemented dairy cows after an intramammary challenge with Staphylococcus aureus. J Vet Med A Physiol Pathol Clin Med. 54, 478-483.
14. Larsen HJS 1993. Relation between selenium and immunity. Nor J Agric Sci, 11, 105-119.
15. Maas J, Galey FD, Peauroi JR, Case JT, Littlefield ES, Gay CC, Koller LD, Crisman RO, Weber DW, Warner DW and Tracy ML 1992. The correlation between serum selenium and blood selenium in cattle. J Vet Diagn Invest, 4, 48-52
16. Malard BA, Dekkers JC, Ireland JM, Leslie KE, Sharif S, Lacey VC, Wacter L and Wilkie BN 1998. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. J Dairy Sci, 81, 585-595.

17. McKenzie RC, Rafferty TS and Beckett GJ 1998. Selenium: an essential element for immune function. Immunol Today 19, 342-345.

18. Miller GY, Bartlett PC, Erskine RJ and Smith KL 1995. Factors affecting serum selenium and vitamin E concentration in dairy cows. JAVMA 206, 1369-1373.

19. Miller JK, Brzezinska-Slebodzinska E and Madsen FC 1993. Oxidative stress, antioxidants and animal function. J Dairy Sci 76, 2812-2823.

20. Mukherjee R 2008. Selenium and vitamin E increases polymorphonuclear cell phagocytosis and antioxidant levels during acute mastitis in riverine buffaloes. Vet Res Commun 32, 305-313.

21. Pechová A, Pavlata L, Dvorák R and Lajková E 2008. Contents of Zn, Cu, Mn and Se in Milk in Relation to their Concentrations in Blood, Milk Yield and Stage of Lactation in Dairy Cattle. Acta Vet Brno 77, 523-531.

22. Pilarczyk B, Jankowiak D, Tomza-Marciniak A, Pilarczyk R, Sablik P, Drozd R, Tylkowska A and Skólmowska M 2012. Selenium concentration and glutathione peroxidase (GSH-Px) activity in serum of cows at different stages of lactation, Biol Trace Elem Res 147, 91-96.

23. Rabiee AR, Lean IJ, Stevenson MA and Socha MT 2010. Effects of feeding organic trace minerals on milk production and reproductive performance in lactating dairy cows: A meta-analysis. J Dairy Sci 93, 4239-4251.

24. Ran L, Wu X, Shen X, Zhang K, Ren F and Huang K 2010. Effects of selenium form on blood and milk selenium concentrations, milk component and milk fatty acid composition in dairy cows. J Sci Food Agric 90, 2214-2219.

25. Smith KL, Hogan JS and Weiss WP 1997. Dietary vitamin E and selenium affect mastitis and milk quality. J Anim Sci 75, 1659-1665.

26. Weiss WP 2002. Relationship of mineral and vitamin supplementation with mastitis and milk quality. In: Proc. Annual Meeting, National Mastitis Concil, Orlando, Florida, USA, 37-44.

27. Weiss WP, Hogan JS, Todhunter DA and Smith KL 1997. Effect of vitamin E supplementation in diets with a low concentration of selenium on mammary gland health of dairy cows, J Dairy Sci 80, 1728-1737.

Original paper

SWINE DYSENTERY: PRACTICAL OBSERVATIONS, CONTROL AND DIAGNOSTICS

Prodanov-Radulović J. [*]*, Došen R., Stojanov I., Polaček V., Milanov D., Pušić I., Marčić D.*

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Abstract

Swine dysentery is a severe mucohemorrhagic enteric disease of pigs which has a large impact on pig production, with important losses caused by mortality and suboptimal performance. The causative agent is *Brachyspirahyodysenteriae*. The aim of the paper was to evaluate all the available data on *B. hyodysenteriae* presence on swine farms in Vojvodina region. The material for this research included five swine farms, where certain disorders and health problems in weaned, grower and fattening pigs were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: anamnestical and clinical evaluation, gross pathological examination, standard bacteriological testing for detection of the presence of aerobic and anaerobic bacteria in the tissue samples derived from diseased and/or died pigs. Besides this, in some cases the molecular diagnostic method (RT-PCR) was included. Swine dysentery is a common and important endemic problem in many swine farms in Vojvodina. On endemically infected swine farms transmission mainly occurs by ingestion of infected faeces. All the observed factors affecting disease persistence and transmission on the farm are thoroughly analysed and discussed. Finally, current prophylactic and therapeutic approaches to fight against disease are described.

Key words: *control, diagnostics, swine dysentery*

Introduction

Swine Dysentery (SD) is a severe mucohemorrhagic enteric disease of pigs which has a large impact on pig production, with important losses caused by mortality and suboptimal performance (Alvarez-Ordóňez et al., 2013). The causative agent is *Brachyspirahyodysenteriae,* a gram negative, motile, anaerobic bacterium which belongs to the *Brachyspiraceae* family, phylum *Spirochaetes* (Hampson, 2012) and naturally infects pigs (including feral pigs) and occasionally some other species (Jacobson et al., 2004). On infected farms it has been isolated from mice, rats, dogs and feral birds (Alvarez-Ordóňez et al., 2013). The disease primarily affects pigs during the growth and finishing periods, but it may also occur in adults and occasionally in suckling pigs (Hampson, 2012). Clinical signs may vary in range, from mild mucous diarrhea with unaltered general condition to severe hemorhagic diarrhea with a mortality rate of 50-60% (Alvarez-Ordóňez et al., 2013). The disease is primarily transmitted by ingestion of faecal

material either from clinically affected pigs or from clinically normal pigs that carry *B. hyodysenteriae* (Novotna and Škardova, 2002).

Swine dysentery is a relatively common endemic problem in many countries in the European Union, South America and Southeast Asia (Hampson, 2012).The incidence varies in different countries and regions and changes with time. Variations in prevalence can be due to the use of different diagnostic methods or to differences among countries in housing management and feeding regimens (Novotna and Škardova, 2002). In many countries the prevalence may be concealed by the use of antimicrobials as feed additives, in others the ban of antibiotics as growth promoters may have resulted in an increase in SD prevalence (Alvarez-Ordóňez et al., 2013; Pringle et al., 2012).

The aim of the paper is to summarize the available data on *B. hyodysenteriae* presence on swine farms in Vojvodina region. The factors affecting disease transmission are thoroughly discussed (husbandry factors - production system, production stage, farm management). Finally, current prophylactic and therapeutic approaches to fight this disease are described.

Materials and methods

The material for this research derived from five swine farms, where certain disorders and health problems in different swine categories (weaners, growers and fattening pigs) were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: anamnestical and clinical evaluation, gross pathological examination, standard bacteriological testing to detect the presence of aerobic and anaerobic bacteria in the organs and tissue samples (spleen, liver, kidney, lung, mesenterial and mediastinal lympho nodes) derived from diseased and/or died pigs. In one case, the microbiological feed testing, in order to examine the presence of fungi and mycotoxins by the method of thin layer chromatography, was included. Besides this, the molecular diagnostic methods, real time reverse transcriptase-polymerase chain reaction (RT-PCR) for detection of *Mycoplasma hyopneumoniae* (Strait et al., 2008) and a multiplex RT-PCR for detection of *Brachyspira hyodysenteriae* (DNA extracted from faeces) (La et al., 2006) were applied.

Results and discussion

In the first examined farm, by anamnestical evaluation, health problems in weaners and finishers were discovered: loss of weight, insufficient weight gain and dehydration. Clinically, at the end of the weaning period, the bloody diarrhoea in most of weaned piglets was detected. In some cases, the diarrhoea was greyish black, with blood and mucus flecks. Perineal staining was also present. Analysing the existing data on the farm, the high incidence of morbidity and mortality in weaners was noticed. Therapeutic treatment of piglets only temporarily improved health problems. In addition, a reappearance of SD often occurs after removal of antimicrobials from the water or feed. Clinically, in the finishers, the prominent signs of bronchopneumonia (dyspnea, cough and purulent nasal discharge, fever) and intermittently bloody diarrhoea have been registrated lately. By applying gross pathological examination on dead weaners and finishers, the prominent changes on the digestive tract (*Gastroenteritis haemorrhagica, Typhlocolitis haemorrhagica, Ulcus oesophagogastricum*) and respiratory tract (*Pleuritis adhaesiva diffusa, Pneumonia fibriosa in statu hepatisationis rubrae et griseae*) were detected. By bacteriological testing from the tissue samples derived from the dead fatteners, *Escherichia coli haemolytica* and *Actinobacillus suis* were isolated. By applying RT-PCR method on

the faecal samples derived from weaners and finishers, *B. Hyodysenteriae* was detected. By applying real time RT-PCR method on the lung tissue derived from dead finishers, *M. hyopneumoniae* was detected.

Swine dysentery occurs mainly in grower and finisher pigs, and less frequently in weaners. It is often seen a few weeks after animals are moved from the nursery, coinciding with a dietary change and removal of antimicrobials used to control respiratory and enteric diseases (Alvarez-Ordóňez et al., 2013). The first evidence of SD is usually soft, yellow to gray faeces. A few days after infection, large amounts of mucus and often flecks of blood are found in the faeces. This progresses to watery stools containing blood, mucus, and shreds of white mucofibrinous exudate (Hampson, 2012). Where antibiotic medication is routine, any cause of loss of appetite (such as pneumonia) stops the intake of antibiotics and the animal may then succumb to SD (Novotna and Škardova, 2002). Outbreaks of SD are often associated with stressful conditions such as crowding, transportation or dietary changes and composition of the diet (Hampson, 2012).The pig diet has been proposed as one of the most important factors that can influence spirochaete colonization and the occurrence of mucohemorrhagic diarrhea (Jacobson et al., 2004). The intestinal tract of pigs is densely populated with bacteria and the intestinal microbiota has important influence on animal health and growth performance (Hampson, 2012). Leser et al. (2000) indicated that the pig intestinal microbial ecosystem responds fast and dynamically to perturbations such as dietary changes or infection with intestinal pathogens.

In the second evaluated swine farm, the feed from a different source was introduced in weaners-growers production stage. After that, the problem of high morbidity and mortality in weaned pigs was detected. Clinically, in the weaners and growers, a weaker food intake and bloody diarrhea associated with pronounced apathy and decumbency was observed. By applying gross pathological examination of dead weaners and growers the prominent changes on the digestive tract (*Gastroenteritis haemorrhagica, Enteritis colibacillosa, Typhlocolitis haemorrhagica)* were detected. Pigs with clinical dysentery had gross lesions, compatible with SD in the large intestine (Jacobson et al., 2004). A predominant gross change in most pigs was moderately to markedly increased mucous secretion and superficial necrotic foci with pseudo membranes. By bacteriological testing from the tissue samples deriving from dead pigs the following bacteria were isolated: *Escherichia coli haemolytica, Clostridium perfringens*. By applying RT-PCR method on the faecal samples derived from weaners and growers, *B. Hyodysenteriae* was detected.

The clinical and gross pathology findings suggested the possibility that change in the feed components influences the health condition of examined swine farms. It is well known that on endemically infected swine farms, clinical signs often occur after change in feed composition (Hampson, 2012).The influence of diet composition on the appearance of SD might be mainly related to the digestibility of their ingredients, which, in turn may have effect on the composition and equilibrium of the large intestine microbiota (Jacobson et al., 2004). The composition of the microbiota is relevant because *B. hyodysenteriae* executes its pathogenic action in association with other anaerobic members of the large intestinal microbiota (Alvarez-Ordóňez et al., 2013).

On the third examined swine farm, the health problems in finishers were connected with the recent purchasing of breeding animals from 3 different sources. Clinically, the bloody diarrhoea was continually present in finishers, especially in the first 10-15 days in fattening phase. Diarrhea was also detected after moving to new pens and mixing with different animals. At the same time, breeding animals (sows, boars) were only occasionaly affected. Therapeutic treatment of finishers by oral and parenteral antibiotics application only temporarily improved health condition. Applying gross pathological examination on the

dead finishers, typical changes SD were found: swollen colonic mucosa, with loss of typical rugose appearance, covered by mucus and fibrin, with flecks of blood. In some animals lesions become more chronic i.e. mucosal surface was covered by a thin, dense, fibrinous exudate. However, lesions were found also in clinically healthy pigs as reddened areas of the colonic mucosa, covered with mucus, but with normal colonic contents. By applying RT-PCR method on the faecal samples derived from breeding animals and finishers, *B. Hyodysenteriae* was detected.

Biosecurity aspects are important for the prevention of infective disease transmission. New outbreaks of SD usually occur following introduction of asymptomatic carrier pigs that are not quarantined and/or treated prophylactically (Alvarez-Ordóňez et al., 2013). Introduction of new stock is the greatest risk, so a reliable history of the source herd is essential. Purchased animals should be quarantined for at least 3 weeks, and treated to eliminate *B. hyodysenteriae* (Hampson, 2012). Oubreaks of SD can also occur in herds following introduction of contaminated feed, tracks, or by visitors who have had contact with infected pigs. Asymptomatic pigs may develop diarrhea following stressful management procedures, such as moving to new pens, mixture of animals from different origin, inadequate stocking densities or changes in feed (Alvarez-Ordóňez et al., 2013).

On the fourth examined swine farm, the health problems in fattening stage in the last 7 days were noticed. Clinically, pigs became depressed, manifested clinical signs of apathy and inappetence. Also, decreased interest for food was recorded with the signs of respiratory disease (cough) and bloody diarrhoea. The clinical signs were noticed after some changes in feed components, i.e. the new source of corn and barley was introduced. By applying gross pathological examination on dead fatteners the prominent changes on the digestive tract indicative for SD (diffuse mucohemorrhagic typhlocolitis) were observed. With an aim to exclude alimentary intoxication, the laboratory feed testing was applied. The presence of mycotoxin (Ochratoxin A) in sunflower pellets (0.1mg/kg) and in the barley (0.02mg/kg) was discovered.

It may be supposed that as a consequence of immunosuppresive action of mycotoxins (Kabak et al., 2006), clinical and gross pathological lesions that correspond to SD occurred on the examined farm. The gastrointestinal tract represents the first barrier against ingested food contaminants and natural toxins. Stability of the intestinal flora appears to be an important factor for animal health. Thus an impaired balance of the intestinal microbiome could have many adverse effects on the health of the host. However, data on the influence of toxins on the intestinal microflora are still limited (Greinier et al., 2013). The biggest challenge with mycotoxicoses is the non-specific nature of symptoms in the affected animals (Kabak et al., 2006). Consequently, the health disorders due to mycotoxins in the feed are difficult to diagnose (Prodanov-Radulović et al, 2011).

On the last examined swine farm, certain irregularities in the implementation of all-in/all-out system were found. Analysing the existing data on the farm, it was concluded that the basic health problem and the high mortality in growers and fatteners is a consequence of respiratory diseases and SD. Clinically, mucohemorrhagic diarrhoea occurs dominantly in the growers and sporadically in fattening pigs. Despite the fact that the growers and fatteners were therapeutically treated, there was no evident response to applied medication. Besides this, in the fatteners the canibalism and signs of respiratory disease were detected. The main problem on the farm is the existing production technology: at the same time there are always 5 different age groups of pigs situated in the same object. Despite separated pigs with the clinical signs of SD, the animals faeces can be easily carried out by workers boots through the corridors and stables. By applying gross pathological examination on the dead fatteners the prominent changes on the digestive tract

(*Gastroenteritis haemorrhagica, Typhlocolitis haemorrhagica*) and respiratory tract (*Pleuropneumonia, Pneumonia fibriosa in statu hepatisationis rubrae et griseae, Pericarditis fibrinosa*) were detected. By applying RT-PCR method on the fecal samples derived from weaners and finishers, *B. Hyodysenteriae* was detected. By applying real time RT-PCR method on the lung tissue derived from dead finishers, *M. hyopneumoniae* was detected.

Brachyspira hyodysenteriae is shed in faeces for variable periods of time. On endemically infected swine farms transmission mainly occurs by ingestion of infected faeces (Jacobson et al., 2004). Transmission between pens may occur in housing systems where there are open channels between pens (Hampson, 2012). All-in/all-out management with cleaning and desinfection between batches reduces the risk of reinfection of medicated pigs and limits spread of SD infection (Alvarez-Ordóňez et al., 2013). Ideally, affected batches of pigs should be moved to clean buildings after medication to break the cycle of infection. Careful disposal of infected bedding, the use of boot scrubbers and desinfectant foothbaths, cleaning and desinfection of equipment used in infected areas, and changing of protective clothing are vital measures (Hampson, 2012). To a great extent, the production system is a decisive factor in the control and prevention of SD. In farrow-to-finish herds, the pathogen can persist in endemic infected sows, which have overcome the infection and developed protective immunity but still shed the pathogen in their faeces. The proximity of facilities and continuous flow of animals in this sort of production system will facilitate the transmission of infection to non-infected animals. Depending on the herd immune status and measures taken to control the disease, animals will be more or less severely affected and the disease will affect principally pigs at the growing or finishing period when the medication used to control respiratory infections is removed, favoring the expression of SD (Alvarez-Ordóňez et al., 2013).

The treatment and control of SD are based mainly on the use of antimicrobials, as no commercial vaccine against *B. hyodysenteriae* is available (Hidalgo et al., 2011). Antimicrobial agents such as pleuromutilins, macrolides and lincosamides are used in the control of SD (Novotna and Škardova, 2002). However, decreased susceptibility to tiamulin among *B. hyodysenteriae* isolates has been reported in several countries. Such isolates have been detected in many pig-producing countries, for example in Spain and Czech Republic, and they represent a serious threat to the pig industry (Hidalgo et al., 2011). Continuous treatment with antibiotics in herds with SD over extended periods of time is the probable explanation to the situation of resistance reported (Pringle et al., 2010).

In Serbia, many herds are preventively or curatively treated against SD. When started with treatment, it is advised to treat the whole group in the stable. Parenteral injection of, for example, tiamulin, tylosin and lincomycin can be used. Besides this, water/feed medication is given for in-contacts (e.g. tiamulin or valnemulin). However, development of resistance to antimicrobial agents is an increasing threat to the treatment options (Novotna and Škardova, 2002). Depending on the herd structure, the production system, and economical considerations, SD eradication can be performed in several ways. These vary from intensive medication of all pigs for a short period to introduction of medicated early weaning and multisite production, through an ongoing program performed by emptying and desinfection of each herd unit in a cycle, and introducing medicated animals to cleaned and disinfected units (Hampson, 2012).

The recommendation for biosecurity plan includes some basic principles for the prevention and control of SD, such as maintaining a closed herd with no contact with outside animals. In the case of new introductions, the farmer should isolate, test and treat incoming animals

for SD. The measures should also include control of visitors and vehicles, and prevention of direct and indirect contact with other domestic species and wildlife. The producer should ensure that swine feed and water are free of contamination.When introducing new animals to the herd, attemps should be made to establish the following: the disease status of the resident herd and the farm of origin, the available diagnostic tests, prophylactic treatments and their efficacy. All principles mentioned above apply equally to intra-farm biosecurity. External security of building; all-in/all-out policies, cleaning and desinfection between houses, and treatment prior moving or mixing may be important in controlling the spread of SD between different ages, groups and houses where pigs are kept. Especially important is the existence of strategically placed, well-maintained effective disinfectant foot baths and cleaning equipment; washing facilities and separation of clean and dirty areas; separated way for carcass removal and appropriate facilities and protocols for slurry or manure collection and disposal.

Conclusion

In order to control SD in endemically infected swine herd, improved basic hygiene measures and use of strategic medication are necessary. Eradication by depopulation is the preffered method of control if practically and economically possible. Accordingly, the antimicrobial susceptibility testing of clinical isolates of *B. hyodysenteriae* has become essential in selecting SD treatment strategies. Moreover, a monitoring program may help to detect new resistance trends and to evaluate the usefulness of the available antimicrobials on a national level. Nowadays, a natural solution for prevention and treatment of SD could be the use of plant extracts. These include a natural product added to swine feed (it is not a medication) and can be used freely (no waiting period) in all production phases.

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References

1. Alvarez-Ordóňez A, Martinez-Lobo FJ, Arguello H, Carvajal A and Rubio P 2013. Swine Dysentery: Aetiology, Pathogenicity, Determinants of Transmission and the Fight against the Disease. Int. J. Environ. Res. Public Health 10, 1927-1947.
2. Greinier B and Applegate TJ 2013.Modulation of Intestinal Functions following Mycotoxin Ingestion: Meta-Analysis of Published Experiments in Animals. Toxins 5, 396-430.
3. Hampson DJ 2012. Brachyspiral Colitis. In Diseases of Swine (eds JJ Zimmerman, LA Karriker, A. Ramirez, KJ Schwartz, GW Stevenson), pp. 680-696. Wiley-Blackwell Publishing, Iowa, USA.
4. Hidalgo A, Carvajal A, Vester B, Pringle M, Naharro G and Rubio P 2011. Trends towards lower antimicrobial susceptibility and characterization of acquired resistance among clinical isolates of *Brachyspirahyodysenteriae* in Spain. Antimicrobial agents and Chemotherapy, 55(7), 3330-3337.
5. Jacobson M, Fellstrom C, Lindberg R, Wallgren P and Jensen-Waern M 2004. Experimental swine dysentery: comparison between infection models. Journal of Medical Microbiology 53, 273-280.

6. Kabak B, Dobson AD and Var I 2006. Strategies to Prevent Mycotoxin Contamination of Food and Animal Feed. Food Science and Nutrition 46, 593-619.
7. La T, Collins AM, Phillips ND, Oksa A and Hampson DJ 2006. Development of a multiplex-PCR for the rapid detection of the enteric pathogens *Lawsonia intracelullaris*, *Brachyspira hyodisenteriae* and *Brachyspira pilosicoli* in porcine faeces. Letters in Applied Microbiology 42, 284-288.
8. Leser TD, Lindecrona RH, Jensen TK, Jensen BB and Moller K 2000. Changes in Bacterial Community Structure in the Colon of Pigs Fed Different Experimental Diets and after Infection with *Brachyspirahyodysenteriae*. Applied and Environmental Microbiology 66 (8), 3290-3296.
9. Novotna M and Škardova O 2002. Brachyspirahyodysenteriae: detection, identification and antibiotic susceptibility. Vet. Med.-Czech. 47(4), 104-109.
10. Pringle M, Landen A, Unnerstad HE, Molander B and Bengtsson B 2012. Antimicrobial susceptibility of porcine Brachyspirahyodysenteriae and Brachyspirapilocicoli isolated in Sweden between 1990 and 2010. ActaVeterinariaScandinavica 54:54.
11. Prodanov-Radulović J, Došen R, Stojanov I, Pušić I, Živkov-Baloš M and Ratajac R 2011.Interaction between the mycotoxins and causative agents of swine infective diseases. Proc. Nat. Sci. Matica Srpska 120, 251-259.
12. Strait EL, Melissa L, Madsen F, Minion C, Christopher-Hennings J, Dammen M, Jones KR and Thacker EL 2008. Real-Time PCR Assays To Address Genetic Diversity among Strains of *Mycoplasma hyopneumoniae.* Journal of clinical microbiology, 2491–2498.

Original paper

LITTERS HEALTH STATUS AND GROWTH PARAMETERS IN THE SOWS FEEDING DIETS SUPPLEMENTED WITH PROBIOTIC Actisaf Sc 47® WITHIN PREGNANCY OR LACTATION

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Abstract

The aim of this study was to investigate the effect of supplementing standard diets for pregnant and lactating sows with live yeast culture (*Saccharomyces cerevisiae*) on their health status, as well as the health status and growth parameters of their litters during lactation. A total of 120 sows were divided into three groups: the first group was fed diets supplemented with probiotics during pregnancy (G+P, n=40), the second group was fed these diets during lactation (L+P, n=40), and the third group was the control group which was not fed diets supplemented with probiotics (C, n = 40). During the lactation period, a significantly ($p<0.01$) smaller proportion of probiotic treated sows (G+P=7.5%, L+P=12.5%) manifested clinical signs of the uterus and/or the udder disease in comparison with the control sows (22.5%). The incidence of infectious diarrhea in the nursing piglets was significantly ($p<0.05$) lower in the treated sows (12.5%) compared to the control sows (27.5 %). The average number of weaned piglets per litter (p/l) and average litter weight at weaning (lw) (G+P=11.6 p/l and 103.6 kg lw, L+P=11.1 p/l and 102.8 kg lw, C=10 p/l and 79 kg lw) were significantly higher ($p<0.01$ or $p<0.05$) in sows treated with probiotic compared to the control sows. These results clearly show that the use of probiotic significantly improves the health status of sows and nursing piglets, as well as the piglets growth parameters.

Key words: *diets, performance, piglets, probiotic, sows*

Introduction

Under the intensive production conditions, sows are exposed to numerous chronically stressogenic conditions (Hyun et al., 1998) which reduce their natural immunity (Kick et al., 2011; Potočnjak et al., 2012) and increase susceptibility to various infectious agents

(Sutherland, 2006). In addition, the long-term application of conventional antimicrobial preparations in the prevention and treatment of infectious diseases significantly increase infectious agent resistance to these antimicrobial preparations (Cromwell, 2002; Pugh, 2002; Gagrčin et al., 2003; Le Coz, 2012). Reduced natural immunity and increased resistance of microorganisms to antimicrobial agents result in the increase of numerous infectious diseases, and consequently in the reduced reproductive performance of sows (Hogg and Lewis, 1997; Floss and Tubbs, 1999; Gagrčin, 2003; Sutherland, 2006; Yeskim, 2007; Stančić et al., 2010). Recently, the problem of lower sow reproductive performance, as a result of reduced natural immunity and increased infective agents resistance to conventional antimicrobial preparation, has been frequently solved by using probiotic preparations (natural immunomodulators) (Blecha, 2001; Pavičić et al., 2003; Zvekić, 2006; Gallois and Oswald, 2008; Pragathi et al., 2011; Zvekić et al., 2012; Apić and Zvekić, 2013). Yeast culture was one of the most active natural immunomodulators added to food in order to prevent infectious diseases of the udder and the uterus, as well as to increase the reproductive performance of sows and their litters (Bonneau and Laarveld, 1999; Davis et al., 2004; Gallois et al., 2009; Pragathi et al., 2011). However, the results of previous studies on the effectiveness of replacing conventional antimicrobial agents with probiotics for the prevention and treatment of infectious diseases, as well as their impact on the reproductive performance of sows, are not entirely consistent (Gallois et al., 2009; Plante et al., 2011; Zvekić et al., 2012; Ferroni et al., 2012; Faldyna et al., 2012; Apić et al., 2013). According to the studies of Gagrčin et al. (2002), (2003), Stančić et al. (2010) and (2012), in more than 50% of Serbian pig farms, there is a problem of udder and uterus infectious diseases (MMA - syndrome), resulting in significantly reduced reproductive performance of sows.

Therefore, the aim of this study was to investigate the effect of adding probiotic Actisaf Sc 47® in gestating or lactating diets on the performance of sows and their litters.

Material and methods

Experimental sows. The experiment was carried out on a commercial pig farm, with about 1,200 sows in the reproductive herd, located in the AP Vojvodina, Serbia. A total of 120 experimental sows (between the first and the sixth parity) were divided into three separate groups, immediately after artificial insemination. The sows in each experimental group were equalized according to body condition, parity and health. The pregnant sows were housed in group pens (10 sows per group) and equalized by age, body condition, parity, health and the stage of pregnancy. Between 7 and 10 days before the scheduled date of farrowing, the sows were moved into the farrowing building with individual pens, where they stayed with their litters during lactation. The average lactation duration was 33 days.

Feeding sows. Standard feed diets for gestating and lactating sows were used as complete basic diets (produced by Veterinary Institute, Subotica, Serbia). These diets were supplemented with the probiotic preparation "Actisaf Sc 47", which contains live culture of *Saccharomyces cerevisiae 47* (produced by Lesaffre Group, France), in the amount of 600 g per ton of the basic feed diet for gestating or lactation sows. During the first half of gestation, all sows received 3.2 kg of complete basic diets per day, and during the second half of gestation, 3.5 kg per day. During lactation, sows were fed ad libitum with complete basic diets

for lactating sow, twice a daily (morning and afternoon). Water was available ad libitum for pregnant and lactating sows.

Experimental groups of sows. The first group (n=40 sows) was fed diets supplemented with probiotic within gestation (G+P group), the second group (n=40 sows) was fed diets supplemented with probiotic only within lactation (L+P group), and the third control group (n=40 sows) was fed only basal diets without probiotics (C group).

Estrus detection and artificial insemination. The detection of estrus was performed twice per day by direct contact with the sexually mature teaser boar, starting from the first day after weaning. The double artificial insemination (AI) was performed in estrus detected sows within first 7 days after weaning.

Data recorded: (a) liter size at farrowing, (b) litter weight at farrowing, (c) infectious diarrhea and preweaning piglets mortality and (d) litter size and litter weight at weaning. The measurement of litter weight was done immediately after farrowing and weaning and was performed with digital scales. Sows rectal temperature was measured first three days after farrowing. Elevated rectal temperature (>39.3^{o}C) and manifestation of specific clinical signs, were assumed as postpartum metritis, mastitis, hypogalactia or agalactya. The diagnosis of piglets diarrhea was established on the basis of specific clinical symptoms.

Statistical analysis. The evaluation of phenotypic parameters of the research results was done by the "Statistic 12" software package according to the average, minimum and maximum values and standard deviation of the experimental results.

T-test was used to test the difference between the arithmetic means of the results.

Results and discussion

The average live born piglets per litter were 12.22 in the G+P sows, 11.53 in the L+P sows, and 11.42 piglets in the control sows. These values were significantly ($p<0.05$) higher in the G+P sows in comparison with the control and L+P group of sows, but average number of live born piglets per litter in L+P and C control group of sows was not significantly different ($p>0.05$) (Table 1).

The average weight of a live born piglet in the litter. in both treatment groups (G+P = 16.4 kg. L+P = 16.1 kg) was significantly higher ($p<0.01$) than in the control group (13.5 kg). The significantly higher average number of weaned piglets per litter was estimated in G+P sows (11.65 piglets. $p<0.01$). and in L+P sows (11.15 piglets. $p<0.05$) than in the control group of sows (10.0 piglets). Infectious diarrhea was clinically manifested in 12.5% litters of probiotic treated sows (G+P and L+P groups). and in the significantly higher ($p<0.05$) proportion of litters (27.5%) of untreated (control-C) sows. Within the first three days after farrowing averaged piglets mortality per litter was 0.43 piglets in the G+P sows and 0.65 piglets in the L + P sows. and this difference was not statistically significant ($p>0.05$). The average number of dead piglets per litter. within the first three days after birth in the control group of sows was 0.97 piglets. and was significantly higher ($p<0.05$) only compared with treatment groups G+P sows. Preweaning piglet mortality was approximately 10% lower in the probiotic-treated sows (G+P = 4.9%. L+P = 3.4%) compared to the control sows (C = 14.2%). The average weaned litter weight was significantly higher ($p<0.01$) in both treatment groups (G+P = 103.6 kg. L+P = 102.8 kg) in comparison with the control group of sows (79.1 kg) (Table 1).

Table 1. *Litter parameters from farrowing to weaning ($\bar{x} \pm SD$)*

Parameters		Period of treatment with probiotic "Actisaf Sc 47"		Control group, without probiotic (C)
		Gestation (G+P)	Lactation (L+P)	
Number of litters		40	40	40
Total piglets born (n)		520	505	515
Aver. live born piglets per litter (n)		$12.23^{a}\pm1.66$ (9-16)	$11.53^{b}\pm1.50$ (9-14)	$11.42^{b}\pm1.88$ (8-16)
Aver. weight of live born piglets per litter (kg)		$16.43^{A}\pm2.17$ (12-21)	$16.07^{A}\pm2.26$ (13-21)	$13.47^{B}\pm2.74$ (7-21)
Litters with infectious diarrhea	n	5 ± 0.33^{a}	5 ± 0.33^{a}	11 ± 0.45^{b}
	%	12.5	12.5	27.5
Piglets mortality within first 3 days after farrowing	Total (n)	17	26	39
	A/L	$0.43^{a}\pm0.87$	$0.65^{ab}\pm0.80$	$0.97^{b}\pm1.23$
Total preweaning piglets mortality (%)		4.9	3.4	14.2
Aver. weaned piglets per litter (n)		$11.65^{A}\pm2.09$ (7-16)	$11.15^{a}\pm1.76$ (7-14)	$10.0^{Bb}\pm2.50$ (4-16)
Aver. litter weight at weaning (kg)		$103.6^{A}\pm15.66$ (51-128)	$102.8^{A}\pm13.53$ (63-103)	$79.1^{B}\pm16.9$ (34-102)

Minimal and maximal values in parenthesis. A/L – Average per litter
Values with different superscripts significantly differ: ^{ABC} ($p<0.01$); ^{abc} ($p<0.05$); Not significant: ^{NS} ($p>0.05$)

The studies carried out on large farms in Serbia show that the average farrowing number per sow was 3.5 with the average 2.1 annual farrowing index (Radović et al.. 2006; Stančić et al.. 2010). The average farrowing rate is 78.9%. and the average number of live born piglets per litter was 10.9. Total sows culling rate was 38.4% per year. About 42% of total culled sows were culled due to the health problems. Diseases of the uterus and/or udder were the reasons for culling 30.4% of the total sows culled due to the health problems (Radović et al.. 2006; Stančić et al.. 2010; Maletić. 2012; Stančić et al.. 2012). The authors also found permanent decreasing in the efficacy of treating sow uterus and/or udder diseases. as well as newborn piglet infectious diarrhea by using conventional antimicrobial preparations. primarily due to resistance of infectious agents on increasing number of antimicrobial preparations. Similar problems related to increasing microbial resistance to conventional antimicrobial preparations have been shown by other authors (Wray and Gnanou. 2000; McEwen and Fedorka-Cray. 2002; Ozawa et al.. 2012). Therefore. the aim of this study was to solve this problem by using natural probiotic preparations.

In recent years. natural probiotics have been used as substitutes for traditional antimicrobial preparations in animal production (Spring, 2004; Roseboom et al., 2005; Gallois and Oswald, 2008; Giang. 2010; Bass et al., 2012). The results obtained by experimental studies in the present paper clearly show that feeding the sows during pregnancy or lactation period by standard complete diets supplemented with probiotics (live culture of *Saccharomyces cerevisiae*) significantly improve their health status and health status of their piglets.

preweaning piglet mortality within lactation. and increase the average number of weaned piglets per litter and the average litter weight at weaning. Similar results after application of probiotic preparation. were obtained by other authors. Namely. although not clearly consistent (Jacela et al.. 2010). most researches show that the use of probiotic preparations. containing live yeast culture. in the diets of pregnant and lactating sows. can significantly reduce sow puerperal uterus and/or mammary gland diseases. newborn piglet infectious diarrhea. and increase the preweaning piglet performance (Blecha. 2001; Davis et al.. 2004; Zvekić. 2006; Gallois et al.. 2009; Williams. 2010; Kenny et al.. 2011; Zvekić et al.. 2012; Potočnjak et al.. 2012; Lazarevic et al.. 2012 Apić et al.. 2013; Apić and Zvekić. 2013). In addition. the occurrence of microbial resistance to conventional antibiotics can be avoided by the application of probiotics (Wray and Gnanou. 2000; McEwen and Fedorka-Cray. 2002; Ozawa et al.. 2012). Consequently. this prevents the appearance of residual antibiotics in the feed of animal origin used in human nutrition. and their harmful impact on the health of the human population (Perreten. 2005; Marshall and Stuart. 2011; Kjeldgaard et al.. 2012).

Results of other authors show that treatment with preparations containing live yeast culture. increases natural sows immunity (effect of Glucans + Mannan Oligosaccharide). This results in the significant decrease of sows puerperal uterus and/or udder diseases (Blecha. 2001; Maxwell et al.. 2003; Kogan and Kocher. 2007; Gallois and Oswald. 2008; Gallois et al.. 2009; Bass et al.. 2012; Sallamon. 2012). Furthermore. adding a live yeast culture (*Saccharomyces cerevisiae*) in diets of sows during gestation and lactation increases the gamma globulin (IgG and IgA) in colostrum and early milk of sows (Jurgens and al.. 1997; Hung and Lindemann. 2011; Zanello et al.. 2012; Jang et al.. 2013). Sufficient milk production of healthy sows and increased content of immunoglobulins in colostrum and milk. significantly reduce piglets mortality from starvation and infectious diarrhea during the first days after farrowing (Miguel et al.. 2004; Zvekić. 2006; Wilcock. 2011; Shen et al.. 2011; Apić and Zvekić. 2013).

Conclusion

The results of this study clearly show that the use of probiotic preparation "Actisaf 47" (live yeast culture of *Saccharomyces cerevisiae*) in the diets of pregnant and lactating sows significantly improves the health status of their piglets in the lactation period. In addition. litter productive parameters (the number of weaned piglets per litter and litter weight at weaning) were also significantly higher in treated than in untreated (control) sows.

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References

1. Apić I, Zvekić D, Apić J, Savić B and Stančić I 2013. Litter parameters after newborn piglets peroral treatment with "Hokovit" immunomodulator preparations. Kafkas Universitesi Veteriner Fakultesi Dergisi 19, 1007-1010.

2. Apić I and Zvekić D 2013. Litter parameters in primiparous sows treated with immunomodulators supplemnted in diets. 23rd International symposium „New Technologies in Contemporary Animal Production". Serbia. Novi Sad, 169-171.
3. Bass B, Perez V, Yang H, Holzgraefe D, Chewning J and Maxwell C 2012. Impact of a whole yeast product on sow. litter. and nursery performance. Arkansas Anim Sci Dep Rep 1, 104-115.
4. Blecha F 2001. Immunomodulators for prevention and treatment of infectious diseases in food-producing animals. Vet. Clin. North. Am. Food Anim Pract 17, 621-33.
5. Bonneau M and Laarveld B 1999. Biotechnology in animal nutrition. physiology and health. Livestock Prod Sci 59, 223–241.
6. Cromwell GL 2002. Why and how antibiotics are used in swine production. Animal Biotechnology 13, 7–27.
7. Davis ME, Maxwell CV, Erf GF, Brown DC and Wistuba TJ 2004. Dietary supplementation with phosphorylated mannans improves growth response and modulates immune function of weanling pigs. J Anim Sci 82, 1882-1891.
8. Faldyna M, Göpfert E, Alexa P and Sramkova Z 2012. The effect of live yeast on clinical model of swine colibacillosis. Retrieved March 12. 2014. from http://www.allaboutfeed.net/Nutrition
9. Ferroni M, Agazzi A, Invernizzi G, Savoini G, Chevaux E and Le Treut Y 2012. Inclusion of live yeast S. cerevisiae boulardii (CNCM I-1079) in sow lactation diets: effects on sows and nest performances. Retrieved March 12. 2014. from http://www.feedstuffs.com/Media
10. Floss JL and Tubbs RC 1999. Infection Causes of Inferility in Sows. Agricultural Publ G2315, 1-6.
11. Gagrčin M. Kovčin S and Stančić B 2002. Zdravstveni i proizvodni rezultati u farmama svinja na području Vojvodine za 2001. godinu. Savremena poljoprivreda 51. 265-268.
12. Gagrčin M, Stančić B, Božić A and Orlić D 2003. Intrauterine infections in pigs. Lucrari Stiintifice Medicina Veterina Timisoara XXXVI, 479-482.
13. Gallois M and Oswald IP 2008. Immunomodulators as efficient alternatives to in-feed antimicrobials in pig production? Archiva Zootechnica 11, 5-32.
14. Gallois M, Rothkötter HJ, Bailey M, Stokes CR and Oswald IP 2009. Natural alternatives to infeed antibiotics in pig production: can immunomodulators play a role? Animal 3, 1644-1661.
15. Giang HH 2010. Impact of bacteria and yeast with probiotic properties on performance. digestibility. health status and gut environment of growing pigs in Vietnam. PhD. Swedish University. Uppsala.
16. Hogg A and Levis GD 1997. Swine Reproductive Problems: Infectious Causes. Neb Guide Edu G89-926-A 1-9.
17. Hung FI and Lindemann DM 2011. Evaluation of Celamanx® on performance of sows and their weaned pigs. Research Bulletin Swine S-62, 21-23.
18. Hyun Y, Ellis M, Riskowski G and Johnson RW 1998. Growth performance of pigs subjected to multiple concurrent environmental stressors. J Anim Sci 76, 721–727.
19. Jang DY, Kang WK, Piao GL, Jeong ST, Auclair E, Jonvel S, D'Inca R and Kim YY 2013. Effects oflive yeast supplementation to gestation and lactation diets on reproductive performance. immunological parameters and milk composition in sows. Livestock Science. www.elsevier.com/locate/livsci

20. Kenny M, Smidt H, Mengheri E and Miller M 2011. Probiotics – do they have a role in the pig industry? Animal 5, 462–470.

21. Kick AR, Tompkins MB and Almond GW 2011. Stress and immunity in the pig. Vet Med Resource 1, 1-17.

22. Kjeldgaard J, Cohn TM, Casey GP, Hill C and Ingmer H 2012. Residual Antibiotics Disrupt Meat Fermentation and Increase Risk of Infection. M Bio 3, 1-4.

23. Kogan G and Kocher A 2007. Role of yeast cell wall polysaccharides in pig nutrition and health protection. Livestock Science 109, 161–165.

24. Lazarević M, Milovanović A, Barna T, Miljas N and Milanov D 2012. Endometritis therapy in sows by intrauterine infusion of yeast cell wall solution. Acta Vet Bgd 62, 611-626.

25. Le Coz P 2012. The effect of live yeast on colibacillosis in field conditions. Retrieved March 12. 2014. from www.allaboutfeed.net/Nutrition

26. Maletić Z 2012. Reproductive efficiency of sows depending on the model of nutrition in pregnancy and lactation. PhD. University of Novi Sad. Serbia.

27. Marshall MB and Stuart BL 2011. Food Animals and Antimicrobials: Impacts on Human Health. Clin Microbiol Rev 24, 718-733.

28. Maxwell CV, Ferrell K, Dvorak RA, Johnson ZB and Davis ME 2003. Efficacy of mannan oligosaccharide supplementation through late gestation and lactation on sow and litter performance. J Anim Sci 81(Suppl. 2), 69.

29. McEwen SA and Fedorka-Cray PJ 2002. Antimicrobial use and resistance in animals. Clin Infect Dis 34, 93-106.

30. Miguel JC, Rodriguez-Zas SL and Pettigrew JE 2004. Efficacy of a mannan oligosaccharide (Bio-Mos) for improving nursery pig performance. J. Swine Health Prod 12(6), 296–307.

31. Ozawaa M, Makitab K, Tamurab Y and Asaia T 2012. Associations of antimicrobial use with antimicrobial resistance in Campylobacter coli from grow-finish pigs in Japan. Preventive Vet Med 106, 295– 300.

32. Pavičić Ž, Vučemilo M, Tofant A, Vijtiuk N, Popović M, Balenović M and Balenović T 2003. Effect of immunostimulator Baypamun on plasma cortisol concentration in gilts regrouped during the late stage of pregnancy. Acta Vet Brno 72, 509-514.

33. Perreten V 2005. Resistance in the food chain and in bacteria from animals: relevance to human infections. In Frontiers in Antimicrobial Resistance: Atribute to Stuart B. Levy. (eds DG White. MN Alekshun. and PF McDermott). pp. 446–464. ASM Press. Washington. DC. USA.

34. Plante AP, Laforest P-J and Farmer C 2011. Effect of supplementing the diet of lactating sows with NoPro. on sow lactation performance and piglet growth. Can J Anim Sci 91, 295-300.

35. Potočnjak D, Kezić D, Popović M, Zdolec N, Valpotić H, Benković V, Mršić G, Janjatović A, Lacković G and Valpotić I 2012. Age-related changes in porcine humoral and cellular immune parameters. Veterinarski Arhiv 82, 167-181.

36. Pragathi D, Vijaya T, Anitha D, Mouli KC and Sai Gopal DVR 2011. Botanical immunomodulators - potential therapeutic agents. J. of Global Pharma Technology 3, 1-14.

37. Pugh DM 2002. The EU precautionary bans of animal feed additive antibiotics. Toxicology Letters 128, 35–44.

38. Radović I, Stančić B, Popov R, Trivunović S and Teodorović M 2006. Reproductive performance of first litter and sows of higher parity sows. Contemporary Agriculture 55, 83-90.

39. Rozeboom DW, Shaw DT, Templeman R, Miguel JC, Pettigrew JE and Connoly A 2005. Effects of mannan oligosaccharide and an antimicrobial product in nursery diets on performance of pigs reared on three different farms. J Anim Sci 83, 2637-2644.

40. Salmon H 2012. The effect of live yeast on sow-mediated immunity. http://en.engormix.com/MA-pig-industry

41. Shen YB, Carroll JA, Yoon I, Mateo RD and Kim SW 2011. Effects of supplementing *Saccharomyces cerevisiae* fermentation product in sow diets on performance of sows and nursing piglets. J Anim Sci 89, 2462-2471.

42. Spring P 2004. Impact of mannan oligosaccharide on gut health and pig performance. In Interfacing immunity. gut health and performance (eds LA Tucker and JA Taylor-Pickard). pp.93-105. Nottingham University UK.

43. Stančić B, Gagrčin M, Stančić J, Stevančević O and Potkonjak A 2011. Infective and non-infective etiology of sow infertility. Contemporary agriculture 59, 180-193.

44. Stančić I, Radović I, Dragin S, Erdeljan M and Apić I 2012. Veterinary and zootechnical situation in artificial insemination at swine farm units in Vojvodina (Serbia). Contemporary Agriculture 61, 54-60.

45. Stančić J, Gagrčin M and Dragin S 2010. Seasonal differences in sow reproductive performances. Contemporary Agriculture 59, 37-48.

46. Sutherland MA, Niekamp SR, Rodriguez-Zas SL and Salak-Johnson JL 2006. Impacts of chronic stress and social status on various physiological and performance measures in pigs of different breeds. J. Anim Sci 84, 588–596.

47. Williams NT 2010. Probiotics. Am J Health Syst Pharm 67, 449-58.

48. Wilcock P 2011. Piglets performing better with yeast during lactation. Pig Progress. May. 31. 2011. http://www.pigprogress.net/

49. Wray C and Gnanou CJ 2000. Antibiotic resistance monitoring in bacteria of animal origin: analysis of national monitoring programmes. Int J Antimicrob Agents 14, 291–294.

50. Yeske P 2007. Health Problems That Affect Fertility. Nat Hog Farmer 15, 21-32.

51. Zanello G, Meuren F, Serreau D, Claire Chevaleyre C, Melo S, Berri M, D'Inca R, Auclair E and Salmon H 2012. Effects of dietary yeast strains on immunoglobulin in colostrum and milk of sows. Vet Immunol Immunopathol 152, 1. 20-27.

52. Zvekić D, Apić I and Gagrčin M 2012. Dietary supplemenation with natural immunomodulators and sows fertility. Contemporary Agriculture 61, 199-204.

53. Zvekić D 2006. Effect fo immunomodulators on sows reproductive performance parameters in productive conditions. PhD. University of Novi Sad. Serbia.

Original paper

EFFECT OF LITTER TREATMENT ON THE OCCURRENCE OF FOOT PAD LESIONS

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Abstract

Pododermatitis (foot pad lesions) is one of the main welfare problems in modern broiler production in countries with developed poultry farming. Factors contributing to the occurrence of these lesions are nutrition, stocking density and material used for litter. There are a number of different procedures that can be applied in order to prevent and reduce the number of lesions with the most pronounced defects. The experiment was conducted on broiler chickens grown in 10 buildings of 240 m^2 each. The stocking density was 35 kg/m^2. The experiment was set up in five treatments with two replicates. Treatment one (T1) - control with a straw litter, treatment two (T2) - litter treated with microbial preparation Micropan®, treatment three (T3) – litter with addition of lignin, treatment four (T4) – litter with addition of lignin and Micropan® and treatment five (T5) - chopped straw without supplements. At the end of the experiment, on day 42 the intensity of the lesions was scored on the slaughter line. The presence of lesions was scored using scale from 0 (no lesions) to 3 (plantar pads with more than 50% damage). Based on the results of the trial it can be concluded that litter has a significant impact on the presence and the degree of foot pad lesions. The lowest score of foot pad lesions was observed in the treatment T5 (chopped straw). Different treatments of litters may also contribute to the solution of the problem of pododermatitis since the results in all treated groups (T2, T3, T4 and T5) were better when compared to the control.

Key words: *broiler, litter treatment, pododermatitis*

Introduction

Foot pad lesions are a type of contact dermatitis affecting the plantar region of the feet in poultry and other birds. At an early stage, discoloration of the skin is seen. Hyperkeratosis and necrosis of the epidermis can develop, and in severe cases, these changes are followed by ulcerations with inflammatory reactions of the subcutaneous tissue (Ekstrand et al., 1997).The lesions are commonly named “ammonia burns” and are thought to be caused by a combination of moisture, high ammonia content, and other not yet specified chemical factors in the litter (Berg, 2004). By definition, the chicken paw is the portion below the spur, while chicken feet include the lower leg as well as the foot. The terms paw and foot are interchangeable and both terms will be used in this paper. Due to the market value of this product and increasing welfare

issues, it is in the best interest of the poultry industry to reduce paw down grades and condemnations. Therefore, factors associated with increased incidence of foot pad lesions and methods to reduce it need to be evaluated. Revenue from chicken paws in 2008 alone was worth $280 million. Harvesting large, unblemished paws has become a priority to poultry companies all over the world. Research on this subject has been ongoing since the 1940s and has looked into many different areas including nutrition, environment, and genetics (Shepherd and Fairchild, 2010).The FPD condition is an important aspect of poultry welfare that in severe cases can cause pain (Berg, 1998) resulting in unsteady walk (Harms and Simpson, 1975; Hester, 1994). Selection for rapid growth rate in broilers is accompanied by a decrease in walking ability, and there is a high unfavorable phenotypic correlation (0.8) found between BW and overall walking ability (Kestinet al., 2001). The FPD condition is a part of a general walking ability problem, but specific knowledge of genetic effects on FPD is very scarce.

Litter serves several functions that include thermal insulation, moisture absorption, protective barrier from the ground, and it allows for natural scratching behavior. Bedding material must not only be a good absorber of moisture but also to have a reasonable drying time (Grimes et al., 2002; Bilgili et al., 2009). Although litter refers to the mixture of bedding material, fecal droppings and moisture, the term is used interchangeably with bedding materials. The most commonly used litter material in Europe is straw (Grimes et al., 2002). The best-performing material was pine shavings followed by: rice hulls, ground corncobs, stump chips, pine sawdust, bark and chips, pine bark, and clay (Grimes et al., 2002). Differences in particle size of straw were suggested to be the most important factor. The emergence of the foot pad lesions is multifactorial problem influenced by a large number of factors among which the most significant are ventilation, feeding, watering, microclimate and health status of animals. Consequences of the development are significant both in terms of animal welfare and in terms of economic feasibility of production (Bjedov et al., 2013). Differences in particle size of these materials were proposed to be the most important factor. The aim of this study was to determine the effect of litter on the occurrence of foot pad lesions.

Material and methods

The experiment was conducted on broiler chickens grown in 10 buildings of 240 m^2 each. The stocking density was 35 kg/m^2. The experiment was set up in five treatments with two replicates. Treatment one (T1) - control with a straw litter, treatment two (T2) - litter treated with microbial preparation Micropan®, treatment three (T3) – litter with addition of lignin, treatment four (T4) – litter with addition of lignin and Micropan® and treatment five (T5) - chopped straw without supplements. Lighting program provided 23 hours of light + 1 hour of dark. Birds were vaccinated against Newcastle disease (NCD) and infectious bursal disease (IBD) as per commercial recommendations. Feed and water supply were *ad libitum.* At the end of the experiment, on day 42 the intensity of the lesions was scored on the slaughter line. These labeled birds were examined weekly for the presence of foot dermatitis and given a lesion score using the methodology described by Martrenchar et al. (2002). Briefly, the scores were 0: no lesion; 1: lesion on <25% of the pads; 2: lesion on 25 to 50% of the pads; 3: lesion on >50% of the pads. Data were analyzed by ANOVA using the GLM procedure and the means separated by Duncan *post* hoc test using StatSoft computer package (STATISTICA 11, 2012).

Results and discussion

The results indicate that different litter treatments have an impact on the occurrence of foot pad lesions. Table 1 shows the results of the experiment. The best results were obtained by using a chopped straw litter as compared to the other treatments that were used in the experiment. The results reached by De Baere et al. (2009) show that the use of large quantities of chopped straw of 1.5 kg/m^2 produced a significant increase in the intensity of the FPD in broiler chickens. In their work, they state that the use of chopped straw at a rate of 2.0 kg/m^2 leads to an increase in the number of lesions with the highest degree of damage.

Table 1. *The number of scored broiler (No) and values (%) of foot pad damage by applying different treatments*

Treatments	T1		T2		T3		T4		T5	
Footpad lesions	No	%	No	%	No	%	No	%	No	%
0	1	0.06	24	0.88	6	0.27	30	1.13	39	1.59
1	3	0.18	128	4.71	22	0.97	238	8.98	212	8.62
2	547	32.54	924	34.02	945	41.76	1042	39.32	1002	40.76
3	1130	67.22	1640	60.38	1290	57.00	1340	50.57	1205	49.02
Total/Average	1681	2.66^{a}	2716	2.53^{b}	2263	2.55^{bc}	2650	2.39^{d}	2458	2.37^{d}

($^{a,b,c,d;}$), Means in the same row with different letters are significantly different (P<0.05)

The results in this experiment are consistent with previous studies (Grimes et al., 2002; Bilgili et al., 2009) in which the use of different litter materials caused a reduction in the occurrence and intensity of foot pad damage. There was a statistically significant difference between the chopped straw and a straw litter, litter treated with microbial preparation Micropan®, and litter with addition of lignin. No statistically significant difference on the occurrence of foot pad lesions between litter with addition of lignin and Micropan® and chopped straw without supplements was observed.

Conclusion

The emergence of the FPD is a multifactorial problem that is influenced by a large number of factors among which the most significant are ventilation, feeding, watering, microclimate and health status of animals. Consequences of the development are significant both in terms of animal welfare and in terms of economic feasibility of production. It is possible that different litter treatments significantly prevented the occurrence and reduced the intensity of foot pad lesions. Good knowledge of all factors of the occurrence of FPD significantly contribute to the prevention of these types of dermatitis and reduce intensity damage in flocks where the disease has occurred.

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References

1. Berg C 1998. Foot-pad dermatitis in broilers and turkeys. PhD Swedish University of Agricultural Sciences, Uppsala.
2. Berg C 2004. Measuring and Auditing Broiler Welfare. C. A.Weeks and A. Butterworth pp 37 - 49. CABI Publishing, Wallingford, UK.
3. Bilgili SF, Hess JB, Blake JP, Macklin KS, Saenmahayak B and Sibley JL. 2009. Influence of bedding material on footpad dermatitis in broiler chickens. The Journal of Applied Poultry Research 18, 583–589.
4. Bjedov S, Žikić D, Perić L, ĐukićStojčić M and Milošević N 2013. Effect of different litter treatments on production performance of broiler chickens. Biotechnology in Animal Husbandry 29, 4, 625-630.
5. De Baere K, Cox M, Vervaet E, Zoons J and Fiks-Van Nierkek T 2009. Effect of litter material on foot pad dermatitis and hock burn in broilers. 8th European Symposium on Poultry Welfare, Cervia, Italy, 18-22 May, pp: 52.
6. Ekstrand, C, Algers B, and Svedberg J 1997.Rearing conditions and foot pad dermatitis in Swedish broiler chickens. Preventive Veterinary Medicine 31, 167–174.
7. Grimes JL, Smith J and Williams CM 2002. Some alternative litter materials used for growing broilers and turkeys. World's Poultry Science Journal 58, 515–526.
8. Harms RH and Simpson FC 1975. Biotin deficiency as a possible cause of swelling and ulceration of foot pads. Poultry Science 54, 1711–1713.
9. Hester PY 1994. The role of environment and management on leg abnormalities in meat-type fowl. Poultry Science 73, 904–915.
10. Kestin SC, Gordon S, Su G, and Sørensen P 2001.Relationship in broiler between lameness, liveweight, growth rate and age.Veterinary Record 148, 195–197.
11. Martrenchar A, Boilletot E, Huonnic D and Pol F 2002. Risk factors for foot pad dermatitis in chicken and turkey broilers in France. Preventive Veterinary Medicine 52, 213–226.
12. Shepherd EM and Fairchild BD 2010. Footpad dermatitis in poultry. Poultry Science Journal 89, 2043–2051.

Original paper

CORRELATIONS BETWEEN INCIDENCE OF FOOT PAD LESIONS AND BODY WEIGHT OF BROILERS IN DIFFERENT REARING SYSTEMS

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Abstract

The incidence of foot pad lesions of broilers of moderate growth was investigated in order to establish correlations with body weight. Broilers were reared until the age of 42 days in the floor system in the poultry house and then were divided into two groups. The first group continued growing in the poultry house until the age of 84 days and the second group was growing in the free range system until the same age. Individual measurements of body weight and evaluation of the incidence of foot pad lesions of broilers were carried out at the end of the experiment. In a correlation analysis of previously transformed data on the percentage of broilers with lesions and body weight within each weight group, data were obtained that showed an association between these traits depending on the rearing system.

System of rearing had significant impact on the strength and direction of correlation between body weight and the incidence of foot pad lesions, in light of the determined correlation coefficient r = -0.95 at the significance level p=0.01 in the free range system, and r=0.56 (p>0.05) in chickens reared in the poultry house.

Key words: *body weight, broilers, lesions, rearing system*

Introduction

The lesions on the foot pads of broilers usually represent the type of contact dermatitis, and depending on the degree of damage they can range from the level of surface inflammatory processes to necrotic changes in the deep layers of tissue. Their incidence is associated with rearing conditions (Meluzzi et al., 2008), stocking density (Ferrante et al., 2006; Škrbić et al., 2010), nutrition (Eichner et al., 2007; Škrbić et al., 2012), which exert their effects through litter quality considered as the most important factor for the development of various forms of dermatitis (footpad, hock burns, breast blisters) (Allain et al., 2009). Footpad dermatitis causes pain and affects the incidence of lameness (Sorensen et al., 2000) which can result in a lower gain and final body mass of chickens. In general, the footpad lesions pose a problem to the welfare and economic efficiency of broiler production. Research results of Kjaer et al. (2006) show significant differences in the incidence of footpad dermatitis in fast-growing and slow growing broiler hybrids. Đukić-Stojčić et al. (2013) have found differences in the frequency of footpad lesions in moderate growing broilers depending on the rearing system. Body mass of broilers is a potential

predisposing factor for the incidence of footpad lesions, given that male broilers showed a greater tendency towards more frequent incidence and more severe degrees than the female birds (Bilgili et al., 2006).

The aim of this study was to investigate the presence and nature of phenotypic correlations between body mass and frequency of broiler footpad lesions in the hybrids of moderate growth in terms of extensive rearing in the house and the traditional free range rearing system.

Materials and methods

The experiment was conducted on a total of 300 chickens of genetic potential for a moderate rate of growth (Redbro). By 42 days the chicks were reared in the poultry house in the floor rearing system. Conditions of feeding and breeding were typical for standard fattening. The chickens were housed in boxes, 50 birds/box or 12 birds/m^2. After 6 weeks, 150 broilers were transferred to the traditional free range rearing facility to the slaughter age (84 days), which was the same for chickens from each rearing system. Throughout the experiment, the diet was based on complete mixtures. The chicks were fed starter (22.2 % CP; 3100Kcal) grower (19.4 % CP; 3110Kcal) and finisher (17.3 % CP; 3170Kcal) diets, while the broiler in the free range system had available pasture area of 10m^2/bird. By individual measuring of chicks at the end of the experiment, in both rearing systems, the final body mass was determined. At the same time, the incidence and the degree of lesions on the foot pads were visually evaluated according to the method of Thomas et al. (2004), in which score 1 indicates the absence of lesions, score 2 presence of moderate lesions and score 3 the presence of severe lesions. Based on the measured body mass, chickens were divided into six weight groups : <2.0 kg, 2.0-2.5 kg, 2.5-3.0 kg, 3.0-3.5 kg, 3.5 - 4.0 kg and > 4.0 kg. Also, for the purpose of the data processing, the summary frequency of the incidence of foot pad lesions was taken into account, regardless of the degree of damage and the score.

Statistical analysis was performed using the software program Statistica (Stat.Soft, Inc. 8 version) and was based on the determination of the parameters of descriptive statistics and correlation analysis of body mass of broilers and the incidence of lesions on the foot pads of broilers within weight groups depending on the rearing system. Data expressed in percentages were previously transformed to *arc sin*.

Results and discussion

Based on the average scores for footpad lesions we can state in general that the condition of leg pads in each rearing system was satisfactory (Table 1), which is partly the effect of the genotype of moderate growth broilers. In the free range rearing system, average scores, depending on the weight group, ranged from 1.0 to 1.25, and in the rearing system in the poultry house, from 1.0 to 1.20. Results obtained by Kjaer et al. (2006) point out the important role of genotype for the development of dermatitis, in terms of less sensitivity to their development in the slow growing hybrids. As for the rearing system in the poultry house, in the first two groups of broilers with the lowest body mass the incidence of footpad lesions was not recorded. In contrast, in the free range rearing system, in these groups, the highest percentage of broilers with footpad lesions was determined (33.33% and 18.75%).

Table 1. *Frequency of incidence and average score of footpad lesions in broiler weight groups and rearing systems*

Weight group	Rearing system	Average body weight, kg (mean ±SD)	Incidence of footpad lesions, %	Average score of footpad lesions
< 2.0 kg	free range	1.71 ± 0.18	33.33	1.25
	poultry house	1.77 ± 0.06	0	1.0
	average	1.73 ± 0.15	22.22	1.17
2.0-2.5 kg	free range	2.33 ± 0.13	18.75	1.16
	poultry house	2.25 ± 0.14	0	1.0
	average	2.30 ± 0.14	10.71	1.09
2.5-3.0 kg	free range	2.79 ± 0.16	5.66	1.05
	poultry house	2.78 ± 0.15	10.34	1.12
	average	2.78 ± 0.15	7.32	1.07
3.0-3.5 kg	free range	3.21 ± 0.15	4.17	1.04
	poultry house	3.26 ± 0.15	16.0	1.2
	average	3.23 ± 0.15	8.22	1.09
3.5-4.0 kg	free range	3.77 ± 0.14	2.13	1.04
	poultry house	3.76 ± 0.13	11.43	1.13
	average	3.77 ± 0.14	6.10	1.08
> 4.0 kg	free range	4.26 ± 0.20	0	1.0
	poultry house	4.35 ± 0.29	7.69	1.12
	average	4.31 ± 0.25	4.25	1.06

Different conditions in the studied rearing systems influenced the opposite direction of the correlation between body mass of broilers and frequency of lesions of the foot pads. In the free range rearing system, correlation coefficient of r = -0.95 (p=0.01) indicates a strong negative correlation, i.e. significantly lower incidence of foot-pad dermatitis in broilers of greater body mass (fig. 1). In the floor rearing system, in the poultry house, a medium strong positive correlation was found (r=0.56, p>0.05). However, given the lack of statistical significance of determined correlation coefficient, regardless the strength of correlations, it can be argued that there is a correlation between body mass of broilers and the incidence of footpad lesions in the floor system of rearing in the poultry house (Figure 2). In accordance, the weak, insignificant correlation between the foot pad dermatitis and the body mass of broilers was determined also by Kjaer et al. (2006).

The quality of litter or bedding is a crucial factor for the development of dermatitis. In terms of free range rearing it is anticipated that this problem is less pronounced. Physical activity of broilers, or freedom of movement, exposure to natural rearing conditions (grassy range, air, sun), contribute to the overall better shape of legs (Kestin et al., 1992). In such conditions, footpad lesions were more common in groups with lower body mass maybe due to "inherited" condition of the poultry house, where the chickens were reared the first six weeks. Footpad lesions cause a painful condition that affects the ability to move and thereby reduce competition for food and weight gain. In the floor system of rearing, in the poultry house, the condition of foot pads continuously deteriorated with the increase in body mass due to intense and prolonged contact with wet litter until the age of slaughter. In this sense, the management of production is of great importance for the maintenance of environmental conditions in the house (air temperature, relative humidity), satisfactory quality of litter (Wang et al., 1998, Allain et al., 2009). A negative correlation between the serious footpad dermatitis and live mass of broilers, i.e. meat yield, is determined by Hashimoto et al. (2013) and indicates the possibility of improving these production parameters through control of the incidence of footpad dermatitis.

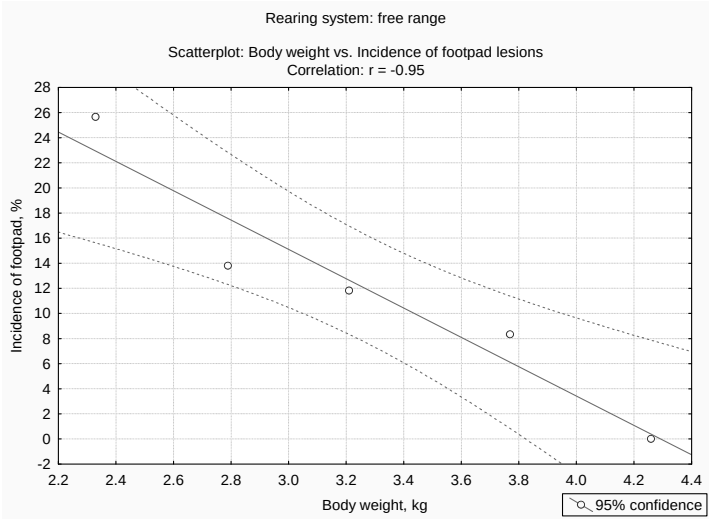

Figure 1. *Correlation between body mass and incidence of footpad lesions in the free range rearing system*

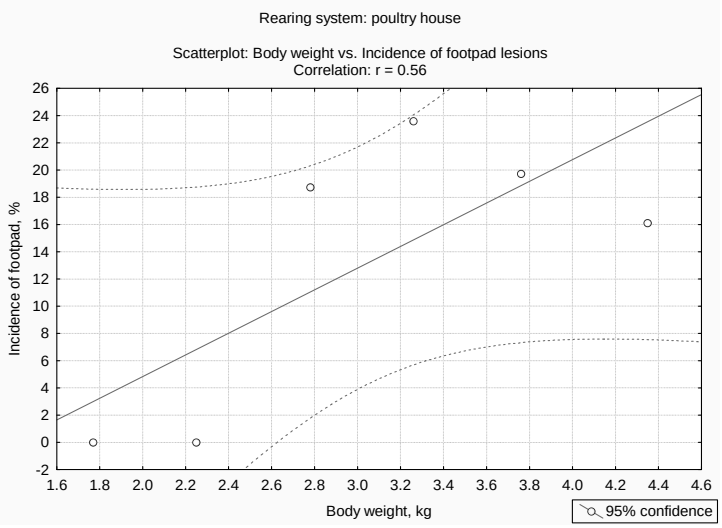

Figure 2. *Correlation between body mass and incidence of footpad lesions in the floor rearing system*

Conclusion

The system of rearing of broilers exhibited a significant effect on the correlation between the body mass and incidence of footpad lesions. In the free range rearing system, the body mass and the incidence of footpad lesions were in strong negative phenotypic correlation, while in the floor rearing system, in the poultry house, phenotypic correlation between these traits was not statistically confirmed.

Low average footpad lesion scores, i.e. satisfactory condition of foot pads of broilers in both rearing systems, provide confirmation of the importance of genotype for the development of dermatitis.

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References

1. Allain V, Mirabito L, Arnould C, Colas M, Le Bouquin S, Lupo C and Michel V 2009. Skin lesions in broiler chickens measured at the slaughterhouse: relationships between lesions and between their prevalence and rearing factors. British Poultry Science 50, 407-417.
2. Bilgili SF, Alley MA, Hess JB and Nagaraj M 2006. Influence of Age and Sex on Footpad Quality and Yield in Broiler Chickens Reared on Low and High Density Diets. Journal Applied Poultry Research 15, 433–441.
3. Đukić Stojčić M, Milošević N, Perić L and Bjedov S 2013. Plumage condition and foot pad lesions of medium growing broilers reared extensively in the poultry house and in traditional free range. 10th International Symposium Modern Trends in Livestock Production, Belgrade, Serbia, 6 pp.
4. Eichner G, Vieira SL, Torres CA, Coneglian JLB, Freitas DM and Oyarzabal OA 2007. Litter Moisture and Footpad Dermatitis as Affected by Diets Formulated on an All-Vegetable Basis or Having the Inclusion of Poultry By-Product. Journal Applied Poultry Research 16, 344-350.
5. Ferrante V, Lolli S, Marelli S, Vezzoli G, Sirri F and Cavalchini LG 2006. Effect of light programmes, bird densities and litter types on broilers welfare. XII European Poultry, Conference, Verona, Italy. Word's Poultry Science Journal 62 (supplement), 586.
6. Hashimoto S,Yamazaki K, Obi T and Takase K 2013. Relationship between Severity of Footpad Dermatitis and Carcass Performance in Broiler Chickens. The Journal of Veterinary Medical Science 75, 1547–1549.
7. Kestin SC, Knowles TG, Tinch AE and Gregory NG 1992. Prevalence of leg weakness in broiler chickens and relationship with genotype. Veterinary Record 131, 190-194.
8. Kjaer JB, Su G, Nielsen BL and Sorensen P 2006. Foot pad dermatitis and hock burn in broiler chickens and degree of inheritance. Poultry Science 85, 1342–1348.
9. Meluzzi A, Fabbri C, Folegatti E and Sirri F 2008. Survey of chicken rearing conditions in Italy: effects of litter quality and stocking density on productivity, foot dermatitis and carcase injuries. British Poultry Science 49, 257-264.
10. Škrbić Z, Pavlovski Z, Lukić M and Petričević V 2010. Assessment of Broiler Welfare in Different Stocking Densities. XIII European Poultry Conference, Tours, France, 4 pp.
11. Škrbić Z, Pavlovski Z, Lukić M, Petričević V, Miljković B and Marinkov G 2012. The effect of the diet on incidence of footpad lesions and productivity of broilers. Biotechnology in Animal Husbandry 28, 353-360.
12. Sorensen P, Su G and Kestin SC 2000. Effects of Age and Stocking Density on Leg Weakness in Broiler Chickens. Poultry Science 79, 864-870.

13. Thomas DG, Ravindran V, Thomas DV, Camden BJ, Cottam YH, Morel PCH and Cook CJ 2004. Influence of stocking density on the performance, carcass characteristics and selected welfare indicators of broiler chickens. New Zealand Veterinary Journal 52, 76-81.

14. Wang G, Ekstrand C and Svedberg J 1998. Wet litter and perches as risk factors for the development of foot pad dermatitis in floor-housed hens. British Poultry Science 39, 191-197.

Original paper

FEED QUANTITY EFFECT ON CARP JUVENILES' PLASMA PROTEIN AND IMMUNOGLOBULIN LEVELS

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Abstract
Plasma protein and immunoglobulin (Ig) levels in fish can be influenced by stress reaction, caused by some nutritional factors. This paper shows effects of different feed quantity on total protein and total Ig levels in blood of common carp (*Cyprinus carpio*) juveniles exposed to chronic stress conditions.
The study was carried out in a recirculation aquaculture system (RAS) during 96 days. Carps (initial weight 6.43±0.02 g) were distributed into four groups in three replicate tanks, 40 fish per tank. The groups were formed according to the feed quantity applied: 2, 3, 4 and 5% of commercial extruded mixture in relation to the ichthyomass (i.e. groups I, II, III and IV respectively).
According to the results, the mean values of total plasma proteins and Ig were highest in group IV and the lowest in group II ($p<0.05$). Total protein levels were influenced by feed quantity ($p<0.05$), and sampling time ($p<0.001$). Total Ig levels were influenced by duration of the experiment i.e. sampling time ($p<0.001$). In all groups the mean values of plasma proteins after two month of the experiment was increased ($p<0.01$), and the mean values of Ig in less fed groups I and II ($p<0.05$ and $p<0.01$, respectively). Total plasma proteins and Ig were not significantly affected by water quality parameters only in the group fed 2% of feed (group I). Significant positive correlation between total proteins and Ig was determined in each experimental group ($p<0.01$).
In terms of reducing stress and ensuring welfare in carp juveniles, applied feed amount should match to the length of fish growing in the fish tanks, stocking density and capacity of the system for efficient water purification.

Key words: *common carp, feed quantity, RAS, total immunoglobulins, total proteins*

Introduction

Growing carps in tanks of recirculation aquaculture systems (RAS) implies the absence of natural food and entirely dependence on added feed. Commercial feed must be compliant with the needs of fish, regarding its quality, pellet size, shape and quantity. In deciding the optimal feed quantities for carp juveniles Stanković et al. (2011) considered production and economic indicators (e.g. weight gain, feed conversion ratio and feed price). However, producers' goals must also be directed towards ensuring the welfare of fish.

The level of nutrition is one of the main factors that affect water quality in RAS. Decomposition of uneaten feed and its retention in tanks led to the deterioration of water

quality in the system (Isla Molleda, 2007), which triggers stress response mechanisms in fish (Conte, 2004; Hastein et al., 2005).

Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms (Wendelaar, 1997). Total plasma protein and immunoglobulin levels were used in the studies of stress response in fish by Cœurdacier and Dutto (1999), Harikrishnan et al. (2003), Ardó et al. (2009), Patriche et al. (2009), Hajibeglou and Sudagar (2010), and Noori (2013). Effects of the feed quantity on these blood parameters in common carp juveniles (*Cyprinus carpio L.*), which are grown in RAS, are not sufficiently examined.

The aim of this paper is to show effects of different feed quantity on total plasma protein and immunoglobulin levels in common carp juveniles kept in tanks over a period of three months.

Material and methods

The study was carried out in recirculation aquaculture system (RAS) of the CEFAH (Center for Fishery and Applied Hydrobiology of the Faculty of Agriculture, University of Belgrade, Serbia). During 96 days 480 specimens of common carp (*Cyprinus carpio*) with the initial weight of 6.43±0.02 g were held in 120-litre tanks with constant water flow of 0.5 l/min. They were distributed in four groups in three replicate tanks, 40 fish per tank. The groups were formed according to the feed quantity applied: 2, 3, 4 and 5% of commercial extruded mixture (38% of proteins and 12% fat, "VZ Subotica", Serbia) in relation to the ichthyomass (i.e. groups I, II, III and IV, respectively). Fish feed was weighed by digital scale (KERN PLS 2100-2, Germany) and distributed by hand, twice a day (08:00–08:30 and 14:00–14:30 hrs).

Water quality in the tanks was checked three times a week (temperature, pH, and dissolved oxygen i.e. DO) by appropriate probe (MULTI 340i/SET, WTW, Germany), and twice a month (unionized ammonia i.e. NH_3-N, and organic substances content based on $KMnO_4$ consumption) by analyzing water samples (Anon., 1985; Čoha, 1990). Water sampling and direct measurements of the water parameters were conveyed in the morning, before feed distribution.

The study had three periods of parameters' examination, and each period was finished at the day of blood sampling. Blood samples were collected from three fish per tank at 32, 64 and 96 day of the experiment. Prior to blood sampling fish were anaesthetized in MS-222 (Sigma-Aldrich®). Methods of sampling blood from caudal vein and blood samples preparation for analyses were described by Roberts (1989), and by Ardó et al. (2009). Blood plasma was isolated by centrifugation (1400G, 15 min), and samples were stored at -20°C before the measurements.

Total protein concentration in the blood was determinated colorimetrically by Fluitest TP (Analyticon® Biotechnologies AG, Germany), as well as total immunoglobulins, with addition of polyethylene glycol (PEG) (Sigma-Aldrich®), as described by Ardó et al. (2008). Both parameters were measured using microplate reader Multiskan Spectrum (Thermo Labsystems; Waltham, MA, US).

As factors affecting blood parameters in this study, the feed quantity and duration of exposure to the same conditions (periods between two consecutive sampling) were considered. Data were analyzed using parametric tests (two-way factorial ANOVA, LSD and Fisher LSD test) at significance level of $p<0.05$. For immunoglobulin levels square root data transformation was applied. Relation between blood and water parameters was

evaluated according to the value of Spearman's rank correlation coefficient. The results were analyzed by STATISTICA 8.0 Software (StatSoft, Inc. 2007), and Microsoft Office EXCEL 2007.

Results and discussion

Based on the results of all blood samplings during the experiment, the highest mean value of total proteins was obtained in group IV, and the lowest in group II ($p<0.05$) (Table 1).

Table 1. *Statistical parameters for total plasma proteins (g/l)*

Groups	Statistical parameters					
	N	Mean*	SE**	Min	Max	C_V (%)**
I	27	38.88 ^{ab}	1.73	19.92	48.18	23.16
II	27	38.12 ^{a}	1.59	22.41	51.62	21.67
III	27	38.94 ^{ab}	1.41	20.26	49.18	18.77
IV	27	42.75 ^{b}	1.15	33.82	56.41	13.96

*a, b – significant at $p<0.05$; the same letter - no significant difference ($p> 0.05$); **SE - standard error; Cv - coefficient of variation

The mean values of total protein were high in all groups, but in group IV was slightly above the upper physiological limit for carp of 40 g/l (according to Svobodová and Vykusová, 1991).

Protein levels could be influenced by the nutritional status (Love, 1980). Patriche et al. (2009) and Noori et al. (2013) found that level of total plasma proteins varies depending on the protein concentration in the feed. In our study, high mean values of the total protein in all groups could be influenced by the high protein level in the feed.

According to results of two-way ANOVA, total protein levels were significantly influenced by feed quantity ($p<0.05$), and period of observation ($p<0.001$), but there was no interaction between these two factors ($p>0.05$). This indicates that blood protein concentration was also influenced by factors other than feed quantities and duration of the experiment. In Table 2 differences of total proteins mean values in each group are shown.

Table 2. *Mean values of the total plasma proteins at the end of the each period (g/l)*

Periods	Groups*			
	I	II	III	IV
first (1^{st} - 32^{nd} day)	28.20 ^{A}	29.17 ^{A}	32.36 ^{A}	37.74 ^{aA}
second (33^{rd} - 64^{th} day)	43.24 ^{B}	42.80 ^{B}	43.58 ^{B}	46.61 ^{B}
third (65^{th} - 96^{th} day)	45.20 ^{B}	42.38 ^{B}	40.90 ^{B}	43.90 ^{b}

*a, b –significant at $p<0.05$; A, B –significant at $p<0.01$; the same letter - no significant difference ($p> 0.05$)

Result shows significant increase in total protein levels in all groups after two months of the experiment i.e. in the second period ($p<0.01$), while these values were not significantly changed in the third period ($p>0.05$).

Increased synthesis of proteins could be associated with tertiary stress response. Stress could be caused by changes in water quality and increased stocking density due to growth of the fish. Plasma composition could be also changed by handling and anaesthetics applied at blood sampling (Ross and Ross, 2008). Smit et al. (1979) found that tricaine methanesulfonate (TMS or MS-222) can induce haemo-concentration in common carp.

This common effect of stress was monitored in several research by measuring plasma protein levels (cited in the paper by Bystriansky et al., 2006).

As in plasma proteins, the highest mean value of total immunoglobulins was in group IV, and the lowest in group II ($p<0.05$). However, in all groups coefficients of variation were greater than in total proteins (Table 3). Largest variations of immunoglobulin levels in group II may result from sporadic occurrence of high concentrations of NH_3-N that was recorded mostly in this group, and which could adversely affect the activity of the carps' immune system (Wlaslow et al., 1990). Cœurdacier and Dutto (1999) found in their study significantly lower level of total immunoglobulins in fish exposed to high level of ammonia compared with fish from the control group.

Table 3. *Statistical parameters for total immunoglobulins (g/l)*

Groups	Statistical parameters					
	N	Mean*	SE**	Min	Max	C_V (%)**
I	27	18.01 ^{ab}	0.90	4.83	25.91	25.89
II	27	15.85 ^{a}	1.18	3.88	25.85	38.83
III	27	17.34 ^{ab}	1.13	1.99	31.33	33.93
IV	27	19.17 ^{b}	0.99	4.64	29.74	26.88

*a, b –significant at $p<0.05$; the same letter - no significant difference ($p> 0.05$); **SE - standard error; Cv - coefficient of variation

Feed quantity (itself and in interaction with sampling) had no significant influence on total immunoglobulin levels ($p>0.05$), unlike to the period of observation ($p<0.001$). Similar to plasma proteins, absence of the interaction between feed quantity and period of observation could indicate the influence of other factors, including quality of the water in tanks, increased stocking density and the presence of bacteria in the water.
Primary and secondary stress responses are short-term effects of acute, short-lived challenges. When these responses are prolonged or repeated and fish has no way to avoid or escape the challenge, a series of tertiary effects become apparent, including changes in immune function (cited by Broom DM in EFSA, 2008). Influence of the observation periods was manifested in all groups except in group III (Table 4).

Table4. *Mean values of the total plasma immunoglobulins at the end of the each period (g/l)*

Periods	Groups*			
	I	II	III	IV
first (1st - 32nd day)	14.83 ^{a}	11.27 ^{a}	16.84 ^{a}	17.00 ^{a}
second (33rd - 64th day)	19.45 ^{ab}	19.07 ^{B}	19.38 ^{a}	22.89 ^{b}
third (65th - 96th day)	19.76 ^{b}	17.20 ^{B}	15.81 ^{a}	17.61 ^{ab}

*a, b – significant at $p<0.05$; A, B – significant at $p<0.01$; the same letter - no significant difference ($p> 0.05$)

Results from the table indicate that in the less fed groups I and II a significant increase in the amount of immunoglobulin occurred ($p<0.05$ and $p<0.01$, respectively). On the other hand, although decrease of the values in groups II, III and IV in the third period was not significant, it could be result of chronic exposure to stressors (Dobšíková et al., 2009; Magnadottir et al., 2010), considering the suppressive effects on the immune response of fish.

According to previously published data from the same experiment, increased feed quantity was affected fish average body mass (Stanković et al., 2009), and the water quality was gradually deteriorated (Relić, 2011; Relić et al., 2011) especially in groups III and IV in

the third period. Table 5 shows that total protein and immunoglobulin concentration was significantly affected by at least one water quality parameter in all experimental groups, except for group I.

Table 5. *Significant correlation between water and blood parameters at group level*

Parameters	Groups**							
	I		II		III		IV	
	ρ*	p-level	ρ	p-level	ρ*	p-level	ρ	p-level
temp & prot	-0.163	0.418	*-0.409*	**0.034**	-0.214	0.285	-0.223	0.263
temp & Ig	-0.233	0.241	*-0.430*	**0.025**	-0.296	0.134	-0.072	0.723
DO & prot	0.345	0.078	*0.392*	**0.043**	*0.425*	**0.027**	0.117	0.562
NH_3-N & prot	-0.486	0.329	*0.886*	**0.019**	-0.486	0.329	0.543	0.266
organic sub. & Ig	0.754	0.084	0.319	0.538	0.086	0.872	*-0.886*	**0.019**
prot & Ig	*0.600*	**0.001**	*0.857*	**<0.001**	*0.604*	**0.001**	*0.572*	**0.002**

*ρ – Spearman's rank correlation coefficient**;** **bolded values are statistically significant

Total proteins were in the significant negative correlation to the water temperature in group II, and positively correlated to DO (groups II and III) and NH_3-N concentration (group II). Total immunoglobulin values were significantly and negatively correlated to the water temperature (group II) and organic matter content (group II). Correlation between total proteins and immunoglobulins was significant and positive in the each experimental group ($p<0.01$), and strongest in group II.

Conclusions

According to the results from this study, feed quantity has showed influence on total protein levels, while period of observation i.e. time of sampling had influence on both parameters, total protein and immunoglobulin levels.

Rearing conditions (limited space, increase of feed quantity with increasing of the ichtyomass, increasing of stocking density, and consequently deterioration of the water quality over the time) have created a state of chronic stress.

In terms of reducing stress and ensuring welfare in carp juveniles, applied feed amount should match to the length of fish growing in the fish tanks, stocking density and capacity of the system for efficient water purification.

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References

1. Anon 1985. Standard Methods for the Examination of Water and Wastewater, 16th Edition, APHA. 1268 pp.
2. Ardó L, Yin G, Xu P, Váradi L, Szigeti G, Jeney Z and Jeney G 2008. Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (Oreochromis niloticus) and resistance against *Aeromonas hydrophila*. Aquaculture 275, 26-33.

3. Ardó L, Relic R, Csengeri I, Jeney Z and Jeney G 2009. Effect of fish feeds with different fatty acid contents on stress response of common carp (preliminary results). Conference proceedings – IV international Conference „Fishery", May, 27-29.2009, Faculty of Agriculture, Belgrade – Zemun, 191 – 196.
4. Bystriansky JS, Le Blanc PJ and Ballantyne JS 2006. Anaesthetization of Arctic charr Salvelinus alpines (L.) with tricaine methanesulphonate or 2-phenoxyethanol for immediate blood sampling. Journal of Fish Biology 69, 613–621.
5. Dobšíková R, Svobodová Z, Bláhová J, Modrá H and Velíšek J 2009. The effect of transport on biochemical and haematological indices of common carp (Cyprinus carpio L.). Czech J. Anim. Sci. 54, 11, 510–518.
6. Conte FS 2004. Stress and the welfare of cultured fish. Appl. Anim. Behav. Sci. 86, 205–223.
7. Cœurdacier JL and Dutto G 1999. Effect of chronic exposure to ammonia on alterations of proteins and immunoglobulins in sea bass (*Dicentrarchus labrax*) serum. Aquat. Living Resour. 12, 4, 247-253.
8. Čoha F 1990. Drinking water – Standard methods for the examination of the hygienic quality (in Serbian). Privredni pregled. Beograd.
9. EFSA 2008. Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on Animal welfare aspects of husbandry systems for farmed fish: Carp. The EFSA Journal, 843-Annex II, 1-19.
10. Hajibeglou A and Sudagar M 2010. Immune Response of Common Carp (*Cyprinus carpio*) Fed with Herbal Immunostimulants Diets. Journal of Animal and Veterinary Advances 9, 13, 1839-1847.
11. Harikrishnan R, Nisha Rani M and Balasundaram C 2003. Hematological and biochemical parameters in common carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophila* infection. Aquaculture 221, 1-4, 41-50.
12. Håstein T, Scarfe AD and Lund VL 2005. Science-based assessment of welfare: aquatic animals. Rev. sci. tech. Off. int. Epiz. 24, 2, 529-547.
13. Isla Molleda M 2007. Water quality in recirculating aquaculture systems (RAS) for Arctic Charr (*Salvelinus alpinus* L.) culture. The United Nations University. Fisheries training programme. Iceland. 54 pp.
14. Love RM 1980. The Chemical Biology of Fishes. London Academic Press, 541 pp.
15. Magnadottir B, Gisladottir B, Audunsdottir SS, Bragason BT and Gudmundsdottir S 2010. Humoral response in early stages of infection of cod (*Gadus morhua* L.) with atypical furunculosis. Icel. Agric. Sci. 23, 23-35.
16. Noori AN and Al-Sraji AY 2013. Effect of Different Dietary Protein and Fats on Some Biochemical Blood Parameters in Common Carp Fingerlings (*Cyprinus Carpio* L) Reared in Float Cages. Asian J. Exp. Biol. Sci. 4, 2, 293-296.
17. Patriche T, Patriche N and Tenciu M 2009. Cyprinids total blood proteins determination. Lucrări ştiinţifice Zootehnieş i Biotehnologii 42, 2, 95 – 101.
18. Relić RR 2011. Study of rearing conditions' effects on carp (*Cyprinus carpio* L., 1758) welfare. PhD thesis. University of Belgrade, Faculty of veterinary medicine. Belgrade, Serbia. 253 pp.
19. Relic R, Ciric M, Vucinic M, Radenkovic-Damnjanovic B, Poleksic V and Markovic Z 2011. Assessment of the Risk Level for Carp Welfare Based on the Water Quality in RAS. „Prospects for the 3rd Millennium Agriculture", 29th September-1st October 2011. Bulletin UASVM, Veterinary Medicine 68(1)/2011, 8-14. Plenary paper.

20. Roberts RJ 1989. Fish Pathology. W B Saunders Co; 2nd edition, 480 pp.

21. Ross LG and Ross B 2008. Anaesthetic and Sedative Techniques for Aquatic Animals. Third Edition. Blackwell Publishing, UK, 240 pp.

22. Smit GL, Hattingh J and Burger AP 1979. Haematological assessment of the effects of the anaesthetic MS 222 in natural and neutralized form in three freshwater fish species: interspecies differences. Journal of Fish Biology 15, 633–643.

23. Stanković M, Grubišić M, Poleksić V, Vukojević D, Lakić N, Relić R and Marković Z 2011. Effect of feed quantity on the growth rate of carp juveniles reared in tanks. V International Conference „Aquaculture & Fishery“, Institute of animal science, Faculty of agriculture, University of Belgrade, June 1-3. 2011, Coference proceedings, 504 – 509.

24. Svobodová Z and Vykusová B 1991. Haematological Examination of Fish. In Diagnostics, prevention and therapy of fish diseases and intoxications. Manual. FAO. 270 p. http://www.fao.org/docrep/field/003/AC160E/AC160E00.htm

25. Wendelaar BSE 1997. The stress response in fish. Physiol. Rev. 77, 591-625.

26. Wlasow T, Dabrowska H and Ziomek E 1990. Hematology of carp exposed to prolonged sublethal ammonia intoxication. Pol Arch Hydrobiol. 37, 429–438.

Original paper

BASIC BLOOD ANALYSIS OF RABBITS IMMUNIZED WITH VACCINE AGAINST MYXOMATOSIS

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Abstract

The aim of this preliminary study was to assess the immune response of rabbits triggered by vaccination against myxomatosis. In experiments, 14 New Zealand White rabbits (7 does – D and 7 bucks – B at the age of 1 to 3 years) were used. Samples of rabbit peripheral blood (PB) were collected from *a. auricularis centralis* to heparinised tubes 2 weeks before and 4 days after the subcutaneous injection (0.5 mL) of vaccine against myxomatosis (Pharmavac MXT). Mononuclear cells from peripheral blood (PBMCs) were isolated using Ficoll centrifugation. Isolated PBMCs were then frozen and stored at -192 °C. For phenotyping, the frozen cells were thawed and stained with the following anti-rabbit monoclonal antibodies (mAbs): anti-IgM (NRBM, IgG1), anti-CD4 (RTH1A, IgG1), anti-CD8 (ISC27A, IgG2a), anti-pan T2 (RTH21A, IgG1) and anti-CD45 (L12/201, IgG1). As the secondary immunoreagent, fluorescein isothiocyanate (FITC) or R-phycoerythrin (R-PE) labelled anti-mouse conjugates of appropriate subisotypes were used. We found significantly ($P<0.05$) increased percentage of either T-cells (does D5 and D7, and bucks B5, B6 and B7), or B-cells (bucks B2 and B7) in the rabbit peripheral blood. In conclusion, fast and adequate immune response to antigen (vaccine against myxomatosis) was indicated by the increase in T lymphocyte subsets 4 days after immunization. Thus, rabbit does (D5 and D7) and bucks (B5, B6 and B7) might be selected to create F1 generation for the future experiments.

Key words: *immune response, lymphocyte subsets, rabbit*

Introduction

Rabbit (*Oryctolagus cuniculus*) is one of the animal species often used as an experimental model in human and veterinary research. The rabbit model served for the study of infectious diseases such as syphilis (Gamboa and Miller, 1984), tuberculosis (Dannenberg, 1991), human T lymphotropic virus-I (Sawasdikosol et al., 1993) and human immunodeficiency virus (Filice et al., 1988). Rabbit is also useful for studies of various non-infectious diseases such as atherosclerosis (Jayo et al., 1994) or eye disorders (Peiffer et al., 1994) and is still the animal of choice for production of many polyclonal antibodies (Mage, 1998). These antibodies are typically produced by inoculation of a suitable mammal, such as a mouse, rabbit or goat. An antigen injected into the mammal triggers an immune response.

The three key interacting elements in immune responses are antigen-presenting cells (APCs), thymus-derived lymphocytes (T cells), and bone-marrow derived lymphocytes (B cells). T cells emerge from the thymus as $CD4^+$ or $CD8^+$ cells. The former are involved in a helper function to B cells, in cell-mediated immune responses in lymphokine secretion. The latter are specialised for cytotoxic killing of other cells, particularly virus-infected cells or, at least in experimental circumstances, tumour cells (Nossal, 1997). B cells are responsible for antibody formation (Nossal et al., 1968). The early IgM response of B cells may be triggered directly by antigen in a T-cell-independent manner, but most long-lasting immune responses, involving IgG, IgA, or IgE antibodies, need the help of activated T cells (Miller, 1972).

Determination of lymphocyte subset distribution in the peripheral blood is a routine part of laboratory tests in human patients suspected on immunodeficiency. At present, flow cytometry is also becoming a useful tool in veterinary medicine, particularly in small animal practice. The knowledge of physiological values is necessary for recognition of changes in lymphocyte subset distribution (Faldyna et al., 2001).

Objective of this preliminary study was to assess the immune response of rabbits triggered by vaccination against myxomatosis.

Materials and methods

Adult (1 - 3 years old) and clinically healthy rabbits of New Zealand White (NZW) line (n = 14; 7 does – D and 7 bucks – B) reared in a partially air-conditioned hall of a local rabbit farm at RIAP Nitra were used in the experiments. The animals were housed in individual cages, under a constant photoperiod of 14 h of day light. Temperature and humidity in the building were recorded continuously by means of a thermograph positioned at the same level as the cages (average relative humidity and temperature during the year was maintained at 60 ± 5% and 17 ± 3°C). The rabbits were fed *ad libitum* with a commercial diet (KV; TEKRO Nitra, s.r.o.) and water was provided *ad libitum* with nipple drinkers. The treatment of the animals was approved by the Ministry of Agriculture and Rural Development of the Slovak Republic, no. SK P 28004 and Ro 1488/06-221/3a.

Samples of rabbit peripheral blood (PB) were collected from *a. auricularis centralis* to heparinised tubes 2 weeks before and 4 days after the subcutaneous injection (0.5 mL) of vaccine against myxomatosis (Pharmavac MXT; Pharmagal Bio, Slovak Republic). One dose of vaccine contained live attenuated virus of myxomatosis min. 10^3 $TCID_{50}$. Mononuclear cells from peripheral blood (PBMCs) were isolated using Ficoll centrifugation according to the original protocol: Isolation of mononuclear cells from human peripheral blood by density gradient centrifugation (Miltenyi Biotec, 2008). Isolated PBMCs were then frozen as follows. Cells were resuspended in 1.5 mL of the appropriate 4 °C cooled RPMI 1640 medium (Gibco, Life Technologies, Grand Island NY, USA) containing L-glutamine, 10% FCS and 10% dimethyl sulfoxide (DMSO). Once aliquoted, cryovials were placed on ice and then transferred into a "Mr. Frosty" freezing container (Thermo Scientific Nalgene, Rochester, NY, USA), and stored at -80 °C for 24h. Cryovials were then transferred into liquid nitrogen for long-term storage at -192 °C. For phenotyping, the frozen cells were then thawed as follows. Five mL of RPMI 1640 medium containing 10% FCS, warmed to temperature at 37 °C, was aliquoted into 50 mL centrifuge tubes. No more than 2 cryovials were thawed at the same time. The cryovials were thawed in a 37 °C water bath until the cell suspension was almost completely melted. Two mL of the appropriate medium was slowly added to thawed cells and then slowly transferred drop by drop to the tubes containing corresponding medium with simultaneous

mixing of the cell suspension. Then another 20 mL of appropriate medium was added to the tubes. The tubes were two times centrifuged at 488x g for 10 min in order to remove DMSO from the cell suspension and then the cells were resuspended in 2 mL of the Dulbecco's PBS without Ca and Mg (PAA Laboratories GmbH, Pasching, Austria). Cell count was evaluated using the Bürker-Türk counting chamber (Brand, Germany).

Frozen-thawed cells were divided into prepared tubes and stained with different clones of anti-rabbit monoclonal antibodies: anti-IgM (NRBM; Bio-Rad AbD SerotecGmbH, Germany), anti-CD4 (RTH1A; WSU, Pullman, WA), anti-CD8 (ISC27A; WSU, Pullman, WA), anti-pan T2 (pT2; RTH21A; WSU, Pullman, WA) and anti-CD45 (L12/201; Bio-Rad AbD SerotecGmbH, Germany) according to the producer's manual. As the secondary immunoreagent, FITC or R-PE labelled anti-mouse conjugates of appropriate subisotypes (eBioscience, Austria) were used. To assess contamination of the lymphocyte gate by other cell types, the cross-reactive FITC labelled mAb against human CD14 antigen (TUK4; Bio-Rad AbD SerotecGmbH, Germany) was used. In each sample, 10000 - 50000 cells were measured using flow cytometer FACS Calibur (Becton Dickinson, Mountain View, CA). 7-AAD Staining Solution (BD Biosciences, USA) was used to exclude dead cells from analysis. The common leukocyte antigen CD45 and CD14 expression was used for the ''lymphogate'' set up and lymphocyte purity determination as described by Jeklova et al. (2007). Results obtained for the other surface markers were recalculated to 100 % of $CD45^+$and $CD14^-$ cells in the''lymphogate''.

Observed results were evaluated statistically using χ^2-test in SigmaPlot software (Systat Software Inc., Germany) and expressed as the means ± standard error of means (SEM). P-values at $P<0.05$ were considered as statistically significant.

Results and discussion

In this study, we evaluated the changes in lymphocyte subsets of rabbit peripheral blood collected from seven does (D1-7) and seven bucks (B1-7) before and after immunization with vaccine against myxomatosis. After immunization, significant decrease ($P<0.05$) in the percentage of B lymphocytes (IgM^+ cells) was observed in almost all rabbit does (except in D1; Table 1). On the other hand, the percentage of T lymphocytes subsets increased significantly ($P<0.05$) in does D5 ($pT2^+$, $CD4^+$ and $CD8^+$ cells) and D7 ($pT2^+$ and $CD8^+$ cells), what was reflected in higher pT2/IgM ratio value for doe D5 (Table 1). Higher percentage of $CD8^+$ cells ($P<0.05$) was also found in doe D6 after immunization (Table 1). We noticed significantly decreased ($P<0.05$) value of CD4/CD8 ratio only in doe D3 (Table 1). There were no changes in the percentage of double positive lymphocytes ($CD4^+CD8^+$) in any doe.

On the contrary, inconsistent values in the percentage of B lymphocytes were obtained in rabbit bucks after immunization. Significant increase ($P<0.05$) of IgM^+ cells were found in bucks B2 and B7, whereas decreased ($P<0.05$) percentage of IgM^+ cells had bucks B3 and B4 (Table 2). The percentage of T lymphocytes subsets increased significantly ($P<0.05$) in bucks B5 ($pT2^+$, $CD4^+$ and $CD8^+$ cells), B6 ($pT2^+$ and $CD4^+$ cells) and B7 ($CD4^+$ and $CD8^+$ cells) after immunization (Table 2). On the other hand, buck B1 had lower ($P<0.05$) percentage of $pT2^+$ cells (Table 2). We observed higher ($P<0.05$) pT2/IgM ratio value only in buck B4 (Table 2). Similarly, no changes in the percentage of double positive lymphocytes ($CD4^+CD8^+$) or CD4/CD8 ratio values were found in any buck after immunization.

Table 1. *Lymphocytes subsets in peripheral blood of rabbit does before and after immunization*

Sample	Blood collection	pT2 (%)	CD4 (%)	CD8 (%)	$CD4^+CD8^+$ (%)	IgM (%)	pT2/IgM (ratio)	CD4/CD8 (ratio)
D1	Before IM	26.96	22.33	4.20	0.60	25.73	1.05	5.32
	After IM	21.66	15.38	4.40	0.71	17.74	1.22	3.49
D2	Before IM	40.19	36.52	4.57	0.89	28.50^a	1.41	8.00
	After IM	33.68	27.08	4.59	0.58	15.01^b	2.24	5.90
D3	Before IM	34.57	29.15	2.50	0.21	30.51^a	1.13	11.66^a
	After IM	25.58	19.01	3.94	0.21	15.23^b	1.68	4.83^b
D4	Before IM	56.53	22.85	27.22	2.05	24.17^a	2.34	0.84
	After IM	57.26	22.51	30.33	1.83	14.05^b	4.08	0.74
D5	Before IM	32.67^a	27.14^a	6.08^a	0.79	41.16^a	0.79^a	4.46
	After IM	55.38^b	37.53^b	14.48^b	0.81	14.43^b	3.84^b	2.59
D6	Before IM	47.07	30.13	11.51^a	1.73	31.48^a	1.50	2.62
	After IM	38.51	24.10	40.39^b	1.36	16.01^b	2.41	0.60
D7	Before IM	29.67^a	31.07	2.61^a	0.87	35.72^a	0.83	11.90
	After IM	43.29^b	40.57	5.88^b	1.17	15.16^b	2.86	6.90
Average values	Before IM	38.24	28.45	8.38	1.02	31.04	1.29	6.40
	After IM	39.33	26.59	14.86	0.95	15.37	2.62	3.58

IM – immunization; Results are expressed as means ± SEM; ^{a} vs ^{b} within the same column and sample were statistically significant at $P<0.05$.

Table 2. *Lymphocytes subsets in peripheral blood of rabbit bucks before and after immunization*

Sample	Blood collection	pT2 (%)	CD4 (%)	CD8 (%)	$CD4^+CD8^+$ (%)	IgM (%)	pT2/IgM (ratio)	CD4/CD8 (ratio)
B1	Before IM	46.45^a	34.11	5.48	0.93	21.37	2.17	6.22
	After IM	30.5^b	23.35	5.28	0.53	17.35	1.76	4.43
B2	Before IM	31.98	29.28	5.75	0.44	21.62^a	1.48	5.10
	After IM	27.87	21.58	7.25	0.38	31.55^b	0.88	2.98
B3	Before IM	39.58	28.42	4.91	0.53	28.27^a	1.40	5.79
	After IM	30.04	23.51	8.27	0.25	14.68^b	2.05	2.84
B4	Before IM	42.85	32.82	5.39	1.45	28.23^a	1.52^a	6.09
	After IM	45.89	39.73	6.80	1.51	2.11^b	21.75^b	5.85
B5	Before IM	29.43^a	27.51^a	4.07^a	1.05	29.38	1.00	6.76
	After IM	60.77^b	49.80^b	8.51^b	1.44	27.59	2.20	5.85
B6	Before IM	43.72^a	32.98^a	5.23	0.95	38.53	1.13	6.31
	After IM	64.74^b	53.75^b	8.60	2.26	30.29	2.14	6.25
B7	Before IM	38.43	31.91^a	3.40^a	1.09	13.18^a	2.92	9.40
	After IM	49.65	48.18^b	8.62^b	2.25	27.18^b	1.83	5.59
Average values	Before IM	38.92	31.00	4.89	0.92	25.79	1.66	6.52
	After IM	44.21	37.13	7.61	1.23	21.53	4.66	4.83

IM – immunization; Results are expressed as means ± SEM; ^{a} vs ^{b} within the same column and sample were statistically significant at $P<0.05$.

The average percentages of specific lymphocyte subsets (pT^+, $CD4^+$, $CD8^+$, $CD4^+CD8^+$ and IgM^+) in peripheral blood of rabbits before immunization (Table 1 and 2) are similar to those observed by Jeklova et al. (2007) (40.1%, 29.4%, 10.4%, 2.0% and 41.9%, respectively), thus confirming the normal health status of rabbits used for experiment. Although these authors found more than 10% higher value of B-cells in comparison to our results (Tables 1 and 2) this could be due to the use of CD79α antibody for enumeration of B-cells. However, the anti-rabbit IgM mAb has been already successfully used as B-cell marker also in other studies (Vajdy et al., 1998; Lanning et al., 2000; Tokarz-Deptula and Deptula, 2005).

According to observed results after immunization, we found significantly ($P<0.05$) increased percentage of either T-cells (does D5 and D7, and bucks B5, B6 and B7), or B-cells (bucks B2 and B7) in the rabbit peripheral blood (Tables 1 and 2). An increase in lymphocyte concentration is usually a sign of a viral infection, in this case caused by the attenuated virus of myxomatosis. Adequate immune response in observed rabbits might be indicated by the increased percentage of $CD4^+$ (D5, B5, B6 and B7) or $CD8^+$ (D5, D7, B5 and B7) cells. Although, both $CD4^+$ and $CD8^+$ lymphocytes are very important in the reaction cascade of immune response, they differ in their function. The most important distinction is that $CD4^+$ cells see antigenic peptides in association with MHC class II molecules whereas $CD8^+$ cells react with peptide plus MHC class I (Nossal, 1997). $CD4^+$ T cells, when activated, develop into T cells secreting a large variety of cytokines (Kelso et al., 1991). However, as the immune response matures, there are many instances where either a T helper (Th)-1 response or a (Th)-2 responses become dominant (Mosmann and Coffman, 1989). The Th-1 response leads to inflammatory phenomena and the Th-2 response to antibody formation, including IgG1 and IgE formation (Finkelman et al., 1990).

Conclusion

In conclusion, fast and adequate immune response to antigen (vaccine against myxomatosis) was indicated by the increase in T lymphocyte subsets 4 days after immunization. Thus, rabbit does (D5 and D7) and bucks (B5, B6 and B7) might be selected to create F1 generation for the future experiments.

Acknowledgments

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References

1. Dannenberg Jr AM 1991. Delayed-type hypersensitivity and cell-mediated immunity in the pathogenesis of tuberculosis. Immunology Today 12, 228–233.
2. Faldyna M, Levá L Knötigová P and Toman M 2001. Lymphocyte subsets in peripheral blood of dogs – a flow cytometric study. Veterinary Immunology and Immunopathology 82, 23-37.
3. Filice G, Cereda PM and Varnier OE 1988. Infection of rabbits with human immunodeficiency virus. Nature 335, 366-369.

4. Finkelman FD, Holmes J, Katona IM, Urban JF Jr, Beckmann MP, Park LS, Schooley KA, Coffman RL, Mosmann TR and Paul WE 1990. Lymphokine control of in vivo immunoglobulin isotype selection. Annual review of immunology 8, 303-333.
5. Gamboa D and Miller JN 1984. Experimental neonatal syphilis, I: evidence of resistance to symptomatic infection in neonatal rabbits following intradermal inoculation with *Treponema pallidum* (*Nichlos* strain). Pediatric Research 18, 965–971.
6. Jayo JM, Schwenke DC and Clarkson TB 1994. Atherosclerosis research. In the biology of the laboratory rabbit (Manning, PJ, Ringler, DH and Newcomer, CE eds), 367-376 pp. Academic Press, San Diego, CA.
7. Jeklova E, Leva L and Faldyna M 2007. Lymphoid organ development in rabbits: Major lymphocyte subsets. Developmental and Comparative Immunology 31, 632-644.
8. Kelso A, Troutt AB, Maraskovsky E, Gough NM, Morris L, Pech MH and Thomson JA 1991. Heterogeneity in lymphokine profiles of CD4+ and CD8+ T cells and clones activated in vivo and in vitro. Immunological reviews 123, 85-113.
9. Lanning D, Sethupathi P, Rhee KJ, Zhai SK and Knight KL 2000. Intestinal microflora and diversification of the rabbit anti-body repertoire. Journal of Immunology 165, 2012–2019.
10. Mage RG 1998. Immunology of lagomorphs. In Handbook of vertebrate immunology (Pastoret PP, Griebel P, Bazin H and Govaerts A eds.), 223–260 pp. Academic Press, San Diego, CA.
11. Miller JFAP 1972. Lymphocyte interactions in immune responses. International Review of Cytology 33, 77-130.
12. Mosmann TR and Coffman RL 1989. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual review of immunology 7, 145–173.
13. Nossal GJV 1997. Host immunobiology and vaccine development. Lancet 350, 1316–1319.
14. Nossal GJV, Cunnigham A, Mitchell GF and Miller JFAP 1968. Cell to cell interaction in the immune response. III. Chromosomal marker analysis of single antibody-forming cells in reconstituted irradiated or thymectomized mice. The Journal of Experimental Medicine 128, 839-853.
15. Peiffer RL, Pohm-Thorsen L and Corcoran K 1994. Models in ophthalmology and vision research. In The biology of the laboratory rabbit (Manning PJ, Ringler DH and Newcomer CE eds), 410–430 pp. Academic Press, San Diego, CA.
16. Sawasdikosol S, Hague BF, Zhao TM, Bowers FS, Simpson RM, Robinson M and Kindt TJ 1993. Selection of rabbit CD4- CD8- T cell receptor-γ/δ cells by in vitro transformation with human T lymphotropic virus-I. The Journal of Experimental Medicine 178, 1337-1345.
17. Tokarz-Deptula B and Deptula W 2005. Values of selected immune and haematological parameters in healthy rabbits.Polish Journal of Veterinary Sciences 8, 107-112.
18. Vajdy M, Sethupathi P and Knight KL 1998. Dependence of antibody somatic diversification on gut-associated lymphoid tissue in rabbits. Journal of Immunology160, 2725-2729.

Original paper

EFFECT OF ACUTE HEAT STRESS ON SOME HEMATOLOGICAL PARAMETERS, TRACE ELEMENTS AND MEAT QUALITY IN RABBITS

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Abstract

The aim of the present study was to investigate the effect of acute heat stress on some hematological parameters, trace elements and meat quality traits in rabbits. Twelve male rabbits at the age of 4 months were allocated into 2 groups: control and experimental. The experimental rabbits were exposed to intermittent solar radiation for 4 hours at 36 °C. The following parameters were determined: white blood cells (WBC) count, red blood cells (RBC) count, hematocrit, neutrophil and lymphocyte percentages, liver and meat content of chromium (Cr) and selenium (Se), pH of meat at 60 min and 24 h *post mortem*, meat color, myoglobin and water holding capacity (WHC). Exposure to heat increased neutrophil to lymphocyte ratio ($P<0.01$) and liver content of Cr ($P<0.01$), while WBC count, RBC count, hematocrit, liver Se, muscle Cr and Se, pH of meat at 24 h, meat color, myoglobin and WHC were not affected by the applied heat load. The experimental rabbits had significantly lower meat pH at 60 min after slaughter relative to the control rabbits ($P<0.05$).

It was suggested that experimental rabbits had sufficient muscle glycogen at the time of slaughter needed to produce the lactic acid that reduced the ultimate pH of post-mortem muscle within the range observed in the control rabbits.

Key words: *chromium, heat, hematocrit, lymphocytes, neutrophils, selenium*

Introduction

Rabbits are more susceptible to high than to low ambient temperatures. Recent studies on chickens and pigs have presented controversial results concerning metabolic effects of high ambient temperatures (Lin et al., 2006; Hicks et al., 1998). The research papers about the effect of acute thermal stress in rabbits are scarce and incomplete (Amici et al., 2000). Heat is known to cause oxidative stress, which leads to generation of free radicals. Reactive oxygen species can initiate lipid peroxidation and cause cellular damage to tissues (Aitken et al., 1989).

The effect of thermal stress on the quality of rabbit meat has been studied less intensively as compared with transportation stress, feed ratios and other biological and zootechnical factors (Zotte, 2002).

The available literature provides little data about the content of Cr and Se in tissues and blood. Cromium is known to increase liver glycogen and glycogen synthetase (Samanta et al., 2008) and acts as an antagonist to the glycolitic effect of stress hormones. Selenium is an essential component of the body's antioxidant system.

The aim of this study was to investigate the effect of heat stress on some hematological parameters, liver and muscle content of Cr and Se and meat quality in farm rabbits.

Materials and methods

The experiment comprised 12 New Zealand White male rabbits (*Oryctolagus cuniculus*) at the age of 4 months and average weight of 3 kg, divided in two groups (control and experimental), consisting of 6 rabbits each. Rabbits were reared in an enclosed building under summer conditions with variable natural temperatures within the range of 24 to 27 °C. They were housed individually in wire-floor cages, provided with feeders and automated drinkers – feed and drinking water were supplied *ad libitum*.

The experimental group was exposed to direct sunlight for 4 hours at ambient temperature of 36 °C without any access to food and water. Blood samples were collected by ear venepuncture before exposure to heat (basal level) and 2 hr later, when heat exposure was interrupted for 15 min for the second blood sampling. The following hematological parameters were determined: white blood cell (WBC) count, hematocrit, neutrophil to lymphocite ratio (N:L), and red blood cell (RBC) count. Total erythrocyte and leukocyte counts were determined by manual haemacytometer chamber count. Haematocrit was measured by the microhaematocrit method. Peripheral blood leukocytes were counted on smears. The smears were stained using May-Grunvald and Gisma stains (Lucas and Jamros, 1961). At the end of the stress period the animals of both groups were slaughtered. Liver and muscle (m. biceps femoris) samples were taken and analyzed for trace elements – Cr (Chromium) and Se (Selenium). Trace elements were measured by Inductively coupled plasma mass spectroscopy method – ICP-MS, using Agilent 7500 cx.

The following physicochemical characteristics of m. longisimus dorsi were determined: pH measured at 60 min and 24 h post mortem by pH-meter, color-measured by Specol 11 at 525 nm, water holding capacity (Grau and Hamm, 1952), and myoglobin (Hornsey et al., 1956).

The results of one factor statistical analysis are expressed as means ±S.E.M. and were analyzed by ANOVA.

Results and discussion

White blood cells (WBC) count in the experimental rabbits remained unchanged after exposure to heat (Table 1). Exposure to heat has been reported to decrease WBC count in laying hens (Mashaly et al., 2004), cocks (Nathan et al., 1976) and growing female rabbits (Ondruska et al., 2011). Glucocorticoids seem to play a certain role in the maintenance of leukocyte counts (Deutsch et al., 2007). Exposure to acute heat stress, unlike other stressors, results in a quick decline of glucocorticoids level following an initial nonspecific increase in the adrenal response (Gudev et al., 2004). The unaffected WBC count in our study is in agreement with the reported lack of change in WBC of growing and adult male rabbits exposed to 36 °C for 4 weeks (Ondruska et al., 2011).

The experimental rabbits in our study had higher neutrophil to lymphocyte ratio (N: L) after exposure to heat relative to that in the control rabbits (Table 1).

It has long been known that the increase in neutrophil to lymphocyte ratio is due to glucocorticoids-induced release of polymorphonuclear leukocytes from bone marrow, delayed apoptosis, and reduced egress of polymorphonuclear leukocytes into tissue (Nakagawa et al., 1997). Neutrophil to lymphocyte ratio is widely used as an indirect marker of stress-elicited increase in adrenal activity.

Table 1. *Effects of acute heat stress on the hematology of New Zealand rabbit*

HEMATOLOGICAL INDEX	n	CONTROL	EXPERIMENTAL
Erythrocyte no. ($X10^6/mm^3$)	6	5.94 ± 0.046	5.980 ± 0.058
Hematocrit (vol %)	6	39.74 ± 2.913	39.791 ± 3.648
Leukocyte no. ($X10^3mm^3$)	6	6507 ± 113.389	6475 ± 147.478
Neutrophil percentages	6	20.535 ± 4.753	37.541 ± 9.315**
Lymphocyte percentages	6	74.464 ± 4.753	55.791 ± 8.263***

Data are presented as means±standard deviation.
Statistically significant differences are indicated: ** P< 0.01; ***P<0.001 significantly different from the corresponding control value

Heat-induced increase in N:L ratio (from 0.276 in the control to 0.673 in the experimental rabbits) in the present study is not consistent with the reported lack of change in N:L ratio in growing female rabbits exposed to heat (36 °C) for 4 weeks (Ondruska et al., 2011).

This discrepancy could be explained with the different patterns of cortisol dynamics during acute and chronic exposure to heat and its effect on N:L ratio.

Red blood cells count in the experimental rabbits was not influenced by the applied heat load. This data is consistent with the reported slight fluctuation of erythrocyte count in rabbits exposed to heat for 4 weeks (Ondruska et al., 2011). In addition, exposure to heat had no effect on hematocrit value (Table 1). On the contrary, exposure of growing rabbits to 36 °C for 4 weeks was reported to induce a decrease in hematocrit value (Ondruska et al., 2011). The reported decline in hematocrit value was attributed to reduction of cellular oxygen as a requirement for reducing endogenous heat production in order to compensate for the elevated environmental heat. Heat has been reported to decrease corticosterone and ACTH levels in rats (Wang et al., 2009).

Consequently, the observed discrepancy in hematocrit values between our study and that reported by Ondruska et al. (2011) was probably due to the fact that hematocrit value in our study was measured under acute heat exposure, while in their experiment it was registered at the end of 4 weeks long exposure to heat.

Liver content of chromium was significantly higher (P<0.01) in the experimental rabbits relative to that in the control rabbits (Figure 1).

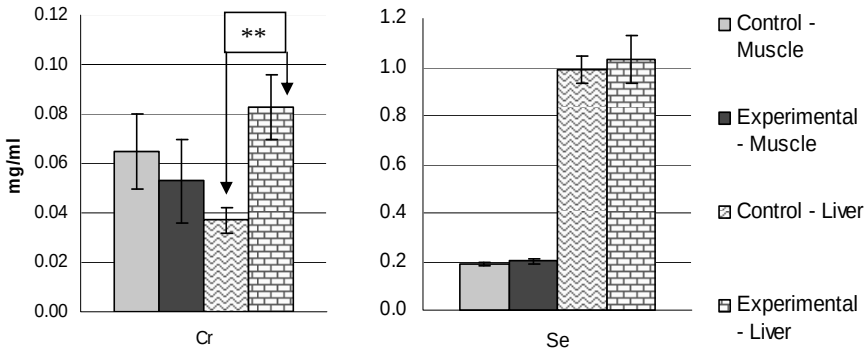

Figure 1. *Effect of heat exposure on selenium and chromium concentration (mg/kg) in rabbit's muscle and liver tissue*
** P<0.01

Chromium is known to potentiate the effect of insulin by facilitating insulin binding to receptors at the cell surface (Pechova and Pavlata, 2007). The demand of chromium is growing as a result of stress factors. High blood glucose concentration stimulates mobilization of tissue Cr and its irreversible loss through urine.

Supplemental chromium increased liver glycogen level and yielded less glucose in the blood under the influence of the catabolic effects of cortisol in broiler chickens kept at 35-36 °C (Samanta et al., 2008). Basal urinary excretion of Cr is related to maximum O_2 consumption (Anderson et al., 1988). Consequently, we expected higher rate of chromium excretion and a decline in liver and muscle Cr stores in the heat stressed rabbits, because of the increased N:L ratio. Contrary to our expectation liver content of Cr was twofold higher in the experimental than in the control rabbits (Figure 1). Muscle content of Cr showed a trend towards lower level in the experimental relative to the control rabbits ($P>0.05$). The lack of significant change in muscle concentration of Cr supports the view that muscle stores of Cr were sufficient to potentiate the effect of insulin thus reverting, at least partly, stress-induced glycogen decomposition during the heat stress episode. Our view is supported by the reported improvement of performance and water holding capacity of meat in pig given supplemental chromium picolinate (Chang Xui, 2009).

Exposure to heat had no effect on both muscle and liver content of selenium (Figure 1). Heat stress is known to increase the level of free radicals (Al-Zahrani et al., 2011). Selenium yeast supplementation improved resistance to oxidative stress in chickens subjected to heat stress (Mahmoud and Edens, 2005).

The unchanged muscle and liver content of Se under the conditions of our study suggests that the experimental rabbits were not Se deficient. It is worth to note that the liver Se was almost 5 times higher than in the muscle.

Muscle pH at 60 min post-mortem was significantly lower in the experimental rabbits in relation to the control rabbits (Table 2). This finding is consistent with the reported acceleration of post-mortem pH decrease in heat-stressed broilers due to the initial high muscle temperature (Wang et al., 2009). The increased post-mortem rate of pH decline in the experimental rabbits in comparison with control rabbits at 60 min was probably due either to pre-mortem increase of glucocorticoid-induced glycogenolysis and build up of lactic acid or to heat-induced alteration in glycolytic activity (Sandercock et al., 2001). On the other hand, it is widely accepted that the ultimate pH of meat (at 24h) is closely related with muscle glycogen concentration at the time of slaughter (Warriss et al., 1989).

Table 2. *Effects of heat exposure on meat quality traits in rabbits*

ITEMS	n	CONTROL	EXPERIMENTAL (EXPOSED TO HEAT)
pH, 60 min	6	7.218 ± 0.080	6.592 ± 0.382 *
pH 24 h	6	5.635 ± 0.079	5.754 ± 0.122
Color, 525 nm/R	6	30.804 ± 1.286	32.362 ± 1.150
Water-holding capacity	6	36.512 ± 1.9	35.588 ± 0.952

Data are presented as means± standard deviation
* $P<0.05$, significantly different from corresponding control level

Muscle pH values at 24 h were similar in both groups although the pH value in the experimental rabbits tended to be higher (Table 2). Our data suggest that experimental rabbits

lactic acid and ensure satisfactory pH at 24 h post-mortem. The control rabbits showed a trend toward better meat quality (P>0.05) as judged by the other meat quality parameters: color, water holding capacity and myoglobin (Table 2).

Conclusion

Exposure of rabbits to intermittent heat stress for 4 h resulted in an increase in N:L ratio and liver content of Cr. It caused higher rate of meat pH decline at 60 min after slaughter. There were no changes in RBC count, WBC count, hematocrit, liver Se, muscle Cr and Se, meat pH at 24h, meat color, myoglobin and water holding capacity.

References

1. Aitken RJ, Clarkson JS and Fishel S 1989. Generation of reactive oxygen species, lipid peroxidation and human sperm function. Biology of Reproduction, 41 (1), 183-197.
2. Al-Zahrani S, Kandeal S, Mohony M and Bodr G 2011. Effect of vitamin E and thymoquinone on physiological and histological characteristics of heat stressed male mice. African Journal of Pharmacy and Pharmacology, 5 19, 2174-2193.
3. Amici A, Franci O, Mastroiacono P, Merendino N, Nardini M and Tomassi G 2000. Short term acute heat stress in rabbits: functional, metabolic and immunological effects. World Rabbit Science, 8, 1, 11-16.
4. Anderson RA, Bryden NA, Polansky MM and Deuster PA 1988. Exercise effects on chromium excretion of trained and untrained men consuming a constant diet. J. Apply Physiology, 64, 249-252.
5. Chang Xui, C 2009. The effect of chromium propionate on production performance and pork quality in finishing pigs. Acta Agricultural Univesitatis Jiangxiensis, 31, 3, 522-525.
6. Deutsch V, Lerner-Geva L, Reches A, Boyko V, Limor R and Gisaru D 2007. Sustained leukocyte count during rising cortisol level. Acta Haematol., 118, 73-76.
7. Grau R and Hamm R 1952. Eine einfache Methode zur Bestimmung der Wasserbindung im Fleisch. Die Fleischwirtschaft, 4, 295-297.
8. Gudev D, Ralcheva S, Moneva P and Johnson HD 2004. Endocrine changes in two behavioral types of cows exposed to heat. Bulgarian Journal of Agricultural Science, 10, 629-636.
9. Hicks TA, McGlone JJ, Whisnaut CS, Kattesh HG and Norman RL 1998. Behavioral, endocrine, immune and performance measures for pigs exposed to acute stress. J. Anim. Sci., 76, 2, 474-483.
10. Hornsey HC 1956. The color of cooked cured pork. Estimation of the nitric oxid- haem pigments. J. Sci. Food Agric., 7, 534-540
11. Lin H, Decnypere E and Buyse J 2006. Acute heat stress induces oxidative stress in broiler chickens. Comparative Biochemistry and Physiology: Part A, 144, 1, 11-17.
12. Lucas AM and Jamros C 1961. Atlas of avian hematology. Agriculture monograph 25. USDA, Washington, DC.
13. Mahmoud, KZ and Edens FW 2005. Influence of organic selenium on hsp70 response of heat stressed and enteropathogenic Escherichia coli-challenged broiler chickens (Gallus gallus). Comparative biochemistry and physiology, Part C: Toxicology and Pharmacology, 141, 1, 59-75.

14. Mashaly MM, Hendricks GL, Kalama MA, Gehad AE, Abbas AO and Patterson PH 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poultry Science*, 83, 889-894.

15. Nakagawa M, Terashima T, Dyachkova Y, Bondy GP, Hogs JC and van Eden SF 1997. Glucocorticoid-induced granulocytosis. Contribution of marrow release and demargination of intravascular granulocytes. Circulation, 98, 2307-2313.

16. Nathan DB, Heller ED and Perek M 1976. The effect of short heat stress upon leukocyte count, plasma corticosterone level, plasma and leucocyte ascorbic acid content. Br. Poult. Sci, 17, 5, 481-485.

17. Ondruska L, Rafay J, Okab AB, Ayoub MA, Jurcik R, Massanyi P, Lukas W and Supuka P 2011. Influence of elevated ambient temperature upon some physiological measurements of New Zealand White rabbits. Veterinarni Medicina, 56, 4, 180-186.

18. Pechova A and Pavlata L 2007. Chromium is an essential nutrient: a review. Veterinarni Medicina, 52, 1, 1-18.

19. Samanta S, Haldor S., Bahadur V and Ghosh TK 2008. Chromium picolinate can ameliorate the negative effects of heat stress and enhance performance, carcass and meat traits in broiler chickens by reducing the circulatory cortisol level. J. Sci. Food Agric., 88, 787-796.

20. Sandercock DA, Hunter RR, Nute GR, Mitchell MA and Hocking PM 2001. Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: Implications for meat quality. Poultry Science, 80, 418-425.

21. Wang JS, Chen SM, Lee SD, Huang CC and Hsien CC 2009. Dehydroepiandrosterone sulfate linked to physiologic response against hot spring immersion. Steroids, 74, 945-949.

22. Wang RR, Pan XY and Peny ZQ 2009. Effect of heat exposure on muscle oxidation and protein functionalities of pectoralis majors in broilers. Poultry Science, 88, 1078-1084.

23. Warriss PD, Bevis EA and Ekins PJ 1989. The relationship between glycogen stores and muscle ultimate pH in commercially slaughtered pigs. Br. Vet. J., 145, 378-383.

24. Zotte AD 2002. Perception of meat quality and major factors influencing the rabbit carcass and meat quality. Livestock Production Science, 75, 1, 11-32.

Review paper

INFLUENCE OF INTENSIVE ANIMAL BREEDING TO THE APPEARANCE OF INFECTIOUS DISEASES (ZOONOSES)

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Abstract

Intensive animal breeding and production is based on farm breeding of animals which represents a major source of raw material for food production. Preserving health of animals requires a good practice during breeding, appropriate feeding and watering, adequate control of pests and wild animals. Animal breeding and production of food of animal origin requires significant engagement of veterinary services within the frame of epizootiological, epidemiological, veterinary and sanitary surveillance. Farm manner of cattle breeding can represent a danger of air, water and ground contamination. In the farms situated in a small space, overcrowded with animals there are ideal conditions for the appearance and spreading of causative agent of infectious diseases (prions, viruses, rickettsiae, chlamydia, bacteria, parasites and fungi), which can be transmitted also to humans and wild animals. From the aspect of public health, special attention should be given to the farms with large number of animals and farms with intensive breeding conditions. This is especially important in pig and poultry breeding, where moderate or high prevalence of infections such as salmonellosis and campylobacteriosis are often present, regardless of the fact that the level of clinical illness caused by these infections is relatively low. Intensive production in animal husbandry leads to increased animal waste, and the richest source of infectious agents represents animal feces.

Key words: *biohazard, epidemiology, farms, zoonoses*

Introduction

Food animal production methods have undergone substantial changes over the past fifty years. Herd health and production management programs are examples of this development. The main focus of these programs is on-farm economics and especially operational management through the application of veterinary and zoo technical skills and a wider knowledge of the commercial and societal consequences of disease in the food-producing animal (Noordhuizen and Collins, 2002; WTO, 1995). Key elements in such a program include risk assessment and priority setting at the start, followed by farm inspections and clinical examination of animals, data monitoring and herd/flock problem analysis and prevention. The approach has to be adapted depending on the species of animal involved and the intensity of the farming enterprise (Brand et al., 1996).

Primary producers must take all reasonable measures to prevent the entry of pathogenic agents onto his/her holding. They are responsible for the health of their stock and must

adopt a positive approach to animal health on the farm with the objective of eliminating or minimizing exposure of food-producing animals to zoonotic agents (Noordhuizen and Collins, 2002). This is an essential component of the longitudinal integrated safety assurance (LISA) schemes now being adopted in many developed countries.

Control of zoonoses on farms

As a starting point, maintenance of the integrity of the herd or flock as a distinct and self-contained entity, if possible, remains a key objective of health control in food animal production. It is essential to ensure the origin of such stock so as to define as far as possible the health status of the herd of origin. Also, it is very important to ensure the traceability of the animals and their production as they move through the food chain. Traceability of all inputs to the farming enterprise, both goods and stock, is important because if a farm manager is unaware of the origin of inputs, he/she cannot be assured that best practices were applied to the production (Noordhuizen and Collins, 2002).

Selective pressure associated with the inappropriate use of antimicrobials can generate multidrug-resistant organisms which can become a public health problem (Abury-Damon et al., 2004). Vaccination can raise herd immunity and reduce the risk of disease and the consequent application of antibiotics. Husbandry practices such as segregating age cohorts, feeding with colostrum's, isolation of sick animals and adequate disinfection can prevent the spread of disease and contribute to maintaining the health of the herd or flock (CEC, 2000). Appropriate ecto and endo-parasites prevent animals from becoming debilitated and more susceptible to other diseases. Policies for the use of animal remedies should be science-based and all usage should be documented and continuously reviewed (Abury-Damon et al., 2004).

In larger enterprises, system of quality management can be established easy, since there are documented procedures and a regimented approach is adopted. Confirmation of the disease-free status of recently purchased animals is best achieved through pre-purchase testing and segregation before such animals are allowed to join the flock/herd (Noordhuizen and Collins, 2002). This concept of quarantine is best continued throughout the production system, with persons other than farm personnel directly engaged on the farm being allowed only limited access to animals or feed (CEC, 2000).

The enforcement of sanitation rules including the use of disinfectants at key points and the wearing of protective clothing and footwear, together with effective controls on the hygienic quality of feedstuffs and water sources, including rodent and pest control, are standard biosecurity practices which are necessary if the integrity of the production unit wants to be maintained. The practice of an "all in/all out" policy of stock movement together with the early segregation of all clinically ill animals offer a direct means of enforcing disease control and prevention at the herd or flock level (Noordhuizen and Welpelo, 1996).

In farm breeding of animals several hazards can be identified: biological, chemical and physical. Biohazard includes biological agents and/or threatening diseases which can be transferred to humans by food or water. Special problem represent zoonoses which are identified as infectious diseases that can be spread by natural way among people, wild and domestic animals. Today, over 1415 different species of infectious organisms are known, which are pathogenic for humans, including 217 viruses and prions, 538 bacteria and rikettsiae, 307 fungi, 66 protozoa and 287 helmints. Out of the total, 868 or 61% are classified as zoonoses and 175 as pathogenic species which can be connected to the

appearance of the disease. From the group of 175 pathogens, 132 species or 75% have a zoonotic potential. There are several factors which can influence the breakout of new zoonotic diseases and also diseases that were under control and reappear again. These factors are: growing number of people in the world, ecological changes, intensive agricultural production, travels, need for more funding, new strains of causative agents, exotic traveling, etc. Actions which are taken for the reduction of risk from zoonoses have to be practical, economically acceptable and flexible.

Many biosecurity measures are general and not specific to particular zoonosis and should be part of good farming practice (Brand et al., 1996; Noordhuizen and Welpelo, 1996). The control of *Campylobacter spp*, but elements of campylobacter control can be applied in principle to the prevention of exposure to other zoonotic agents in a variety of animals, particularly those reared under intensive conditions (Noordhuizen and Schukken, 1996).

Important zoonozes

Tuberculosis in cattle and its importance as a disease in some human populations represents one of the principal reasons for the introduction of direct inspection methods for meat control and the pasteurization of dairy products. As a clinical entity in cattle, this disease has now been almost completely eradicated in most developed countries. Isolated incidences in which there is direct involvement of infected cattle as the source of *Mycobacterium bovis* infection in humans still occur (Hensel and Neubauer, 2002). One of the beneficial effects of national bovine tuberculosis eradication programs has been the effective removal of most infected cattle before they reach the clinical stages of the disease and before the major organs show overt signs of involvement. Animals which show a high responsiveness to bovine tuberculin are the animals most likely to display gross lesions at slaughter. This demonstrates that the tuberculin test is an effective screening test and it may, therefore, provide a basis for the strategic removal of reactors at local or regional level in countries in which financial constraints prevent the implementation of a national eradication program for this disease. Meanwhile, every effort should be made to prevent the entry of such infection into herds of cattle and, in some regions, sheep flocks and herds of goats and deer as well, through the unwitting purchase of infected stock or contact with neighboring infected herds or flocks and wildlife affected with tuberculosis. Segregation of stock of differing ages, effective composting of manure, attention to sanitary issues, including disinfection and a prudent culling program have significant importance in maintaining a tuberculosis-free herd or flock, particularly in regions in which the prevalence of tuberculosis in the animal population is a concern.

*Mycobacterium avium paratuberculosis (*MAP) is the causative agent of paratuberculosis (Johne's disease), a production disease in ruminants, and a hypothesis exists that MAP may be involved in the etiology to Crohn's disease, a chronic inflammatory bowel disease in humans. The hypothesis is not proven, but the general principle that diseased animals should not be used as a source of food for humans should be accepted. However, control to John’s disease in dairy herds is difficult and based on two principles, namely, the identification and elimination of infected animals and the prevention of new infections. Essential to this process is a clear understanding of above mentioned principals from the herd owner. These include next measures: determining the prevalence of the disease in the herd; culling clinical and subclinical cases identified by the available diagnostic tests; reducing the contact between newborn calves and their dams, where the status of the dam is unknown; managing the farm and its environment to ensure that stock do not graze on contaminated pasture; strictly assessing the status of bought-in stock based on serological testing.

Verocytotoxogenic *Escherichia coli (*VTEC) is present in the gut and faces of healthy cattle and sheep and these animals therefore act as reservoirs from which humans can become infected. Livestock is a reservoir for most VTEC, with cattle being the principal source of *E. coli* O157:H7 and other VTEC. Studies from the United Kingdom (UK) and the USA have shown that VTEC is, at least occasionally, present on most farms. The organism can survive in soil for several months (Hensel and Neubauer, 2002). The effects of animal husbandry practices on the prevalence of VTEC in animals are to a considerable extent unknown. Moreover, no defined carrier has been identified which can be targeted for an intervention program. Therefore, eradication of VTEC from the farm livestock or farm environment does not seem to be a reasonable goal.

Brucellosis is an infectious disease of man and animals, mainly cattle, caused by the bacteria, *Brucella spp.* Infection is acquired from infected females during or after abortion, from contaminated milk, sexually transmitted or acquired from a contaminated environment. Infected animals harbor the bacterium in their lymph nodes, udder and uterus, and in the case of bulls, in the male genitalia and related lymph nodes. The bacterium survives for upwards of six months in contaminated slurry and can be spread from farm to farm, and can infect people, during spreading.

Above mentioned disease is or has been the subject of an eradication program in many countries. Control programs can use two principal methods, namely, vaccination and the removal for slaughter of infected and exposed animals based on serological testing. These measures are supported by movement controls and quarantine on infected farms (Noordhuizen and Collins, 2002).

If infected the cows are not identified early, and are not removed immediately, they are a source of infection for other animals and people, either through direct or indirect exposure. Furthermore, farmers and their families, and farm workers, can be exposed to infection by drinking raw milk. Pasteurization makes dairy products safe, but does not deal with the issue of the direct or indirect contact routes of transmission to humans.

Conclusion

Zoonotic hazards that are associated with food animal production and food processing may arise on the farm, in the food plant and in the distribution chain. Awareness on the part of food animal producers and the members of the food industry that such hazards may exist in foods is the first step towards their control. Good hygiene practice throughout processing and, finally, cooking, provides further safeguards for the consumer. The measures currently applied to prevent or control the transmission of zoonotic agents via the food chain in the post-harvest phases are unlikely to be successful in isolation. Effective control relies upon the implementation of a consistently high level of production hygiene at all stages of production both on the farm and in the processing plant, and throughout the remainder of the food chain. In respect to this the role of education remains paramount for all stakeholders at every level of the food industry.

References

1. Abury-Damon H, Grent K, Sall-Ndiaye P, Che D, Cordeiro E, Bougnoux M-E, Riguad E, Le Strat Y, Lemanissier V, Armand-Lefevre L, Delzescaux D, Desenclos J-C, Lienard M. and Andermont A 2004. Antimicrobial resistance in commensal flora of pig farmers. Emerging infectious Diseases 10 (5), 873-879.

2. Brand A, Noordhuizen JPTM and Schukken YH 1996. Herd health and production management in dairy practice. Wageningen Academic Publishers, Wageningen, 543 pp.
3. Commission of the European Communities (CEC) 2000. White paper on food safety. COM 1999. 719 final. CEC, Brussels, 52 pp.
4. Hensel A and Neubauer H 2002. Human pathogens associated with on-farm practices - implications for control and surveillance strategies. In Food safety assurance and veterinary public health, Vol. 1. Food safety assurance in the pre-harvest phase (FJM. Smudlers and JD Collins, Eds). Wageningen Academic Publishers, Wageningen, 125-139.
5. Noordhuizen JPTM and Welpelo HJ 1996. Sustainable improvement of animal health care by systematic quality risk management according to the HACCP concept, Veterinary quarterly 18 (4), 121-126.
6. Noordhuizen JPTM and Collins JD 2002. Pre-harvest health and quality monitoring, risk assessment and their relevance to the food chain. In Food safety assurance and veterinary public health, Vol. 1. Food safety assurance in the pre-harvest phase (FJM Smulders and JD Collins, Eds). Wageningen Academic Publishers, Wageningen, 115-124.
7. World Trade Organization (WTO) 1995. Agreement on the application of sanitary and phytosanitary measures. WorldTrade Organization, Geneva.

Invited paper

MANAGEMENT MEASURES IN BROWN HARE POPULATION IN VARIOUS HABITATS IN SERBIA

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Abstract

Brown hare in Serbia lives in various habitats although the best brown hare habitats are lowland areas and river valleys. Brown hare has successfully adapted to agroecosystems in agricultural areas. In the last two decades in Serbia a number of studies was conducted associated with the analysis of themanagement measures in brown hare population, which recorded some cyclic oscillations in population abundance, density,age structure and exploitationrate. These measures are key elements in management of wildlife population so their implementation should be enforced. Problems related to brown hare population management in Serbia arise because there are neither proper guidelines to determine hare hunting productive areas nor how to define the solvency and capacities of hunting grounds that are not adapted to newly created habitat conditions in certain hunting grounds. Methodology of conducting and defining the number of hare population in various types of hunting grounds does not match given habitat conditions. Taking into account various habitat conditions in hunting grounds of Vojvodina and central Serbia it is very important to define in a correct manner population spring abundance, population density, age structure and exploitation rate for the purpose of sustainable management in hare population.

Key words: *brown hare, management, Serbia*

Introduction

Brown hare (Lepuseuropaeus Pall.) is one of the most widespread and most hunted game species in Europe. It inhabits many various habitats but lowland regions suit him the best (Jennings, 2006; Beukovićet al., 2007). As an original game of open steppe grasslands hare has, during a long time period and due to intensive development of agriculture adapted to agroecosystem. The most abundant hare population can be found in agricultural areas. In Serbia the best hare habitats are lowland areas, primarily in Vojvodina. By intensifying the agriculture typical hare habitats have changed, primarily due to drastic increase of crop farming and intensive use of pesticides and simultaneous reduction in the areas covered with green fodder (BeukovićandMarinković, 1997.).

Despite expressed fertility, in a previous period a significant decline in hare population in Europe and Serbia was recorded what can be explained by drastically changed living conditions (Beukovićet al., 2009a; Popović et al., 1996). Intensive agriculture changed typical hare habitats particularly due to increased crop farming and intensive use of

pesticides as well as decline in areas covered with green fodder (Beukovićet al., 2011a; Đorđevićet al., 2008, 2009, 2010; Laslo, 1996; Popović and Đorđević, 2010). For that reason the abundance of hare population depends mostly on its possibility to adapt to such changes (Katonaet al., 2010). In such changed conditions it is possible to act in two ways - first to protect the hare population by complete prohibition or reduction of hunting, and the second way would be to conduct the process of reintroduction of animals from other hunting grounds or in other words to reintroduce artificially raised animals.

With the intensification of agriculture, predation is the most mentioned cause for hare declines (Schmidt et al., 2004; Panek et al., 2006). In Slovakia in the last 40 years the number of foxes has doubled. In 1970 there were 9900 shot foxes and since 2000 there have been on average 18688 individuals shot annually. In comparison with hare shooting, the fox shooting has been stable since 2002 but the hare shooting has gradually declined so that since 2010 more foxes have been shot compared to hares (Slamečka et al., 2013).

In order to improve management and protect hare population it is necessary to increase their number in hunting grounds. Therefore all factors that can affect hare population should be identified and the possibility to remove or reduce the impact of those factors considered (Popovićet al., 2008; Andrašić, 1971; Vasović, 1971). The effect of climate factors in reproduction period affects the juvenile hare ratio in hare population (Popović et al., 1997; Beuković et al., 2009c; Beuković et al., 2013c). Since we cannot influence certain factors the engagement should be directed to those factors which can be directly or indirectly influenced upon by farmers. Improved results regarding hare population management can be achieved also by improved way of management, or by shifting corresponding parameters (growth rate, losses) within acceptable biological limits (Perišićet al., 2009).

The biggest impact on the number of wildlife population in hunting grounds is exerted by the users of hunting grounds through utilization of populations of game species-i.e. hunting. Population exploitation rate has to correspond to the size of registered game fund and has to follow its oscillations (Popović et al., 2006, 2008, 2011, and 2012). Rational use of hare populations and micropopulations is one of the most important modes of hare protection in impaired ecological conditions and it is completely and exclusively in the hands of the hunting grounds users (Beukovićet al., 2009b, 2011b, 2013b, Popović et al., 1996), while the exploitation rate should be completely controlledby hunting ground users (Beukovićet al., 2000). There is no unique rate of game utilization in different regions or hunting grounds since it varies from one year to another and should depend upon hare population registered fund, growth rate, losses during reproduction, as well as planned winter losses which may vary within certain regions (Popović et al., 2013).

Management measures in thehunting groundsof Serbia

Management in the hunting grounds of Serbia is carried out in accordance with the Law on Game and Hunting (2010), which obliges the users of hunting grounds to develop a 10-year hunting management plan, adopt it, and obtain the approval of the competent Ministry of Agriculture, Forestry and Environmental Protection for it. Apart from the hunting management plan for the current hunting year a hunting ground user must develop an annual hunting management plan. Programme for the development of hunting areas has not been passed yet by the competent ministry and the draft hunting development strategy in the Republic of Serbia (2014) is being compiled.

For conducting the hare population management, the hunting ground user in his hunting management plan must specify the following: hare hunting productive area within the hunting ground, site class of hunting ground for hare hunting and optimal stock of hare in the hunting ground.

According to the Law on Game and Hunting (2010), hare hunting productive areas within the hunting ground are determined by persons licensed for the preparation of planning documents. There are no more specific guidelines for the participants in the project, but there is great difference in % of hare hunting productive area even between similar hunting grounds.

Site class of hunting ground for hare hunting, as an indicator of the necessary complex living conditions for certain game species in Serbia, is determined according to the guidelines of Tomašević et al. (1997).

Optimal stock of game, as optimum population size for certain species of game which should be present in the hunting ground,which is established on the basis of the assessment of the hunting grounds in Serbia, is also determined according to Tomašević et al. (1997). Recommended norms for number of individuals per unit of hunting productive area from these guidelines of Tomašević et al. (1997) are now questionable in the altered habitat conditions.

Determination of the population abundance is carried out in the lowland hunting grounds using method of sample plots. Selected plots are representative of a hunting ground, and the counting is done at 3-4 plots accounting for at least 10-12% of the total hunting ground. In the hilly hunting grounds method of "transects" or lines is applied, with rectangular sample plots 100-300 meters wide and 2000 to 5000 meters long. Counting of game by this method is incomplete as part of the game remains undetected because of the vegetation and terrain configuration. Abundance of hare population in mountain hunting grounds is usually established by identifying tracks and scats of hares, which is quite unreliable method.

Density is the most important element of a population. The number of individuals of a species living per unit of an area (habitat) at certain period of time is the result of various internal and external relationships of species and influence of many abiotic and biotic factors. Therefore the density is an indicator of the overall state of the population. Determination of the density (abundance) of hare populations and their parts at certain periods of the year is of great importance for the planning of harvest. There are two types of density: spring density – at the end of winter and before the start of the breeding season and autumn density – after completion of the breeding season and before the hunting season. Both types of density are important for monitoring population dynamics. Spring density shows how the game survived over winter and the reproductive potential while autumn density shows the reproductive success and what portion of the population may be used. The density of the breeding stock of hare in the Republic of Serbia without provinces in 2012 was 50.76 animals, while in the autonomous province of Vojvodina it was 142.24 per 1000 ha (Hunting Development Strategy of the Republic of Serbia – Draft 2014).

Exploitation rate of a hare population is the most important factor in hunting, as the proper setting of the annual catch limits for hareis significant for the rational exploitation and conservation of the hare population. The utilization rate is under the direct influence of man, and if not applied properly it can cause major damage under all habitat conditions. Determination of the utilization rate was carried out in hunting managementmost often based on the density (abundance) and the actualgrowthrate taken for the purpose of assessing the site class of habitat, which was pre-assessed and graded. Recent research has

shown that the actual growth rate is not positivelycorrelated with the density and that the statistical norms of exploitation based on the site class of habitat are unviable. Actual growth rate varies from year to year and is different for the two neighbouring populations of similar environmental conditions. Realisedhare game bag recordsin hunting year 2012/2013 in relation to breeding stock in the Republic of Serbia without provinces was 11.43 animals, while in the autonomous province of Vojvodinait was 9.43 per 1000ha (Hunting Development Strategy of the Republic of Serbia – Draft 2014).

Determining the age structure of the hare population at the end of the reproductive period is an important indicator of the growth rate and as such it is used in planning the hare exploitation rateona hunting ground. In nature, only 3% of hares survive the age limit of 4 years. Majority of hares do not survive until the hunting season. Yet the leveretsaccount for 50-75% of the autumn population, while theadultsaccount for 25-50%, mainly 1-2 year-old animals. The most reliable method for determining the age of hares is based on the weight of the eye lens. The method is based on the fact that the eye lens grows throughout life (Lord, 1959; and Rieck, 1962). In Vojvodina, this method has been applied for more than 40 years, where the eyes of hunted animals (one eye of each animal) are collected from all hunting grounds users in Vojvodina during the hunting season, followed by treatment of the samplesaccording to a pre-established procedure. Determination of the age structure is not used in Central Serbia or they use it in some hunting grounds on their own initiative used, but without organized collection by hunting organizations. However, recent attempts by Popović et al. (2013) suggest the importance of this measure in the hare management in the hunting grounds of Central Serbia.

Reintroduction of hares into the hunting grounds is carried out in order to increase their breeding stock. Apart from increasing the abundance,thehunting ground users have the view that "bloodrefreshing" will influencethe abundance and quality of the population. Consideringthe fact that a number of environmental factors influence the population and reduction in its abundance and the overall decline, it is necessary to determine the causes and assess the effect of "bloodrefreshing", because otherwise this measure will not give the desired effect.

In Serbia in 2008, 2.20% of the total hare population exploitation was realisedby catching and selling (Popović et al., 2012). During 2011,hares were reintroduced into seven hunting grounds in central Serbia, bringing 709 animals from hunting grounds in Vojvodina. In relation to the total hare abundance in Vojvodina in 2011 amounting to 272,760 animals (Beuković et al., 2012), only 0.26% of the total stock, or 1.94% of the total game bag record, was caught.

Hare population management in lowland habitats of the Autonomous Province of Vojvodina

Observing the period 1989-2008, an average spring hareabundance in Vojvodina amounted to 258,454 animals, the average population density was 13.02 individuals per 100 ha, and the average annual hare game bag record was 41,850 animals. Exploitation rate of the hare population in the above period was 16.23% on the average (Beuković et al., 2009b). Number of hare eyes submitted for examination to the Hunting Association of Vojvodina during the said period amounted to 159,040, i.e., an average of 7,952 per year. The average percentage of young hares in the observed period was 60%, which is very good, and during the observed twelve years (1989-2008) the hunting ground userswere proposed hunting prohibitionin 6.30% of hunting districts each year onaverage, and correction of game bag record plan towards its reduction in 23.72% of hunting districts, while in 54.49% cases

they hunted according to the plan (Beuković et al., 2009b). Based on the SerbianHunting Development Programme for 2000 - 2010 (Group of authors, 2001), in 2009 it was planned to achieve the hare abundance in Vojvodinaof 324,518 animals, and the realization of the plan was 85.02%.

Harebreeding stock in the Autonomous Province of Vojvodinain 2012 was 285,200 animals, with realized game bag record of 27,740 which accounted for 9.73% of the breedingstock (Hunting Development Strategy of the Republic of Serbia - Draft 2014).

Observing certain areas in Vojvodinasuch as Tisa part of Bačka, Beuković et al. (2009a) in his study of the hare population dynamics and exploitationrate pointed out that the abundance of hare population in the hunting grounds of Tisa part of Bačka in the period 2002-2007 increased from 39,371 to 47,398 animals, which is an increase of 20.38%. Population density substantially followed the abundance and increased from 20:09 to 24.23 animalsper 100 ha (Beuković et al., 2009a). Density of haresin Vojvodinain the period 2000-2009 ranged from 12.62 to 15.16 animals per 100 ha (Popović et al., 2012). In Tisa part of Bačka, depending on the year, density of hares ranged from 20.09 to 25.6 animals per 100 ha. In some hunting grounds the attained density was close to 30 animals, and in BačkoPetrovoSeloit was 41.6 animals per 100 ha (Beuković et al., 2007, 2009a, 2011b). When it comes to the exploitation rate of hare populations in the observed period in this region, the same authors suggest that it increased from 0.10 to 0.15. In 2012, the density of the harebreeding stock was 142.24 individuals per 1000ha (Hunting Development Strategy of the Republic of Serbia - Draft 2014).

Based on the research of hare population management in Vojvodina for the period 2003-2012 Beuković et al. (2013) noted that in 2012 the highest percentage of leverets was reported in the area of Srem with 51.75%, followed by Banat with 42.07%, while the lowest percentage of young hares was recorded in Bačkawith 39.76%. Ten-year average (2003-2012) for the percentage of young hares was 53%, which can be assessed as "good". Year with the lowest average percentage of young hares in Vojvodinain the observed ten-year average is 2010 with only 38%, which is rated as "weak", while the highest percentage of leverets was recorded in 2005 with 62% and it is rated as "very good" (Beuković et al., 2013). When it comes to spring hare population abundance in Vojvodina in the period 2003-2012, it was recorded as cyclicwith the average abundance of 278,379 individuals, andthe average annual game bag record of 42,501 animals (Beuković et al., 2013). Also, the same authors pointed out that game bag record in the observed year did nothave statistically significant effect on the population abundance in the next year.

Hare population management in hilly-mountainous habitats of the Republic of Serbia without provinces

Hare abundance in the Republic of Serbia without provinces in 1991 amounted to 311,210, with hare bag record of 25.31% (Popović et al., 1996). In 1998, the hare abundancewas 322,538, while in 2005, the hareabundance increased by 5.4% compared to 1998. The percentage of game bag record in 2000 was 16.64%, while in 2005 it was 20.34% (Popović et al., 2008). In Serbia,hareharvest record in the period 1980-2000 showed a linear declining trend by 1.7% annually, from 8 to 29.4% (average 24.5%) of the breeding stock harvest rate (Ranković and Popović, 2002). In the period from 2000 to 2009,the exploitation rate of hare population in Serbia ranged from 14.26% to 18.02%. Exploitation rate of the hare population in 2008 in Serbia by game bags amounted to 97.80% of the total population use. Tourist hunters participated in this with only 3.95% and domestic hunters with 93.85% (Popović et al., 2012), which is significantly smaller exploitation rate of the

hare population than in the period 1991-1993 when in Serbia this percentage ranged from 21.23% to 25.61 (Popovićet al., 1996).

Hare density in central Serbia in the period 2000-2009 ranged from 6.16 to 6.97 animals per 100 ha, while the average hare density in the Republic of Serbia ranged from 8.17 to 9.10 animals per 100 ha (Popović et al., 2012). Population density in northwest Croatia in hunting season 2004/2005 ranged between 13 and 20.3 animals per 100 hectares of hunting grounds (Pintur et al., 2006), while in Bulgaria, in habitats at up to 600 m above sea level, the density was 1.8 individuals, and only in 5% of the hunting grounds the density was 5 to 8 individuals (Zhelev et al., 2013).

Based on the Hunting Development Strategy of the Republic of Serbia - Draft (2014),hare breeding stock in the Republic of Serbia without provinces in 2012 amounted to 283 760 individuals, which is 79.56% of the optimal fund, with the density of the breedingstock of 50.76 animals per 1000ha and realized game bag record of 11.43% of the breeding stock.

In the research during 2011-2012 in 34 hunting grounds of central Serbia on 862.579 ha, Popović et al., (2013) suggested that the optimal stock of hare is 9.48 animals per 100 ha. Depending on the area, the optimal stock ranges from 8.05 to 13.95 animals per 100 ha of hunting grounds, and breeding stock varies from 6.16 to 12.01 per 100 ha.

Hare breeding stock in the investigated hunting grounds in 2011 was 82.81% compared to the optimal stock that wasprovided for in the hunting management plan for thehunting ground. Haregame bag record varied depending on the area, from 0.76 inDrina-Kolubara area to 1.67 animals per 100 ha in Šumadija area. Haregame bag record in the investigated hunting grounds was 15.54% compared to the breedingstock in 2011. By observing the hareabundance and game bag record in Serbia in different periods it was found that there was a mismatch, i.e. that the increase in population abundance or its reduction was not accompanied by coordinated game bag record (Popović et al., 1996, 2012). Comparing the exploitation rate of hare population in our country with the research on the exploitation rate of hare population in Hungary (Laszlo, 1996; Katona, 2010) amounting to an average of 27.4%, this is a very low exploitation rate.

Based on the analysis of the age structure of the population,there has been established that the exploitation rate of the hare population on the basis of the annual hunting management plan would lead to the reduction of the breeding stock in 66.66% hunting grounds. Forestation of hunting areas did not show statistical significance for the proportion of leverets in the population in the hunting grounds of Central Serbia (Popović et al., 2013).

In 2011, hares were reintroduced into seven hunting grounds in central Serbia, with 709 animals. However, 44 to 249 individuals were introduced into the hunting grounds, or 1.1% to 15.56% compared to the optimal stock of hare for the given hunting ground, which is not enough. The reasons for the reduced introduction of haresinto hunting grounds were the prices of live hares, weakened economic power of hunting ground users, as well as the long period forobtaining approval for the introduction of hares in the hunting ground by the competent ministry, unless it was envisaged in the long term plan at adopting the hunting management plan (Popović et al. 2013).

Since 2011 the campaign of oral vaccination of foxes against rabies in Serbia was initiated. Increasing the abundance of this predator of hareas well as other species of beasts in hunting grounds will affect the abundance of hare in the forthcoming period. A particular problem in the regulation of abundance of foxes and jackals is also the obligatory marking of the shot game, their delivery to specific places in the hunting ground, processing and

evaluation of their trophies, which will affect the indifference of hunters to hunt these predators and their further expansion.

Conclusion

In 2012, hare breeding stock in the Republic of Serbia without provinces amounted to 283,760 individuals, with density of breeding stock of 50.76 animals per 1,000 ha and realized game bag record of 11.43% of the breeding stock.

Harebreeding stock in the Autonomous Province of Vojvodina for 2012 was 285,200 individuals, with realized game bag record of 27,740 which is 9.73% of the breeding stock, while the density of the harebreeding stock per 1000ha was 142.24 animals.

Compared to the year 2000, hare abundance in the Republic of Serbia was reduced; particularly hareabundance and density in Serbia without provinces.

In order to prevent further hare population decline in the Republic of Serbia certain measures must be taken in accordance with relevant habitat conditions in certain hunting grounds:

- Establish hare hunting productive areas, determination of site class and capacity of the hunting ground for this type of game must be carried out by new, established and prescribed criteria and in accordance with the new changes in the habitat conditions in the hunting grounds of Serbia.
- Exercise determinationand control of population abundance:
  - Implementation of adequate methods for determining the abundance.
  - Proper identification of the experimental plots, which were selected according to the site classes of the hunting ground and surface structure of a given hunting ground.
  - Counting control.
  - Transition to other counting methods that would be carried out by hunting professionals.
- It is necessary to regularly conductcontrol of investigation of the age structure of the hare population based on the weight of dried eye lenses from early autumn hunts and provide recommendations on population exploitation as not to jeopardize the hare breedingstock in the given hunting ground. In the hunting grounds in the area of Vojvodina this investigation is carried out regularly, while it is not practiced in other hunting grounds.
- Percentage of introduction of hares in hunting grounds in Serbia is low and it is necessary to introduce hare into hunting grounds with hareabundance below 50% of the optimal stock, but with the previous analysis of the reasons forthe decline and elimination of the causes.
- Reducing losses in the hare population by control of predator abundancein hunting grounds, as well as the prevention of poisoning hares by pesticideuse control.
- Education of hunting staff regarding the hare population management in hunting grounds, with particular stressing the importance of sampling of eyes in the first days of autumn hare hunting as well as education of hunters and population in order to reduce losses in this type of game.

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References

1. Andrašić D 1971. Zaštita protiv šteta na divljači od poplava. Simpozijum o lovstvu, Beograd. Zbornik radova, 9-12.
2. Beuković M, Popović Z, Maletić V, Beuković D, Đoković D 2009c. Влијание на климатските фактори врз процентот на младите во популацијата на зајакот (*Lepus Europaeus* Pall.) во Војводина. IV International Symposium of Livestock Production, Struga, Macedonia, 09-12.09.2009., Proceedings, 232.
3. Beuković M, Đorđević N, Popović Z, Beuković D, Đorđević M 2011a. "Nutrition specificity of brown hare (*Lepus Europaeus*) as a cause of the decreased number of population". Contemporary Agriculture 3-4, 403-413.
4. Beuković M, Popović Z, Beuković D 2013a. Sustainable management in hare populations of Vojvodina during the 2012, year along with a review of the last ten years. Second international symposium on hunting "Modern aspect of sustainable management of game" 17-20.10.2013. Novi Sad, Serbia. Proceedings 29-38.
5. Beuković M, Beuković D, Đorđević N, Popović Z, Đorđević M 2011b. Management of micro-population brown hare (*Lepus europaeus Paal*.) in hunting grounds Potiska Bačka. 22nd International symposium «Safe food production», Trebinje, Bosnia and Hercegovina, 19-25 June, 2011. Proceedings, 16-18.
6. Beuković M, Beuković D, Popović Z, Đorđević N, Đorđević M 2013b. Harvesting mass of brown hare in Vojvodina during seasone 2012. 23 th International symposium "New Technologies in Contemporary Animal production". Proceedings, 294-296.
7. Beuković M, Beuković D, Popović Z, Đorđević N, Đorđević M 2013c. Impact of climatic factors to the percentage of young in the population of brown hare (*Lepus Europaeus* P.) in the Bačka district. Acta Veterinaria 63, 1, 111-122.
8. Beuković M, Beuković D, Popović Z, Perišić P 2009a. Number and usage level dynamics of population brown hare (*Lepus Europaeus*) round of river Tisa in Bačka district. Zbornik naučnih radova Instituta PKB Agroekonomik 15(3-4), 173-179.
9. Beuković M, Bošnjak B, Popović Z 2007.Dinamika populacije zeca u Bačkoj i stepen korišćenja. Simpozijum «Veterinarska medicina, stočarstvo i ekonomika u proizvodnji zdravstveno bezbedne hrane«, Herceg Novi 24. jun – 1.jul 2007. Zbornik sažetaka, str. 111.
10. Beuković M, Marinković B 1997.Effect of changes in Vojvodina agrosistemu on the number of Polish partridge (*Perdix perdix*) and hare (*Lepuseuropaeus*). in: Symposium: Rabbit and partridge in modern agrosistemima, Novi Sad, proceedings, 114-126.
11. Beuković M, Popović Z, Beuković D, Đaković D, Đorđević N 2009b. Management of the mega-population brown hare (*Lepus Europaeus* P.) in Vojvodina. Simpozijum Stočarstvo, veterinarstvo i agroekonomija u tranzicionim procesim, Herceg Novi, 98.
12. Beuković M, Šelmić V, Jović D, Vapa M, Puzović M, Pantelić A, Bognar M, Novkov M, Đaković D, Zeremski M 2000. Dugoročni program razvoja lovstva Vojvodine 2000-2010. godine. Novi Sad.
13. Đorđević N, Grubić G, Popović Z, Stojanović B, Božičković A 2009. Production of feeds and additional feeding of game as a measure of forest and wildlife protection. XIII International Feed Technology Symposium, September, 29th - October, 1th, 2009, Novi Sad. Proceedings, 211-216.

14. Đorđević N, Popović Z, Grubić G, Beuković M 2008. Ishrambeni potencijal lovišta Srbije. XVIII inovacije u stočarstvu, 27-28.11.2008., Poljoprivredni fakultet Zemun. Biotehnologija u stočarstvu, 24 (poseban broj), 529-537.

15. Đorđević N, Popović Z, Grubić G, Beuković M 2010. Uticaj prirodnih izvora hrane i zimskog prihranjivanja na gubitke divljači. 15. Savetovanje o Biotehnologiji, Agronomski Fakultet, Čačak, 26-27. Mart, 2010. Zbornik radova 15, 17, 529-534.

16. Grupa autora (rukovodilac i redaktor Šelmić, V.) (2001):Program razvoja lovstva Srbije 2001-2010. Lovački savez Srbije, Beograd, pp. 1-241.

17. Jennings NV, Smith RK, Hackländer K, Harris S, White PCL 2006. Variation in demography, condition, and dietary quality of hares (*Lepus europaeus*) from high-density and low-density populations. Wildlife biology 12, 2, 179-190.

18. Katona K, Biro Zs, Szemethy L, Demes T, Nyeste M 2010. Spatial, temporal and individual variability in the autumn diet of european hare (*Lepus europaeus*.) in Hungary. Acta zoologica academiae scientiarum Hungaricae 56, 89-101.

19. Laslo K 1996. Razmatranje činjenica koje utiču na gustinu populacije poljskih zečeva na intenzivnom poljoprivrednom području. Savetovanje u Prokuplju i Kikindi 1995., Lovački savez Jugoslavije, Beograd. Zbornik radova, str. 149-153.

20. Lord RD 1959. The lens as an indicator age in cottontal rabbits. J. Wildl. Manage,

21. Panek M, Kamieniarz R, Bresinski W 2006. The experimental removal of red foxes Vulpes vulpes on spring density of brown hares Lepus europaeus in Western Poland. Acta Theriologica 51(2), 187-19

22. Perišić P, Popović Z, Đorđević N, Živković D 2009. Faktori koji utiču na ekonomske efekte gazdovanja važnijim vrstama lovne divljači. Međunarodno savetovanje o lovstvu. Žagubica, april 2009. Zbornik radova, 48-59.

23. Pintur K, Popović N, Alegro A, Severin K, Slavica A, Kolić E 2006. Selected indicators of Brown hare (*Lepus Europaeus* Pallas, 1778) population dynamics in northwestern Croatia. Vet. arhiv 76, 199-209.

24. Popović Z, Beuković M, Milošević G 2013. Analysis of the state of the hare population in the hunting grounds of central Serbia. Second international symposium on hunting "Modern aspect of sustainable management of game" 17-20.10.2013. Novi Sad, Serbia. Proceedings 39-47.

25. Popović Z, Beuković M, Đorđević N 2008. Brojnost i stepen korišćenja populacija divljači u lovištima lovačkog saveza Srbije. XVIII inovacije u stočarstvu, 27-28.11.2008., Poljoprivredni fakultet Zemun. Biotehnologija u stočarstvu 24 (poseban broj), 11-23.

26. Popović Z, Beuković M, Đorđević N 2012. Management brown hare (*Lepus Europaeus* Pall.) population in serbia. International symposium on hunting "Modern aspects of sustainable management of game population". Proceedings, 1-6.

27. Popović Z, Bogdanović V, Gajić I 1996. Analiza promene brojnosti zeca u našoj zemlji. Savetovanje u Prokuplju i Kikindi 1995. godine, Lovački savez Jugoslavije. Zbornik radova, str. 121-132.

28. Popović Z, Bogdanović V, Gajić I 1997. The influence of climatic factors on the participation of young in the hare's population (*Lepus europaeus* Pall.) Ekologija 32(1) 139-144. Beograd.

29. Popović Z, Đorđević N 2010. Gazdovanje populacijama divljači u cilju smanjenje štete. Univerzitet u Beogradu, Poljoprivredni fakultet.

30. Popović Z, Đorđević N, Živković D, Beuković M, Beuković D, Đorđević M 2011. Brojnost i stepen korišćenja populacije zeca i fazana u lovištima Lovačkog saveza Srbije. 16.

međunarodno naučno-stručno savjetovanje agronoma Republike Srpske „Prirodni resursi u funkciji razvoja poljoprivrede i ruralnog područja“, Trebinje, 22-25.03.2011. Zbornik sažetaka, 92.

31. Ranković N, Popović Z 2002. Dinamika odstrela i vrednost izlova nekih vrsta divljači u Srbiji. «Međunarodni simpozijum Stočarstvo i agroekonomija u procesima tranzicije«, Novi Sad. Savremena poljoprivreda 3-4, 195-198.
32. Rieck W 1962. Analyse von Feldhasenstrecken nach Gewicht der Augenlinse. Suppl. Recerche di Zoologie appl. alla Caccia, Vol. IV Bologna
33. Schmidt NM, Asferg T, Forchhammer M 2004. Long-term patterns in European brown hare population dynamics in Denmark: effects of agriculture, predation and climate. BMC Ecology 4, 15.
34. Slamečka J, Sládeček T, Gašparík J, Jurčík R, Paule L 2013. Actual status and development of european brown hare (LEPUS EUROPAEUS) population in Slovakia. Second international symposium on hunting "Modern aspect of sustainable management of game" 17-20.10.2013. Novi Sad, Serbia. Proceedings 48-53.
35. Strategija razvoja lovstva republike Srbije - nacrt (2014). Ministarstvo poljoprivrede I zaštite životne sredine – uprava za šume.
36. Tomašević B, Radosavljević L, Ćernanić A 1997. Bonitiranje lovišta. Lovačka biblioteka Sv. Evstatije, Beograd. st. 1-146.
37. Vasović S 1971. Zaštita divljači od čoveka. Simpozijum o lovstvu, Beograd. Zbornik radova, 27-37.
38. Zakonu o divljači i lovstvu (2010). Službeni glasnik RS br. 18/2010.
39. Zhelev Ch, Ninov N, Mihaylov H, Gruychev G, Stoyanov S, Mirchev R 2013. Density of brown hare (LEPUS EUROPAEUS PALLAS, 1778) in the plain habitats of Bulgaria. Second international symposium on hunting "Modern aspect of sustainable management of game" 17-20.10.2013. Novi Sad, Serbia. Proceedings 54-59.

Review paper

CENTER FOR FISHERY AND APPLIED HYDROBIOLOGY "LITTLE DANUBE", EXPERIMENTAL SCHOOL ESTATE "RADMILOVAC": SYNERGY OF RESEARCH, ECONOMY AND SOCIETY

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Abstract

In every society, initiators and promoters of the development are individuals, teams or institutions with a vision, good ideas, seriousness, but above enthusiasm to work hard. They trace the road and drive activities related to research and/or economy. In the Serbian aquaculture sector such institutions is the Faculty of Agriculture and its Center for Fishery and Applied Hydrobiology (CEFAH) (or the "Little Danube"), which operates within the Experimental school estate "Radmilovac". The Center started to be established in 2007. Unlike other similar Centers built by state resources intended for research and scientific work, the "Little Danube" was created much more as a result of enthusiasm of a group of researchers and their readiness to cooperate with national and international institutions, but also by enthusiasm of individuals ready to financially or materially help some of the Center's programs. The Center's activities are directed towards improving and developing the research and economy in aquaculture. Today the Center in "Radmilovac" and its laboratories located in the building of the Faculty of Agriculture represent an education and research polygon for aquaculture development. The education part is aimed at all levels: from pre-school and elementary school level by educational visits to the "Little Danube", where they get to know different fish species and other aquatic organisms, their culture, fishing, fishing tools; to PhD level students and researchers that increase their knowledge and skills in the Center. CEFAH is a scientific polygon: in its aquaria, tanks, RAS systems, as well as in a number of earthen ponds a range of various experiments in different areas of aquaculture are carried out and knowledge is transferred directly in aquaculture economy. The development of Serbian aquaculture is directly affected by the connection of the Center with the economy through realization of biotechnology projects, fish selective breeding programs, and experiments.

Key words: *aquaculture, CEFAH, economy, research, Serbia*

Introduction

Besides teaching and research, a duty of teachers and teaching assistants working in applied sciences is to contribute to the development of the corresponding economic sector. By linking research and economy a way is paved from faculties and institutes towards reinforced economy.

On the other hand, prerequisite as material basis for research are made: fruitful interaction between research and economy leads to the improvement of both research and economy by creating symbiotic relation between the two.

Bearing this in mind teachers and researchers of the Fishery research group from the University of Belgrade, Faculty of Agriculture, in 2006 started with the creation of the Center for Fishery and Applied Hydrobiology (CEFAH) in order to establish a research and education polygon for aquaculture development, and thus contributing to this agricultural branch reinforcement.

Unlike other similar Centers built by state resources intended for research and scientific institutions, the "Little Danube" was created much more as a result of enthusiasm of a group of researchers and their readiness to cooperate with national and international institutions, but also enthusiasm of individuals ready to financially or materially help some of the Center's programs (Table 1). From the very beginning of its establishment as integral part of research capacities of the Faculty of Agriculture in the area of fishery, CEFAH became a Center for research, dissemination of results , education, and professional activities in the area of fishery and applied hydrobiology, contributing to development of this sector in Serbian economy.

Although in Serbia aquaculture is not one of the main agricultural activities, it is one of the rare animal (cultured and domestic) production type that doubled production in the past 10 years (Marković et al., 2011; Marković and Poleksić, 2011). It should be mentioned that on the global level aquaculture production has been increasing in the past decades (Subasinghe et al., 2009), and aquaculture growth is 8.8% greater than any other food production sector. This is an additional stimulant for efforts to improve Serbian aquaculture.

About the Center

CEFAH occupies a surface area of 13 ha. It consists of upper, middle and lower part linked by the riverbed of the "Little Danube".

The upper Center's part occupies 6 – 7 ha. It was established by creation of 3 wetlands for natural purification of wastewater that arrive on the territory of Radmilovac from the illegal settlement. The built pipeline directs the partly purified water downstream of the school estate. The upper part of the riverbed of the "Little Danube" was created by cleaning and natural setup of the polluted creak " Šugavac" in the area covered by woody and bushy vegetation. Because of the lack of resources and other difficulties this part of the Center is not fully operational; it functions mainly for investigation of the natural process of water purification through wetlands.

Middle part of the "Little Danube" connects the upper and the lower part made from the riverbed of the river Danube model. It is still out of use for the same reasons similar to the upper part.

The main part of the CEFAH is its lower part that occupies approximately 5.5 ha. In this area are located: central building with 2 halls, aquarium building, summer classroom, fisherman's home – fishery museum, a pile-dwelling, a small zoo with aquatic birds, 26 ponds, lower part of the Little Danube with accumulation lake, a peninsula, and the island. The central building consists of: rooms for spawning preparation and spawning, a laboratory, an area with computers for equipment operations monitoring and video surveillance, a room for water preparation (chemical and biological purification), warehouse, washroom, and changing room.

In Hall 1 (surface area of about 90 m^2) there are 60 tanks, and a Zuger egg incubation system with water supply and outlet; in Hall 2 (surface area of about 90 m^2) there are 9 tanks (8 with small RAS, Recirculating Aquaculture System), and 72 experimental aquaria, as well as a system for water supply and water drainage. The aquarium building (surface area 78 m^2) is equipped with a holding construction with 32 aquaria (500L volume each) with filters for water purification and aeration, a cold chamber for cold water fish, a decorative pool and equipment for film projection. CEFAH equipment is modern and includes a robot system for fry feeding, computer system for dissolved oxygen monitoring in the tanks and in experimental fish basins, tagging equipment, RAS systems, video surveillance etc. The water supply for the tanks, aquaria, experimental fish ponds, as well as for the "Little Danube" stream is from springs of 120 – 136m depth. Three deep wells and a system of pumps and pipelines distribute water to each segment of the Center.

CEFAH vision: to use the space of the legacy that will, therefore, always be the property of younger generations, to establish a modern equipped Center for education and research in which new knowledge and skills will be generated and acquired, and to give contribution to development of knowledge, science, economy, better and healthier human society, humanely relationship between people and mankind, and the environment.

Table 1. *List of international and national projects and programs that contributed to establishment and maintenance of the CEFAH*

Project or activity	Types of work	Funds from	Period of realization
Scientific cooperation between Serbia and Norway concerning planning and establishing a genetic improvement program for carp in Serbia, and to transfer knowledge within genetics and selective breeding – a project between Faculty of Agriculture, UB, and AKVAFORSK, Institute of Aquaculture Research, As, Norway	Work on experimental fish farm building, terrain drainage, reconstruction of the central building and Hall 1, equipment purchase	Government of the Kingdom of Norway	2006 – 2007
Building of the summer classroom	Building of the summer classroom	CEFAH team, funds from different sources	2007
Project FP7 "Reinforcement of Sustainable Aquaculture" (ROSA)	Equipment purchase	European Commission	2008
	Reconstruction of Hall 2 and infrastructure building; well drilling and equipment for water supply of the fish farm	Ministry of Science Education and Technological Development of the Republic of Serbia	2008 – 2010
Project "From Šugavac to the Little Danube"	Cleansing of the terrain of the Šugavac creek, installation of pipeline for drainage of the partly purified waste water from the upstream located settlement, naturally shaped riverbed, drilling of 2 wells, and mounting of equipment for pumping and distribution of water for the new riverbed of the Little Danube	Fund for environmental protection of the Republic of Serbia	2011
	Building and equipment of the wooden cabin – public aquarium; building of the fisherman's cabin, purchase and mounting of the plastic pipeline 300 m long, building of two small bridges	Secretariat for environmental protection of the city of Belgrade	2011
	Provision of 765 m concrete tubes Ø 1200 mm, with shipping to "Radmilovac"	Grocka municipality	2010 – 2011
Upgrading of the experimental fish farm	Building of 16 experimental ponds	Donation of particulars	2011
Building of the stone basin in the upper part of the riverbed	Building of the stone basin (surface area 60 m^2)	Donation of particulars	2011

Project "Improvement of production capacities of the carp (Cyprinus carpio) by feeding and selective breeding programs" - TR 31075	Equipment purchase for maintenance of the land area of the farm	Ministry of Science Education and Technological Development of the RS	2012
Project: Final regulation of the educational area "Little Danube"	Building of the outside WC, repair of the summer classroom, classroom equipment	Ministry of Science Education and Technological Development of the RS	2012
Work on upgrading and on arrangement of the Center's area	Building of the small Zoo for aquatic birds, building of the path, fencing of the Center's area, equipment purchase	CEFAH team, funds from different sources	2013
Work on upgrading and on arrangement of the Center's area	Building of a pile-dwelling on the accumulation lake, building and arrangement of small decorative spaces of the Center, building of manholes in the water supply system	CEFAH team, funds from different sources	2014

Education and research programs of the CEFAH are important for the aquaculture economy in Serbia

The Center is an education polygon where new knowledge about fishery waters, plants and animals inhabiting aquatic environment, aquatic organisms' culture, fishery, etc. is acquired. It is intended for all generations: from pre-school and primary school children learning through visit to the Center about different fish species and other aquatic organisms, their culture, fishing methods, fishing gear; to PhD students and scientists generating new knowledge and skills. Particularly important significance of the Center is in the field of practical teaching for students of bachelor, master, specialisation and PhD level. They have a possibility to exercise theoretical lessons learnt and gain practical experience and skills in nearly all fishery activities (spawning, selective breeding, stocking, fish harvesting, feeding, recreational and professional fishery...). By gaining new knowledge and skills students are trained to enter fishery economy sector after completion of studies.

Particular value of the CEFAH is its modern equipment that enables a wide range of research: abiotic and biotic environmental factors in the ponds, in the lower part of the "Little Danube", as well as in experimental, controlled conditions in aquaria and tanks. In the Center, a research is carried out resulting in master, specialization and PhD theses (Poleksić et al., 2013, Marković et al., 2014). It is important to emphasis that due to Center's modern equipment students of different study levels learn the experiment planning, set-up, realization, and results interpretation. This is of upmost importance for their future training for research and in fishery economy.

An important activity of the Center is the realization of experiments in the frame of national and international research projects with the objective of improving the fishery science, and thus the fishery economy. Since inclusion of producers in research projects is increasing in both international and national calls for projects, the results of the CEFAH research projects are quickly transmitted and applied in the economy (Todorčević et al., 2014).

Production doubled in the area where Carp were fed with extruded added (Marković et al., 2012). This is primarily a result obtained by researchers of the CEFAH of the Faculty of

Agriculture. One of the important programs of the Center, developed together with the Center, is the program of selective breeding (Spasić et al., 2010). Its full application in Serbian aquaculture is expected in 4 to 5 years. Besides the carp selection, 3 years ago selective breeding of the rainbow trout started and the results are expected to be applied in 3 to 4 years. An important Activity of the Center is bringing the new knowledge to professionals in the fishery sector through organization of workshops and seminars for fish producers and other users of fishery waters, as well as through individual education visits of producers to the Center. It is worthwhile to mention that the Faculty of Agriculture and the scientists of the CEFAH organize from 2003, every second year, an International Conference that became regionally important and hosted the most important world aquaculture scientists and professionals. That way there is a direct impact on technology improvement on fish farms and on the contribution to aquaculture development in Serbia.

Conclusions

Building, equipping and establishing the Center for Fishery and Applied Hydrobiology (CEFAH) ("Little Danube") on the Experimental School estate "Radmilovac" of the Faculty of Agriculture in Belgrade has created a material basis for improvement of knowledge in the aquaculture sector for all generations, primarily students. This will contribute to development of the fishery economy in the coming future. The Center's particular importance is the fact that a range of research activities have already contributed to the development of Serbia's aquaculture sector, primarily in the area of carp breeding technology enhancement by the use of extruded feed, one of the main factors that contributed to the twofold increase of fish production in the last decade in Serbia. Another important program realized in CEFAH is the selective breeding of carp and rainbow trout that will provide a foundation for quality fry production for stocking in Serbian farms. Networking of the Center with companies, primarily fish farms and feed production companies through research projects and expert collaboration, education, and dissemination activities, represents a solid basis and prerequisite for a symbiotic relations and thus sustainability of the CEFAH and its further valuable influence on aquaculture development in Serbia.

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References

1. Marković Z**,** Stanković M, Dulić Z, Živić I, Rašković B, Spasić M, Poleksić V 2011. Aquaculture and fishery in Serbia - status and potentials. Fifth International Conference "Aquaculture & Fishery" Conference Proceedings. Faculty of Agriculture, June, 1-3.2011, Belgrade-Zemun, Serbia, pp. 36-40.
2. Marković Z, Poleksić V 2011. Akvakultura i Ribarstvo u Srbiji (Aquaculture and Fishery in Serbia). Zoran Marković, pp. 289.
3. Marković Z, Stanković M, Živić I, Trbojević D, Dulić Z, Rašković B, Poleksić V 2012. Improvement of carp feeding technology – a reason for increase of carp (*Cyprinus carpio* l.) production and a chance for increase of carp consumption in Serbia. AQUA 2012, Prague, Czech Republic, September 1-5, 2012, Abstracts pp. 675.

4. Marković Z, Rašković B, Dulić Z, Stanković M, Spasić M, Vukojević D, Relić R, Poleksić V 2014. Impact of the "ROSA" project on improvement of material and human resources in the area of aquaculture at the Faculty of Agriculture, University of Belgrade. EU Project Collaborations: Challenges for Research Improvements in Agriculture. June 2-4, 2014, Belgrade, Serbia. Book of Abstracts, pp. 69.
5. Poleksić V, Dulić Z, Stanković M, Rašković B, Spasić M, Vukojević D, Marković Z 2013. Higher education for aquaculture/fishery at the Faculty of Agriculture University of Belgrade: implementation of the first bologna reform – linking theory and practice. Conference proceedings VI International conference "Water & Fish", Faculty of Agriculture, University of Belgrade – Serbia, June, 12 – 14. 2013, Belgrade, Serbia, pp. 68-73.
6. Spasic M, Markovic Z, Kolstad K, Poleksic V, Stankovic M, Zivic I, Dulic Z, Raskovic B, Ciric M 2010. Selective breeding of common carp (*Cyprinus carpio* L.) in Serbia. Second NACEE Conference of Young Researchers, 30–31 August 2010, Szarvas, Hungary, Conference proceedings, pp. 53–54.
7. Subasinghe R, Soto D, Jia J 2009. Global aquaculture and its role in sustainable development. Reviews in Aquaculture 1, 2–9.
8. Todorčević M**,** Škugor S, Rašković B, Spasić M**,** Stanković M**,** Sørensen M, Kolstad K, Kittelsen A, Dulić Z, Poleksić V, Marković Z 2014. Improvement of Common Carp production in Serbia through EU funded cooperation. EU Project Collaborations: Challenges for Research Improvements in Agriculture. June 2-4, 2014, Belgrade, Serbia. Book of Abstracts, pp. 26.

Original paper

THE EFFECT OF HIVE VOLUME ON EFFICIENCY AND STRENGTH CONSERVATION AND RESTORATION OF FOOD SUPPLIES DURING THE WINTERING IN LANGSTROTH HIVES

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Abstract

The influence of volume of the hive on wintering, colony strength and food supply was observed during the three-year period. Standard LR hives were used for the experiment. Colonies were tested based on the number of hive bodies and the number of frames in the brood hive bodies. The colonies were divided into two groups based on the number of frames: group I had eight frames in the brood bodies, the second group included 10 frames in the brood bodies. Based on the number of hive bodies, colonies were also divided into two groups: colonies with a single hive body and colonies with two hive bodies. The inspections were carried out in the autumn in late August and early September, and in the spring in late March and early April. Colony strength was determined by the amount of brood and food supplies through the amount of honey and pollen. It was found that the number of frames in the brood bodies had almost no impact on the colony strength and the amount of food in the autumn and spring inspections. Percentage ratio in spring and autumn inspections was more favourable in ten-frame hives considering the amount of bees and pollen. The ratio for the amount of brood between the two inspections was higher in eight-frame hives. The colonies replenished bees more rapidly and foraged pollen more efficiently in ten-frame hives while the colonies in eight-frame hives replenished brood quicker. It was found that, during the three-year period, the hive volume had impact on strength and food supply of colonies. Colonies that have wintered in two hive bodies had favourable ratio of spring and autumn inspections for the amount of bees, brood and pollen, while the ratio for the amount of honey was more favourable in the colonies with a single hive body. The colonies with two hive bodies quicker restored strength and pollen supply, while the colonies with a single hive body consumed honey supply more rationally.

Key words: *colony strength, food supply, hive volume, Langstroth hives*

Introduction

A large number of factors affect the successful wintering of honey bees. These factors can be divided into internal and external (Avetisijan, 1982). The external factors include climate, nectar pasture and presence of beneficial or harmful organisms in environment. External conditions

have a decisive impact on the vitality and productivity of honey bee colonies, but beekeepers themselves can not significantly alter most of those conditions.

The internal factors include colony strength, queen bee quality, food supply, honeycomb quality, microclimate and the hive volume. This group of factors is present within the hive and is the result of the life of the very colony. Rinderer and Baxter (1978, 1980), by testing the bees in the laboratory and in field conditions, found that the volume of the honeycomb affects foraging activity of honey bees. Colonies that had more space (empty combs) have processed sugar syrup significantly more and significantly quicker than colonies that had less space.

The aim of this paper is to determine the influence of volume of the hive on overwintering of honey bee colonies in standard Langstroth hives and on the colony strength and food supply during the main inspections.

Materials and methods

The observation of colonies was conducted during the period from 2010 to 2012. Colonies were in standard LR-hives with ten and eight combs. In each group were 5 colonies. In addition to the number of combs, hives had the different number of main bodies. Colonies were prepared for winter in hives with one and two bodies. Autumn inspection was carried out in late August and early September, and spring inspection in late March and early April.

Colony strength (the amount of bees and brood size) and volume of food (honey and pollen surface) were determined on inspections. The amount of bees per hive was determined visually by the amount of bees that occupy combs. It is expressed in parts of the comb that are possessed by the bees (1/10) or as a percentage. Total quantity of bees per hive can be calculated by addition of the amount of bees on combs.

The brood surface is also evaluated in the spring and autumn, by detailed observation of each frame with open and closed brood in the hive. Results are also expressed in parts of the frame (1/10) or as a percentage. Surfaces under honey and pollen were also determined in the same way and at the same time as the two previous traits. The relationships for all observed traits were calculated between spring and autumn inspections, to establish their increase or decrease. At the beginning of the experiment, one year old queens produced in 2009 were in all colonies.

Results and discussion

Hives with eight and ten combs

The amount of bees per hive was more affected by the years of observation than by the number of combs in the hives. In the second year of observation, colonies in ten-comb hives have wintered much better. Although the colonies in eight-comb hives had slightly more bees in autumn survey they wintered worse, and were much weaker in spring with lower number of bees (Table 1). The relationship between wintered and bees after last winter was, in ten-comb hives, increased by 20%. In the next year, the situation has turned around and, in eight-comb hives, the ratio was higher by 10%. Overall, the amount of bees was very similar but colonies in ten-comb hives wintered better (4%).

Table 1. *Inspections of eight- and ten-comb hives (1/10 of combs)*

Year	Hive	Inspection	Bees	Brood	Honey	Pollen
I	Eight-comb	Autumn	6.12	1.98	3.82	0.28
		Spring	3.38	1.50	2.98	0.52
		Relation	**0.55**	**0.76**	**0.78**	**1.86**
	Ten-comb	Autumn	6.44	1.94	3.80	0.16
		Spring	3.36	1.18	2.78	0.36
		Relation	**0.52**	**0.60**	**0.73**	**2.25**
II	Eight-comb	Autumn	7.14	1.74	4.96	0.18
		Spring	2.20	0.96	3.30	0.34
		Relation	**0.31**	**0.55**	**0.66**	**1.89**
	Ten-comb	Autumn	6.56	1.88	4.38	0.34
		Spring	3.50	1.48	3.78	0.38
		Relation	**0.53**	**0.79**	**0.86**	**1.12**
III	Eight-comb	Autumn	7.42	1.50	5.48	0.12
		Spring	5.24	2.20	3.90	0.46
		Relation	**0.71**	**1.47**	**0.71**	**3.83**
	Ten-comb	Autumn	7.80	1.44	4.88	0.08
		Spring	4.74	1.82	2.66	0.74
		Relation	**0.61**	**1.25**	**0.54**	**9.25**
Average	Eight-comb	Autumn	6.89	1.74	4.75	0.19
		Spring	3.61	1.55	3.39	0.44
		Relation	**0.52**	**0.89**	**0.71**	**2.32**
	Ten-comb	Autumn	6.93	1.75	4.35	0.19
		Spring	3.87	1.49	3.07	0.49
		Relation	**0.56**	**0.85**	**0.71**	**2.60**

A similar situation has been also established for the amount of brood, better results were found in eight-comb hives (4%). Variations are expressed per year. Higher amount of brood in the first and third year was determined in eight-comb hives and in the second year in ten-comb hives. Colonies in eight-comb hives had more honey on both inspections. The relationship between spring and autumn inspections was identical in both groups of hives. Colonies in ten-comb hives had slightly more pollen in the spring. Especially, ten-comb colonies foraged large amounts of pollen in spring in the third year of observation (0.74 combs), considering that they were wintered with almost no pollen (Table 1).

Table 2. *Variations of parameters in hives with different number of combs per inspection*

Autumn inspection				
	Number of bees	Brood	Honey	Pollen
CV (%) - 8 combs	28.88	30.23	48.23	60.15
CV (%) - 10 combs	28.39	40.55	35.15	84.011
Spring inspection				
	Number of bees	Brood	Honey	Pollen
CV (%) - 8 combs	55.49	52.63	25.94	53.55
CV (%) - 10 combs	47.11	55.89	48.82	60.97

The number of bees showed very little variation in autumn inspections, and somewhat higher variation in autumn, considering the number of combs per hive (Table 2).

Colonies with one and two bodies

In addition to the fact that colonies with the two bodies had more bees on both fall and spring inspections they had a better relationship between the two examinations (7% more). Colonies with one body wintered badly in the first year of observation. A large area of the brood during the autumn might have had influence on this. Colonies with one body had significantly more brood during this period, which led to the depletion of bees due to its nurturing. These colonies were much more weakened during winter and in spring they very slow rebuild strength and badly fostered brood. In average, colonies with two bodies had almost the same amount of brood in the spring inspections, while companies with one body had almost half as many brood when compared to the autumn inspections. It is interesting to point out that colonies with one body have had larger food supplies in the spring than in the autumn inspection. In colonies with two bodies there was less honey in the spring compared to the autumn inspection. Naturally, greater food supply is determined in colonies with two bodies on each inspection. Wintering of honey bee colonies is very dependent on the amount of food that the bees store before winter (Kulinčević, 1997).

Table 3. *Inspections of hives with one or two bodies*

Year	Hive	Inspection	Bees	Brood	Honey	Pollen
I	One body	Autumn	5.30	2.50	2.44	0.30
		Spring	1.96	0.74	2.68	0.12
		Relation	**0.37**	**0.29**	**1.10**	**0.40**
	Two bodies	Autumn	5.72	1.38	3.40	0.22
		Spring	3.70	1.42	3.24	0.62
		Relation	**0.65**	**1.03**	**0.95**	**2.82**
II	One body	Autumn	5.80	2.26	3.28	0.30
		Spring	3.08	1.52	3.80	0.42
		Relation	**0.53**	**0.67**	**1.19**	**1.40**
	Two bodies	Autumn	6.92	1.94	4.34	0.34
		Spring	3.76	1.82	3.64	0.46
		Relation	**0.54**	**0.94**	**0.84**	**1.35**
III	One body	Autumn	5.20	1.84	3.02	0.14
		Spring	3.42	1.52	2.66	0.60
		Relation	**0.66**	**0.83**	**0.88**	**4.29**
	Two bodies	Autumn	7.14	1.52	5.00	0.16
		Spring	4.30	1.60	3.08	0.66
		Relation	**0.60**	**1.05**	**0.62**	**4.13**
Average	One body	Autumn	5.43	2.20	2.91	0.27
		Spring	2.82	1.26	3.05	0.38
		Relation	**0.52**	**0.57**	**1.05**	**1.41**
	Two bodies	Autumn	6.59	1.61	4.27	0.24
		Spring	3.92	1.61	3.32	0.58
		Relation	**0.59**	**1.00**	**0.78**	**2.41**

Medium-strong and strong colonies survive winter much better than poorer colonies (Jevtić et al., 2005). Wintering of the colonies can be influenced by the quantity of solid food fed to the bees

(Jevtić et al., 2004). In addition, the colony strength depends on the genetic potential of the parent and it depends on the quality and quantity of food that is stored over winter (Mladenović et al., 2002). The presence of a sufficient amount of food affects the amount of royal jelly secreted in the hypopharingeal glands of nursing bees (Taranov, 1986).

Variations between studied traits were higher in spring than in autumn inspections (Table 4).

Table 4. *Variations of parameters in hives with different number of bodies per inspection*

Autumn inspection				
	Number of bees	Brood	Honey	Pollen
CV (%) - one body	14.02	21.70	30.01	74.21
CV (%) - two bodies	20.12	33.85	30.95	97.58
Spring inspection				
	Number of bees	Brood	Honey	Pollen
CV (%) - one body	41.99	51.68	24.67	56.89
CV (%) - two bodies	25.64	38.91	30.08	30.00

After three years of research, it was concluded that the number of combs in the brood (8 and 10) has almost no effect on the colony strength and on the food supply. Colonies quickly replenished bees and foraged larger amounts of pollen in ten-comb hives, while brood was quicker replenished in eight-comb hives. Volume of hives (one or two bodies) has had an impact on colony strength and food supplies. Colonies that have wintered in two bodies had been in more favourable relation between spring and autumn inspections considering the amount of bees, brood and pollen. Colonies with two bodies quickly replenished strength and a pollen supply, while companies with one body consumed stored food more rationally.

Conclusion

After three years of research, it can be concluded that the number of frames in brood box (8 or 10) has almost no influence on the colony strength and the food supply. Honey bees gathered pollen in greater supply in ten-comb hives, while the brood was quicker replenished in eight-comb hives. Volume of the hives (one and two bodies) had impact on colony strength and food supply per. Colonies that have wintered in two bodies had more favorable relation of the spring and autumn inspections for the amount of bees, brood and pollen. Colonies with two bodies quickly renewed strength and a supply of pollen while colonies with one body consumed stored honey more rationally.

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References

1. Аветисян ГА 1982. Пчеловодство. Колос, 320 с.
2. Jevtić G, Mladenović M, Nedić N and Dinić B 2004. Uticaj količine čvrste hrane na zimovanje pčelinjih društava. Biotehnologija u stočarstvu, Vol.20(5-6) 363-368.
3. Jevtić G, Mladenović M and Nedić N 2005. The influence of the quantity of honeybees and honey reserves on wintering of honeybee colonies. Proceedings of the 8th International Symposium Modern Trends In Livestock Production, 5-8 October, Belgrade, Zemun, Serbia and Montenegro, 315-321.
4. Kulinčević J 1997. Pčelarstvo nauka i praksa. IP Potez-Uno, Beograd.
5. Mladenović M, Stanković O, Nedić N and Jevtić G 2002. Uticaj količine hrane na prolećni razvoj pčelinjeg društva. Biotehnologija u stočarstvu, Vol.18(5-6), 339-342.
6. Rinderer TE and Baxter JR 1978. Effect of Empty Comb on Hoarding Behavior and Honey Production of the Honey Bee. Journal of Economic Entomology, Volume 71 (5), 757-759.
7. Rinderer TE and Baxter JR 1980. Hoarding Behavior of the Honey Bee: Effects of Empty Comb, Comb Color, and Genotype. Environmental Entomology, Volume 9(1), 104-105.
8. Taranov GF 1986. Hrana i prihrana pčela. Rossijhozizdat, Moskva.

Original paper

QUALITY OF HONEY BEE BREAD COLLECTED IN SPRING

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Abstract

Pollen is the only source of protein that honey bees collect from nature. It is very important for the development of brood, and consequently, for the development of the colony. Honey bee bread is the pollen which the bees collect from flowers and store in honeycomb cells. Honey bee bread has a modified structure due to the fermentation process under the influence of enzymes it passes through. For this study, ten honey bee colonies were selected. Honey bee bread was sampled from the combs. The quality of pollen and honey bee bread was determined by the chemical composition, using standard methods used in food analysis. Total nitrogen was determined by Kjeldahl method. Micro- and macroelements were determined by spectrophotometric method. The pollen collected from two sites had rich protein content (29.93 % and 27.63 % on average).

Key words: *chemical composition, honey bee bread, pollen*

Introduction

The development of strong and healthy colonies depends on the rearing of strong brood, which itself requires quality nutrients. Regarding honey bee diet, they require similar nutrients as humans. The need for carbohydrates is fully met by the consumption of honey, but other nutrients (primarily proteins, minerals, vitamins, etc.) are far less present in honey, and the need for these is met by the consumption of the pollen (Adekanmbi and Ogundipe, 2009).

Collecting of the pollen is done by forager honey bees, which take pollen from anthers, pack it together with saliva and nectar to their hind legs and bring it to the hive. In the hive, that pollen is, then, stored into the cells of honeycombs and such a stored pollen is called honey bee bread. Pollen is fine to coarse powder that is the microgametophytes of seed plants. Pollen grains have a hard membrane that protects the sperm cells during their movement between the stamens to the pistil of flowering plants or from the male cone to the female cone of coniferous plants. Various plants are pollinated by various ways (wind, insects, etc.). When pollen lands on a compatible pistil of flowering plants, it germinates and produces a pollen tube that transfers the sperm to the ovule of a receptive ovary (Taranov, 2006). Pollen is very rich in protein, which serves as material for tissue growth and tissue regeneration (Kulinčević, 2006).

Pollen and bee bread differ biochemically, due to changes bees afflict upon foraged pollen in the process. Pollen is mixed with saliva and honey and then stored. For example, bee bread contains more reducing sugars than pollen from the same plant species (Casteel, 1912). Also, bee bread contains vitamin K (Haydak and Vivino, 1950) and a milk-digesting enzyme (Hitchcock, 1956); pollen collected from the legs of foraging bees does not. Avetisian (1935) found that bee bread made from birch pollen contained six times as much lactic acid as hand-collected birch pollen. The conversion of pollen to bee bread and the accompanying biochemical changes have often been postulated to be the result of microbial action, principally a lactic acid fermentation caused by bacteria and yeasts (Foote 1957; Haydak, 1958).

The amount of protein in pollen ranges greatly, depending of the plant species. Considering chemical composition, besides protein, pollen also includes free amino-acids, lipids, carbohydrates (sugars, starch and cellulose), minerals (Ca, Mg, P, Fe, Na, K, Al, Mn, S, Cu, etc.), vitamins (pantothenic and ascorbic acid, vitamins of B complex, etc.), various enzymes and coenzymes, etc.

For the honey bees, it is the best if they have access to pollen derived from different plants, because balanced nutrition is of key significance for the development of honey bee colony (Kulinčević, 2006). Pollen, which is the main source of dietary protein and contains essential amino acids for their development (De Groot, 1953), can influence longevity, the development of hypopharyngeal glands (HPG) and ovaries (Pernal and Currie, 2000) and the susceptibility to pathogens (Rinderer and Elliott 1977); but to what extent pollen can affect IC is not known. Finally, honeybee populations have been declining over the last years and a current idea suggests that honeybee colonies may suffer from a compromised immune system, which could be related to poor nutrition commonly associated with colony losses (van Engelsdorp *et al*. 2008).

According to Hrassnigg and Crailsheim (2005), rearing one larva requires 25-37.5 mg of protein, equivalent to 125-187.5 mg pollen. Somerville, (2000), states that the protein levels vary from 6% to 40%. The minimum protein level required for honey bees is 20%. If the aim is rapid breeding and expansion of the hive population to work a heavy honey flow, crude protein levels of 25–30% are required (Somerville, 2000). The period of larval development lasts for about 8 to 9 days.

Material and methods

The aim of this paper was to determine the chemical composition of honey bee bread collected in spring. The experiment was conducted in the months of April and May.

Honey bee bread was sampled from the honey bee colonies of the Institute for Forage Crops Kruševac, directly from the honeycomb. Colonies were stationed at two sites, Makrešane and Vrbnica.

The proximate composition of the bee bread was determined by using standard methods of food analysis (Roma R. B. et al, 1983). According to these authors, ash content was measured in samples that were heated in a muffle furnace at 600°C until a uniform gray-white ash remained. The samples were then used for the subsequent determination of macro- and microelements by using atomic absorption spectrophotometry (AAS-Perkin Elmer 1100 B USA) and total nitrogen in the samples was determined by micro-Kjeldahl method.

Results and discussion

The chemical composition of honey bee bread collected from two sites was somewhat similar when compared (tables 1 and 2). This is due to similar vegetation composition found on these two sites. The analysis of phytocenological composition of plants on two sites showed that the most common plants were dandelion and deadnettle.

When a colony is actively breeding, or during periods of heavy wax production (such as during a heavy honey flow), the demand for pollen is high. Wax glands use a lot of protein and a lack of pollen or pollen with low nutritional values will have significant management implications (Somerville, 2000). During the periods of high bee activity and lower pollen influx (autumn and early spring), honey bees must rely on their store of honey bee bread.

Table 1. *Chemical composition of honey bee bread from Makrešane*

Parameters	Average	SD	CV
Crude ash (%)	3.05	0.2329	7.64
Crude protein (%)	29.93	8.5807	28.67
Crude cellulose (%)	2.64	0.8701	32.95
Crude fat (%)	4.92	0.6975	14.18
NEM (%)	65.26	3.0353	4.65
Ca (%)	0.65	0.1774	27.29
P (%)	0.65	0.0332	5.11
K (%)	0.74	0.0492	6.65
Mg (%)	0.27	0.0250	9.26
Fe (mg/kg)	121.99	31.8625	26.12
Zn (mg/kg)	44.09	3.8639	8.77
Mn (mg/kg)	29.92	0.6006	2.01

Table 2. *Chemical composition of honey bee bread from Vrbnica*

Parameters	Average	SD	CV (%)
Crude ash (%)	2.67	0.2421	9.07
Crude protein (%)	27.63	9.1542	33.13
Crude cellulose (%)	3.23	0.7831	24.24
Crude fat (%)	4.51	0.5873	13.02
NEM (%)	68.28	3.0502	4.47
Ca (%)	0.55	0.0984	17.89
P (%)	0.72	0.0367	5.09
K (%)	0.77	0.0531	6.89
Mg (%)	0.21	0.1012	48.19
Fe (mg/kg)	115.76	29.8394	25.78
Zn (mg/kg)	32.15	2.6571	8.26
Mn (mg/kg)	33.05	0.5975	1.80

In the samples collected in Makrešane, average amount of protein was 0.015 mg for 50g of bee bread sample, while in samples from Vrbnica average was 0.014 mg for 50g of bee bread.

No complete study has been conducted into the role that fats, vitamins and minerals play in honey bee nutrition. Deficiencies or imbalances may well exist (Somerville, 2000).

Honey bees obtain lipids exclusively from pollen, and the lipid content of pollen from various species ranges between 0.8% and 18.9% (Roulston and Cane, 2000). Considering that honey bee bread is derived from pollen, it contains considerable amount of fat.

Honey bees obtain inorganic elements mainly from pollen, and according to Imdorf et al. (1998), bees can obtain minerals from other important sources of minerals like nectar and water or the existence of endogenous mineral pools in adults. During the shortage of fresh pollen, bees will use their stores of honey bee bread to replenish required nutrients.

The authors recommended a diet containing 1000 mg/kg potassium, 500 mg/kg calcium, 300 mg/kg magnesium and 50 mg/kg each of zinc, manganese and iron. Considering data obtained in samples from Vrbnica and Makrešane, honey bee bread do contain relatively large amounts of these elements, and depending on the state of colony (colony strength, brood rearing), this honey bee bread can be used as additional nutrition if there is a shortage of fresh pollen.

The highest variation in samples from Makrešane was determined for the amount of crude cellulose, while in samples from Vrbnica it was for the content of Fe. The variation in the amount of protein was somewhat similar in both sites (tables 1 and 2).

However, the availability of pollen, and subsequently honey bee bread, must be taken into account, and further researches have to be done. Considering obtained results, honey bee bread collected from these sites can be taken from colonies, stored and then used as additional feed for periods of either shortage of natural pollen or in cases of rapid colony development, such is in spring.

Conclusion

Considering that the proteins are among the most vital nutrients required for the development of honey bees, it is important to study the quality of sources of proteins. There were no significant researches considering honey bee bread.

Honey bee bread collected from sites Vrbnica and Makrešane can be considered of good quality, and can be used directly by bees which made it or it can be taken from colonies and used as additional nutrient in spring during the highest activity of brood rearing in colonies. Samples from both sites had relatively high amount of protein (29.93 % and 27.63 % on average), which is the most important nutrient for honey bees. There were no significant researches in the role of other nutrients, so their importance is yet to be determined through future studies.

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References

1. Adekanmbi O and Ogundipe O 2009. Nectar Sources for the Honey Bee (Apis mellifera adansonii) Revealed by Pollen Content. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37, 2, 211-217.
2. Casteel DB 1912 The behavior of the honey bee in pollen collecting. U. S. Dept. Agr. Bull., 1211.

3. Taranov GF 2006. Hrana i ishrana pčela, translation, Partenon, Belgrade.
4. Kulinčević J 2006. Pčelarstvo, Partenon, Belgrade.
5. Hydak M H and Vivino AE 1950.The changes in the thiamine, riboflavin, niacin and pantothenic acid content in the food of female honeybees during growth with a note on the vitamin K activity of royal jelly and beebread. Ann. Entomol.
6. Hitchcock D 1956. A milk-digesting enzyme in pollen stored by honey bees. Amer. Bee J., 96, 487- 489.
7. Avetisan GA 1935.Recent work on the chemical composition of pollen. Bee World 16, 92. Soc. Amer. 43, 361-367.
8. Foote HL 1957. Possible use of microorganisms in synthetic bee bread production. Amer. Bee J. 97, 476-478.
9. Haydak M H 1958. Pollen - pollen substitutes - beebread. Amer. Bee J. 98, 145-146.
10. Roma RB 1983. Composition and Protein Quality of Honeybee-Collected Pollen of Eucalyptus marginata and Eucalyptus calophyllaa, Journal of nutrition 2479-2483.
11. De Groot AP 1953. Protein and amino acid requirements of the honey bee (Apis mellifica L.). Physiol. Comp. Oecol. 3, 197–285.
12. Pernal SF and Currie RW 2000. Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees *(Apis mellifera L.).* Apidologie 31, 387–409.
13. Rinderer TE and Elliott KD 1977. Worker honey bee response to infection with *Nosema apis*. J. Econ. Entomol. 70, 431–433
14. Van Engelsdorp D, Hayes JJr, Underwood RM and Pettis J 2008. A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. PLoS ONE 3, e4071.
15. Hrassnigg N and Crailsheim K 2005. Differences in drone and worker physiology in honeybees (*Apis mellifera* L.), Apidologie 36, 255–277.
16. Doug Somerville 2000. Honey bee nutrition and supplementary feeding, NSW Agriculture, DAI/178
17. Roulston TH and Cane JH 2000 Pollen nutritional content and digestibility for animals, Plant Syst. Evol. 222, 187–209.
18. Imdorf A, Rickli M, Kilchenmann V, Bogdanov S and Wille H 1998. Nitrogen and mineral constituents of honey bee worker brood during pollen shortage, Apidologie 29, 315–325.

Original paper

ANTIBIOTICS RESIDUES AS LIMITING FACTOR OF HONEY QUALITY

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Abstract

Residues of veterinary drugs represent a significant risk to the health of honey consumers. Antibiotics can get into honey by using the antibiotics for treatment and prevention of bees diseases but also through the plant nectar and pollen. In Serbia, the use of antibiotics in beekeeping for bacterial diseases treatment is prohibited and accordingly there is no prescribed maximum permissible concentration for them in honey. The aim of this paper is to monitor the presence of antibiotic residues in honey which necessarily indicate their illegal and uncontrolled use. The presence of antibiotic residues in honey was screened for microbiological method "Modified method 4 plates" (EUR 15127-EN). The total of 135 samples of different honey types has been examined. Five of them (3.7%) were positive to antibiotic residues. The presence of antibiotic residues was found in the acacia honey (0.31%), linden honey (0.33%), sunflower honey (0.19%), mixed honey (0.17%) and honeydew honey (0.10%). Such unprofessional, unconscionable and unlawful use of antibiotics leads to their presence in honey and other bee products, as well as in the highly desirable and valuable products making them unusable.

Key words: *antibiotics, honey, quality, residue*

Introduction

Within the healthy food that nature gives to man, on the list of quality and value, bee products undoubtedly take special place. Honey and other bee products are a real treasure of natural medicinal-prophylactic ingredients (Babic, 2012). Honey is generally considered as a natural and healthy product (Reybroeck, 2003). It has long been known that honey has an antimicrobial activity. Recently the presence of antimicrobial peptide-defensin in honey has been proven which could someday be used for the treatment of antibiotic-resistant infections (Kwakmann et al., 2010). Risks to consumers of honey are highly dependent on the degree of control exercised by the producers, buyers, processors, retailers and authorized bodies for control of honey which prevent or minimize the risk to an acceptable level. The last few decades, which are characterized by a significant increase in industrial production, increased and uncontrolled use of pesticides and antibiotics in agriculture and intense international traffic have increased environmental contamination caused by various pollutants and toxic substances such as heavy metals, pesticides, radionuclides, and antibiotics (Barišićet et al., 1999; Mujić et al., 2011; Roman et al., 2011). Antibiotics can get into honey by using the antibiotics for treatment and prevention of bee diseases, but also through the plant nectar and pollen (Roman, 2005; Roman et al., 2007). Because of that, content of these substances in honey and bee organism is a very good bioindicator of

the use of antibiotics in beekeeping and of their presence in the environment (Celli and Maccagnani, 2003; Porini et al., 2003). Antibiotics, such as streptomycin, tetracyclines and sulfonamides are often used in beekeeping as preventive or therapeutic treatment to protect apiary (Iancu et al., 2012). Other antibiotics such as erythromycin, lincomycin, monensin, enrofloxacin and alike are also used in beekeeping (Johnson et al., 2010). In the EU the use of antibiotics for bacterial infections has been banned, except for the European foulbrood via the "cascade" system, but taking honey from those hives is banned. So there is no MRL (Maximum Residue Limits) for the presence of antibiotics in honey (EEC Regulation 2377/90 and amendments). Codex Alimentarius Standard for Honey (12-1981) in section 4.2. with the defined MRL for pesticides and veterinary drugs does not define the MRL for antibiotics (Johanson et al., 2010). In Serbia, the use of antibiotics in beekeeping for combating bacterial diseases is prohibited and accordingly there is no prescribed maximum permissible concentration of them in honey (Regulation of concentration of pesticides, metals and metalloids and other toxic substances, chemotherapeutics, anabolics and other substances that can be found in food (FRY Official Register, 5/92, 11/92, 32/2002). Although our beekeeping public knows about this the beekeepers still reach for antibiotics in the process of "saving" the American and European foulbrood what results in uncontrolled presence of antibiotic residues in honey and spread of disease. Some countries outside Europe have legalized the use of tetracyclines, sulfonamides and other antibiotics for the treatment of American foulbrood. Systematic use of tetracycline in Canada and the United States has led to resistance strains of Paenibacillus larvae subsp. larvae to tetracycline. Sulfonamides in some countries are used in the prevention of Nosemosis. Chloramphenicol is used in beekeeping in China, and the positive tested samples for chloramphenicol indicate that the honey is of Chinese origin or honey mixed with the Chinese honey (Reybroeck, 2003). Low concentrations of streptomycin can also be found in fruit honey obtained from the nectar collected from the pear orchards treated during flowering with preparations of streptomycin against fire blight (Reybroeck, 2003). Antibiotic residue exhibits a relatively long half–life and can have direct toxic effects on the consumers, such as allergic response and the induction of drug-resistant strains of bacteria (Gunes et al., 2009; Johnson et al., 2010).

In order to organize beekeeping in a way that would reduce the risks of contamination of bee products by harmful substances to a minimum it is necessary to implement a system of self-control in chain "from farm to fork." According to the chain, beekeepers should be included in the introduction of guidelines "good beekeeping practices" that promote technological development and productivity of beekeeping. The best result of this is getting consumer confidence which is the most important on the global market today. Considering the clinical findings and the epizootic situation, the assumption is that the beegarden still uses the antibiotics. The aim of this paper is to monitor the presence of antibiotic residues in honey which necessarily indicate their illegal and uncontrolled use.

Material and methods

In the last 2013,135 samples of different honey types were collected and examined at the Institute of Veterinary Medicine "Novi Sad" in Novi Sad ("NIV NS") Serbia. Honey samples were collected from two sources: honey samples that were brought by the owners at the "NIV NS" and honey samples collected by experts from "NIV NS" on-site production. All the samples were properly packaged in glass or plastic jars and properly labeled. The presence of antibiotic residues in honey was screened for microbiological method "Modified method 4 plates" (EUR 15127-EN). The total of 135 samples of

different honey types has been examined. If the sample contains active antimicrobial residues there will not be growth of the test microorganism in the diffusion zone, ie. it will be a zone of inhibition. The width of the zone of inhibition is measured from the edge of the hole in the agar to the limits of growth of test microorganisms. The results have been statistically analyzed and presented by means of descriptive statistics.

Results and discussion

The total of 135 samples of different honey types has been examined. Five of them (3.7%) were positive to the presence of residues of antibiotics with inhibition of growth of more than 4 mm. The presence of antibiotic residues was found in the acacia honey (0.31%), linden honey (0.33%), sunflower honey (0.19%), mixed honey (0.17%) and honeydew honey (0.10%). Analysis of differences in the frequency of the number of positive samples of different types of honey indicates that there is no statistically significant difference in the frequency of positive samples between different types of honey ($p>0.05$).

The results of our research correspond to the references and indicate that the use of antibiotics is still present in beekeeping. Such unprofessional, unconscionable and unlawful use of antibiotics leads to their presence in honey and other bee products and make highly desirable and valuable products unusable (Plavša et al., 2005).The antibiotics, such as gentamycin, erythromycin, penicillin, tetracycline, streptomycin, ofloxacin and sulphonimides are also reportedly used in beekeeping residues. These antibiotic residues have toxic acute and chronic effects on human health and reduce the efficacy and quality of honey (Zai et al., 2013). "Modified method 4 plates" is a screening method. This method cannot be used for the detection of the types and amounts of antibiotics in honey. Different techniques were used for the detection and quantification of these antibiotics in honey, mostly Biochip array Technology and Thin Layer Chromatography (TLC) were used for the detection, like Elisa method. The latest research has developed valid, simple and rapid method for antibiotics by HPLC method, mass spectrometry and LC/MS (Liquid chromatography–mass spectrometry). These techniques were found sensitive, reproducible and very useful for antibiotics and others drugs detection because by means of these techniques we are able to quantify the presence of a very low amount of drugs in sample. The type detection of antibiotics in honey by using HPLC is fast, valid and specific (Zai et al., 2013). LC / MS method is simple, rapid, reliable and sensitive enough for routine use in laboratory work (Krivoklavek et al., 2005). ELISA technique is simple, sensitive and represents a specific powerful tool for selective detection of a very low amount of substances in physiological, biological and environmental samples (Jeon et al., 2008). Therefore, this method enables the efficient determination of target molecules in a complex sample without extraction of the sample (Jeon et al., 2008).

Conclusion

Considering the facts stated in the introduction of this paper to ban the use of antibiotics in beekeeping, the results indicate that there is still insufficiently controlled use of products in beekeeping and insufficiently developed awareness among beekeepers. The presence of antibiotics residues is a limiting factor in the quality of honey and makes it unfit for human consumption. Food safety is the top priority both in research and in the legislative field. Natural conditions, moderate continental climate and the wealth of flora and fauna are virtually ideal conditions for beekeeping and for getting quality and safe bee products. The potential for obtaining such high valued products is necessary in order to use and store brand quality honey both on the Serbian and foreign market.

References

1. Babić J 2012. Uticaj broja spora *Nosema apis ceranae* na razvoj pčelinjeg društva. Master rad, Univerzitet u Novom Sadu, Poljoprivredni fakultet, Departman za veterinarsku medicinu.
2. Barišić D, Vertačnik A, Bromenshenk J, Kezić N, Lulić S, Hus M, Kraljević P, Šimprag M and Seletković S 1999. Radionuclides and selected elements in soil and honey from Gorski Kotar, Croatia. Apidologia, 3, 277-287.
3. Bogdanov S 2006.Contaminants of bee products. Appidology 37, 1-18.
4. Celli G and Maccagnani B 2003. Honey bees as bioindicators of environmental pollutions. B Insectol 56, 137-141.
5. Gunes M E, Gunes N and Cibik R 2009. Oxytetracycline and sulphonamide residues analysis of honey samples from Southern Marmara region in Turkey. Bulgarian Journal of Agricultural Science, 15, 163-167.
6. Iancu R, Oprean L, Tita M, Lengyel E, Codoi V, Boicean A and Scheider A 2012. Physical-Chemical Analysis and Antibiotic Content of Polyflora Honey in Romania. Bulletin UASVM Animal Science and Biotechnologies 69, 1-2.
7. Jeon M, and Paeng IR 2008. Quantitative detection of tetracycline residues in honey by a simple sensitive immuno assay.AnalitycaChimicaActa 626, 180-185.
8. Johnson S and Jadon N 2010. Antibiotic Residues in Honey.Centre for Science and Environment, www.cseindia.org
9. Krivoklavek A, Šmit Z, Baštinac M, Žuntar I and Plavšic-Plavšic F 2005. The determination of sulfonamides in honey by High Performance Liquid Chromatography – MASS spectromerty method (LC/MS). Journal of Separation Science 28, 1434-1439.
10. Kwakman P, Velde A, Boer L, Speijer D, Vandenbroucke-Grauls C and Zaat S 2010. How honey kills bacteria. The FASEB Journal 24, 2576-2582.
11. Mujić I, Alibabić V, Jokić S, Galijašević E, Jukić D, Šekulja D and Barjamović M 2011. Determination of pesticide, heavy metals, radioactive substances , and antibiotic residues in honey. Polis J Environ Stud 20, 719-724.
12. Plavša N, Đuričić B, Petrović J, Stojanov I and Dovenska S 2005. Epizootiological situation and importance of early detecton of American foulbrood.Proc. Symposium on Veterinary, Clinical Pathology and Therapy ClinicaVeterinaria and Conference for Ovine and Caprine Production, Symposium on Animal Reproduction, Ohrid, 204-209 pp.
13. Porini C, Sabatini AG, Giroti S, Ghini S, Medrzycki P, Grillemzono F, Bortolloti G, Gattavecchia E and Galleti G 2003. Honey bees and bee products as monitors of the environmental contaminations. Apiacta 38, 63-69.
14. Reybroeck W 2003. Residues of antibiotics and sulphonamides in honey on the Belgian market. Apiacta 38, 23-30.
15. Roman A 2005. The influence of environment on accumulation of toxic elements in honey bees' body. ISAH - Warsaw Poland 2, 423-426.
16. Roman A, Bartkowiak A and Reginia M 2007. The accumulation of selected chemical elements of toxic properties in bee honey originating from the industrial and rural-forest areas. ISAH, Tartu Estonia 877-881.
17. Roman A, Madras-Majewska B and Popiela-Preban E 2011. Comparative study of selected toxic elements in propolis and honey. Journal of Apicultural Science 55, 97-106.

Review paper

USE OF PROBIOTICS AS GROWTH PROMOTERS AND IMMUNOSTIMULATORS IN FINGERLINGS OF CYPRINID FISH SPECIES

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Abstract

Intensive aquaculture production has required the development of an individual's resistance to disease rather than depending upon antibiotics or chemotherapeutics. The role of gastrointestinal microflora in disease resistance has been established in many fish species, which has led to the concept of manipulating gastrointestinal microflora for better health management. A number of studies has been conducted in different fish species with various useful microorganisms called 'probiotics' to amplify gastrointestinal microflora to fight against various infectious diseases. Probiotics are beneficial microorganisms which protect the host from diseases. Probiotic protection can be achieved by various mechanisms. Most probiotics used in aquaculture belong to the lactic acid bacteria, the genus *Bacillus*, the photosynthetic bacteria, the yeast, notwithstanding other genera and species have also been used. The immunostimulatory effect of probiotics has been established in many fish species, but their direct involvement in the immune response is not well established. It has also been proven that the application of probiotics in aquaculture has beneficial effects on growth of fish as well as on the environment. At present, data about the efficacy of probiotics in commercial aquaculture of Serbia is still lacking. This review discusses mainly the studies and applications about effects, problems and perspectives of probiotics used in fingerlings of cyprinid fish species, and highlights immunostimulatory effects and growth promotion effects of commercial probiotic products. In the present paper the results that show positive influence of probiotics in cyprinides nutrition on production performance and immune system are summarized. Special accent is given to criteria for proper selection of probiotics in cyprinides production.

Key words: *cyprinids, growth promoters, immune system, probiotics*

Introduction

In the several past years, the usage of some antibiotics has been forbidden by several countries due to serious environmental hazards and various adverse effects in many fish species (Gatesoupe, 2007). Furthermore, resistence of pathogen could increase which often leads to spreading of disease, especially under some stressful culture conditions or if antibiotics are used in lower doses for economical reasons (Suzer et al., 2008). Functional additives, like probiotics, represent a new concept on aquaculture. Probiotics have been

increasingly used in aquaculture after their first admission (Kozasa, 1986). Food and Agriculture Organization (FAO) and World Health Organization (WHO) defined probiotics as live micro-organisms that when administered in appropriate amount confer a health benefit on the host (FAO/WHO, 2001). Probiotics supplemented in fish feed resulted in growth performances and feed utilization better than that of the control basal feed, suggesting that the addition of probiotics reduced the culture cost of cyprinid fish species (Jovanović et al., 2011; Miščević et al., 2011 and 2012). In addition, the application of probiotics in aquaculture as the environment friendly treatments was also increasing rapidly (Gatesoupe, 1999). The immunostimulatory effect of probiotics has been also observed in many studies on different fish species (Salinas et al., 2008). Probiotics should also be considered as potentially useful for the control of fish diseases.

At present, data about the efficacy of probiotics in commercial aquaculture of Serbia are still lacking. This review discusses mainly the studies about applications, effects, problems and perspectives of probiotics used in fingerlings of cyprinid fish species, and highlights immunostimulatory effects and growth promotion effects of commercial probiotic products. In the present paper the results that show positive influence of probiotics in cyprinides nutrition on production performance and immunological system are summarized. Special accent is given to criteria for proper selection of probiotics in cyprinides production.

Probiotics

Probiotics represent components of a micro-organism that is beneficial to the health of the host, so they include certain bacteria strains and yeasts that are not adverse on continued use for a longer period of time (Irianto and Austin, 2002). The mode of action is that good and useful microbes multiply in order to compete with the harmful ones, thus suppressing their growth (Sahu et al., 2008). Wang et al. (2008) suggested that the definition of a probiotic in aquaculture should include the addition of live naturally occurring bacteria to ponds in which fish live. Most probiotics used in aquaculture belong to the genus *Bacillus*, *Lactobacillus* sp., *Bifidobacterium* sp., *Vibrio* sp., *Saccharomyces* sp., *Enterococcus* sp., the photosynthetic bacteria, the yeast, (Kumar et al., 2006) notwithstanding other genera and species that have also been used. Fuller (1987) noted that a probiotic should provide actual benefit to the host, be capable to survive in the digestive tract, be suitable for commercialization and be stable and viable for long-term storage conditions.

Probiotics as growth promoters in fingerlings of cyprinid fish species

Growth process in fish depends on numerous factors (Azaza et al., 2008; Deane and Woo, 2009). One of the most important factors on growth and health of fish is feed supply (Lall and Lewis-McCrea, 2007). According to DeVrese and Marteau (2007), mechanism and function of probiotics effect depend principally on the interactions between probiotic species and microbiota of the host or with immuno competent cell of the intestinal mucous. The growth of fish might have occurred by probiotics interaction that increased minerals absorption (Tewe et al., 1999). Jovanović et al. (2011), Miščević et al. (2011 and 2012) observed that the adding commercial probiotics in the diets for common carp fingerlings improve weight of fish. Results obtained by Faramarzi et al. (2011) indicate that the fingerlings of common carp fed with diets with probiotics supplemented exhibited greater growth than those fed with the control diet. Of the four probiotics treatments in the mentioned trial, the 40% protein diet supplemented with yeast showed the best growth

performance and feed efficiency, suggesting that yeast is an appropriate growth stimulating additive in common carp cultivation. Sahandi et al. (2012) also reported the similar results that demonstrate the effect of *Bacillus* spp on length of silver carp and increased fish growth. Effect of probiotics for common carp (*Cyprinus carpio* L.) and some other warm-water fish species based on growth performance and feed utilization (feed conversion ratio, FCR) were investigated also by Wang and Xu, 2006; Wang et al., 2008; Lara-Flores et al., 2010). The above mentioned authors established that the use of probiotics improve growth performance and show the growth promoting effect. All the probiotics supplemented diets resulted in growth performance and feed utilization better than that of the control diets, suggesting that the addition of probiotics reduced the culture cost of common carp (*Cyprinus carpio*). The use of probiotics was suggested as a tool for improving the growth performance and increasing feed digestibility (Sahandi et al., 2012; Ljubojević et al., 2013). Increase of the weight of fish under the probiotics consumption is not completely understood, but according to Lilly and Stillwell (1965) probiotics improve the gastrointestinal microbial populations and then increase enzyme secretion (Suzer et al., 2008) and consequently increase utilization of nutrients and protein anabolism as the length and weight gain occurs in fish.

Probiotics as immunostimulators in fingerlings of cyprinid fish species

Probiotics are used as dietary supplementations in aquaculture and their role in intestinal microbial balance, growth, nutrition, health status and resistance against infectious agents are already established (Gatesoupe, 1999; Ljubojević et al., 2013). Although, some reports are available on the activation of the immune response by dietary supplementation with probiotics in fish (Aly et al., 2008) their direct involvement in the immune response is not well established (Geijtenbeek and Gringhuis, 2009). Nayak et al. (2007) determined the effect of dietary supplementation with the probiotics bacterium, *Bacillus subtilis*, vitamin C in the form of ascorbyl polyphosphate and their combination on the immune response and disease resistance of Indian major carps. The non-specific immune system can be stimulated by probiotics (Balcázar et al., 2006). In the study conducted by Kumar et al. (2006), an increased growth rate was observed in *L. rohita* (Indian carp) fed diet containing *B. subtilis* compared with control. Furthermore, the survival rate after challenge with *A. hydrophila* was significantly higher in the treatment group compared with the control. Administration of probiotics enhances the survival of carp infected with *A. hydrophila* (Selvaraj et al., 2005). The high rates of establishment of bacterium in the gastro-intestinal tract of fish treated with *B. subtilis* have suppressed the *A. hydrophila* infection, which ultimately resulted in the higher survival (Kumar et al., 2006).

Beneficial effects of probiotics on the environment

Probiotics could help in improvement of the water quality in fish ponds (Panigrahi and Azad, 2007). This is due to the capability of the probiotic bacteria to participate in the turnover of organic nutrients in the ponds, to reduce ammonia, nitrite and total nitrogen levels in water and thus improve the chemical quality of water (Zhang et al., 2013).

Proper selection of probiotics in cyprinides production

For proper selection of probiotics it is essential to understand the mechanisms of their action and to define selection criteria for potential probiotics (Klaenhammer and Kullen,

1999). According to Sahu et al. (2008) methods to select probiotic bacteria for use in the aquaculture should include the following steps: collection of background information, acquisition of putative probiotics, screening of putative probiotics, evaluation of pathogenicity and survival test, *in vivo* evaluation, and evaluation of their effects in rearing conditions.

Future perspectives

Generally, the study of probiotics in fish production of Serbia is still in its early stage, and there have been no commercial probiotics products produced in Serbia so far. In the future, probiotics will gain more popularity in aquaculture of the Republic of Serbia, and the application areas will be expanded. Thus, quality control of probiotics in aquaculture of the Republic of Serbia will become an important issue. The need for the development of adequate technology for the evaluation of the efficiency of microbial agents as probiotics in aquaculture will be also increased.

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References

1. Aly SM, Abdel-Galil Ahmed Y, Abdel-Aziz Ghareeb A and Mohamed MF2008. Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections. Fish & Shellfish Immunology 25, 128-136.
2. Azaza MS, Dhraief MN and Kraiem MM 2008. Effects of water temperature on growth and sex ratio of juvenile Nile tilapia *Oreochromis niloticus*(Linnaeus) reared in geothermal waters in southern Tunisia. Journal of thermal Biology 33, 98-105.
3. Balcázar JL, BlasID, Ruiz-Zarzuela I, Cunningham D, Vendrell D and Múzquiz JL 2006. The role of probiotics in aquaculture. Veterinary microbiology 114, 173-186.
4. Deane EE and Woo NY 2009. Modulation of fish growth hormone levels by salinity, temperature, pollutants and aquaculture related stress: a review. Reviews in fish biology and fisheries 19, 97-120.
5. DeVrese M and Marteau P 2007. Probiotics and Prebiotics: Effects on Diarrhea. Journal of Nutrition 137, 803-811.
6. FAO/WHO 2001. Report on Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. 1–4 October 2001, Cordoba, Argentina. Available at: ftp://ftp.fao.org/ es/esn/food/probio_report_en.pdf.
7. Faramarzi M, Kiaalvandi S and Iranshahi F 2011. The effect of probiotics on growth performance and body composition of common carp (*Cyprinus carpio*). Journal of Animal and Veterinary Advances 10, 2408-2413.
8. Fuller R1987. A review, probiotics in man and animals. Journal of Applied Bacteriology 66, 365–378.

9. Gatesoupe FJ 1999. The use of probiotics in aquaculture. Aquaculture 180, 147–165.

10. Gatesoupe FJ 2007. Live yeasts in the gut: natural occurrence, dietary introduction, and their effects on fish health and development. Aquaculture 267, 20–30.

11. Geijtenbeek TB and Gringhuis SI2009. Signalling through C-type lectin receptors: shaping immune responses. Nature Reviews Immunology 9, 465-479.

12. Irianto A and Austin B 2002. Probiotics in aquaculture. Journal of Fish Diseases 25, 633-642.

13. Jovanović R, Miščević M, Ćirković M, Milošević N, Ljubojević D 2011. Effect of probiotic to the production of one month old carp. 7. Međunarodni gospodarsko – znanstveni skup o ribarstvu; Hrvatsko ribarstvo na pragu EU, Riba kao funkcionalna hrana, Croatia,Vukovar, pp 7-11.

14. Klaenhammer TR and Kullen MJ 1999. Selection and design of probiotics. International journal of food microbiology 50, 45-57.

15. Kozasa M 1986. Toyocerin (*Bacillus toyoi*) as growth promoter for animal feeding. Microbiolial Alimentary Nutrition 4, 121–135.

16. Kumar R, Mukherjee SC, Prasad KP and Pal AK 2006. Evaluation of *Bacillus subtilis* as a probiotic to Indian major carp *Labeo rohita* (Ham.). Aquaculture Research 37, 1215-1221.

17. Lall SP and Lewis-McCrea LM 2007. Role of nutrients in skeletal metabolism and pathology in fish—an overview. Aquaculture 267, 3-19.

18. Lara-Flores M, Olivera-Castillo L and Olvera-Novoa MA 2010. Effect of the inclusion of a bacterial mix (*Streptococcus faecium* and *Lactobacillus acidophilus*), and the yeast (*Saccharomyces cerevisiae*) on growth, feed utilization and intestinal enzymatic activity of Nile tilapia (*Oreochromis niloticus*). International Journal of Fisheries and Aquaculture 2, 93-101.

19. Lilly DM and Stillwell RH 1965. Probiotics: growth promoting factors produced by microorganisms. Science 147, 747-748.

20. Ljubojević D, Ćirković M and Miščević M 2013. Probiotics in carp fish nutrition. Veterinarski glasnik 67, 429-439.

21. Miščević M, Ćirković M, Jovanović R, Ljubojević D, Mašić Z, Novakov N and Marković M 2011. Effect of probiotics to the production of one-year old carp. 22nd International Symposium Food safety production, Bosnia and Hercegovina,Trebinje, pp 50-52.

22. Miščević M, Ćirković M, Jovanović R, Ljubojević D, Novakov N, Mašić Z and Marković M 2012. Effect of probiotics to the production of one-year old tench and common carp. Archiva Zootechnica 15, 29-36.

23. Nayak SK, Swain P and Mukherjee SC 2007. Effect of dietary supplementation of probiotic and vitamin C on the immune response of Indian major carp, *Labeo rohita*(Ham.). Fish & shellfish immunology 23, 892-896.

24. Panigrahi A and Azad IS 2007. Microbial intervention for better fish health in aquaculture: the Indian scenario. Fish physiology and biochemistry 33, 429-440.

25. Sahandi J, Jafaryan H, Roozbehfar R, Babae S, Dehestani M 2012. The use of two enrichment forms (*Brachionus plicatilis* enrichment and rearing water enrichment) with probiotic bacilli spore on growth and survival of Silver carp (*Hypophthalmichthys molitrix*). Iranian Journal of Veterinary Research 13, 289-295.

26. Sahu MK, Swarnakumar NS, Sivakumar K, Thangaradjou T and Kannan L 2008. Probiotics in aquaculture: importance and future perspectives. Indian journal of microbiology 48, 299-308.

27. Salinas I, Abelli L, Bertoni F, Picchietti S, Roque A, Furones D and Esteban MÁ 2008. Monospecies and multispecies probiotic formulations produce different systemic and local immunostimulatory effects in the gilthead seabream (*Sparus aurata*L.). Fish & shellfish immunology 25, 114-123.

28. Selvaraj V, Sampath K and Sekar V 2005. Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (*Cyprinus carpio*) infected with*Aeromonas hydrophila*. Fish & shellfish immunology 19, 293-306.

29. Suzer C, Çoban D, Kamaci HO, Saka Ş, Firat K, Otgucuoğlu Ö and Küçüksari H 2008. *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: Effects on growth performance and digestive enzyme activities. Aquaculture 280, 140–145.

30. Tewe OO, Losel DM and Abu OA 1999. Solid state fermentation of sweet potato using two monoculture fungi: Changes in protei fatty acids and mineral composition. Tropical Journal of Animal Science 5, 219-224.

31. Wang Y and Xu Z 2006. Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Animal Feed Science and Technology 127, 283–92.

32. Wang YB, Li JR and Lin J 2008. Probiotics in aquaculture: challenges and outlook. Aquaculture 281, 1-4.

33. Zhang X, Fu L, Deng B, Liang Q, Zheng J, Sun J and Li W 2013. *Bacillus subtilis* SC02 supplementation causes alterations of the microbial diversity in grass carp water. World Journal of Microbiology and Biotechnology 29, 1645-1653.

Original paper

PROTEIN LEVEL AND EFFICIENCY OF FEED MIXTURE FOR COMMON CARP (*Cyprinus carpio*)

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Abstract

The quality of fish feed and its nutritional value are important for fish production. From an economic point of view it is vital to ensure a cheaper final product while from the nutritional aspects it is essential to fulfill the requirements of fish. The aim of this study was to investigate the digestibility and effect of feed mixtures with different protein content on growth of carp fry. The experiment was carried out at the Laboratory for fish nutrition of the Faculty of Agriculture, University of Belgrade, for 90 days. Fish were fed with concentrate mixtures having 38% (A), 41% (B) and 44% (C) of proteins. The bigger share of the protein part in A were plant proteins (PP), in C fish meal (FM), while in B the share of FM and PP was approximately the same. Results showed significant differences between digestibility of different feed mixture. The digestibility of protein and fat were higher in fish fed diets with higher protein content. Digestibility of nitrogen-free extracts (NFE), energy and digestible energy was not statistically significant ($p > 0.05$) regardless of the level of protein in the diet. The results indicate that the increase in the digestibility of proteins is in accordance with the increase in protein content in the diet e.g. diets with higher content of FM.

Key words: *carp, digestibility, fish meal, plant proteins, protein levels*

Introduction

In fish, as in other domesticated animals, digestibility of feed is one of the most essential attributes in evaluating the efficiency of foodstuffs (Forster, 1999). Determination of apparent digestibility coefficient (ADC) of nutrients in fish feed is important for proper diet formulation (Salim et al., 2004). At the same time, analysis of this parameter helps in estimating the potential pollution of water by wastes produced by fish.

Digestibility of nutrients is variable and depends on a range of factors, where the level and origin of proteins in the diet is one of the most important. Since proteins are the most expensive part of feed mixtures (Wilson, 2003), by decreasing their content or by increasing the digestibility, the price of the final product, fish, can be decreased.

As the aquaculture is in continuous development, expanding and intensifying (Bostock et al., 2009), it is essential to involve different components in fish feed (Tacon, 2005) and provide their maximal utilization. Fishmeal (FM) is a major protein source in compound feeds for intensive fish farming. In an effort to reduce reliance on FM as the primary protein source, most aquaculture diets now use some plant protein ingredients (SOFIA,

2007). From the nutritional aspect, the best potential show components of animal origin, but are the most expensive, while components of plant origin have a lower nutritive value, but have moderate prices and are more available on the market (Storebakken et al., 2000). In order to create better production results, due to high prices of certain components (Aas et al., 2009), it is essential to know requirements of different fish species. In this sense the nutrition and preparation of fish feed is highly important.

Nutrient requirements for growth, reproduction and normal physiological functions are similar to other animals, but fish have much higher requirements in proteins, thus feed mixtures with 25 to 45% of raw proteins are mainly used (Davies and Gouveia, 2010; Firas and Ramadan, 2012). The various studies concluded that digestibility of nutrients and energy from various feedstuffs varies in different fish species (Salim et al., 2004).

From the economical, but also ecological point of view it is important to supply feed that will result in low feed conversion coefficient, high growth rate, good health condition, high quality of the final product – fish meat, and as low as possible load of the aquatic environment with organic matter, phosphorus, and nitrogen (Jahan et al., 2003).

The aim of this study was to determine the apparent digestibility coefficient of concentrate mixtures with different content and origin of proteins for carp yearlings.

Materials and methods

Experimental fish and culture system

Fingerlings of common carp (*Cyprinus carpio*) were obtained from the fish farm "Farmakom" from Dobrić, around Šabac, Serbia. The culture system was developed in the Laboratory for fish nutrition of the Faculty of Agriculture, University of Belgrade, Serbia. Prior to the experiment fingerlings were acclimatized for four weeks in plastics tanks. After acclimation, each tank was stocked with 24 yearlings, average weight 95.6 g.

We used a circulating system consisting of 9 independent tanks (three replicates per treatment), with 120 L of usable water volume and flow rate of 0.34 $Lmin^{1}$ .Water quality and environmental conditions (dissolved oxygen, water temperature, electroconductivity, and pH) were measured in each tank daily using MULTI 340i/SET (WTW, Weilheim, Germany). Air was supplied constantly by a blower to maintain the O_2 concentration around 6 mg/L. The water temperature (23±1°C) was controlled by a thermostat.

Feed ingredients and diet preparation

Fish were fed with concentrate mixtures having different amount of animal protein and plant origin. The bigger share of the protein part in A were plant proteins (PP), in C fish meal (FM), while in B the share of PP and FM was approximately the same (Table 1).

Fish were fed with same percentage of feed depending on the total fish biomass in each tank, i.e. 3.5% of the ichthyomass, using semiautomatic feeders with pendulum. For daily measurements of feed quantity, as well as for control measurements every 30 days, a digital balance CASBEE, model MW 120; Casbee, Samsungm Korea, accuracy 0.01 g was used, while an ichthyometer was employed for length and height measurements.

Table 1. *Composition of experimental diets (% dry matter)*

Feed	A	B	C
Fish meal	26.0	30.0	32.0
Soybean meal	29.0	30.0	31.0
Yeast	2.0	6.0	8.0
Wheat gluten	5.0	5.0	5.0
Wheat	11.5	11.5	11.5
Corn	24.0	15.0	10.0
DCP	1.2	1.2	1.2
Calcium	0.3	0.3	0.3
Min. Vit. premix	1.0	1.0	1.0
Total	100.00	100.00	100.00

Growth and nutrient utilization parameters

Growth performance and diet nutrient utilization were assessed in terms of:

Body weight gain (BWG, g) = final weight (g) - initial weight (g), Buyukcapar and Kamalak, 2006;

Metabolic growth rate (MGR, $gkg^{0.8}day^{-1}$) = BWG_g / (((initial weight / 1000)$^{0.8}$ + (final weight / 1000)$^{0.8}$) / 2) / feeding days, Dabrowski et al., 1986;

Feed Intake (FI, %) = (daily feed intake (g) x 100) / biomass (g), Diler et al., 2007;

Digestibility

Samples of feces were taken daily from the plastic collectors located at the bottom of every fish tank. Protein, carbohydrate, fat, and total energy digestibility were measured using a natural indicator; the Acid-insoluble ash (AIA) method.

ADCs in experimental diets were calculated according to the formula from Maynard and Loosli (1969):

$$Digestibility\ (\%) = 100 - ((F / D) * (Dm / Fm) * 100)$$

Where: F= % nutrient in faeces, D= % nutrient in diet, Dm= % marker in diet, and Fm= % marker in faeces.

Chemical and Statistical analysis

The basal diet was analyzed for dry matter, nitrogen, crude lipid, crude fiber, and ash and the feces for dry matter, nitrogen and ash, using standard methods (AOAC - Association of Official Analytical Chemists, 1990).

Statistical analysis was done using one-factorial analysis of variance with factor type of feed. Individual comparison of average values was carried out using Tukey test.

Results and discussion

Levene's test of experimental data showed that variation in tanks was not significantly different for measured parameters pointing out that the experimental setup was correct and

that differences in growth and nutrient utilization parameters were the result of different feed quality.

During the experiment water temperature was 22.75±0.02°C, with minimal differences in measured values between tanks during the day. Electroconductivity was 524.35±0.48 μS/cm; dissolved oxygen was on average 6.41±0.04 mg/L, and pH value was 7.48±0.01. According to Marković (2010), Flajšhans and Hulata (2007), Hover (1976), all the values monitored were within the optimal range for carp growth.

Proximate composition of feed ingredients is shown in Table 2. Experimental diets contained from 38.10 (A) to 43.72% (C) crude protein and from 19.60 to 19.98 kJ/g of gross energy. Dry matter, crude lipid and ash were in the range of 89.20–93.70%, 8.54–9.64% and 9.50–10.76%, respectively.

Table 2. *Chemical composition of experimental diets (% dry matter)*

Feed	**A**	**B**	**C**
DM gkg^{-1}	937	937	892
Protein	38.10	41.52	43.72
Lipid	8.54	9.07	9.64
Ash	9.50	9.61	10.76
Fiber	2.03	2.45	2.02
[1]NFE	41.83	37.35	33.86
Total	100.00	100.00	100.00
[2]Gross energy	19.60	19.84	19.98
[3]P/E	19.44	20.93	21.88

[1]NFE= 100 –proteins (g) –fat (g) –ash (g) –cellulose (g)
*[2]Gross energy = protein (g) * 23.6 + fat (g) * 39.5 + NFE (g) *17.3*
[3]P/E = Protein-energy ratio (g proteins (kJ)$^{-1}$gross energy

Based on the visual observation during of the experiment, palatability or acceptability of feed was good and the behavior of fish was normal. Daily feed intake was not significantly different between treatments (F=0.812; p=0.497), and was around 1.55-1.65%. After 90 days of feeding, final weight and body weight gain was significantly higher in the fish group fed with C (Table 3).

Fish fed with feed mixture C had a significantly higher (p<0.001) final weight (200.18 ± 6.19 g), BWG (104.60 ± 3.84) and MGR (11.04 ± 0.38) compared to fish fed mixture A and B (Table 3).

Table 3. - *Initial weight (IW), final weight (FW), body weight gain (BWG), metabolic growth rate (MGR), feed intake (FI) in common carp fed experimental diets*

Parameter	**A (***mean±SE)*	**B (***mean±SE)*	**C (***mean±SE)*	ANOVA	
				F	p
IW	95.59 ± 3.80^{NS}	95.33 ± 3.44^{NS}	95.23 ± 2.95^{NS}	0.016	$\approx 1.000^{NS}$
FW	149.79 ± 5.50^{a}	173.56 ± 6.78^{b}	200.18 ± 6.19^{c}	24.031	**<0.001****
BWG	54.64 ± 2.62^{a}	78.07 ± 1.20^{b}	104.60 ± 3.84^{c}	61.027	**<0.001****
MGR	6.29 ± 0.24^{a}	8.52 ± 0.12^{b}	11.04 ± 0.38^{c}	32.893	**<0.001****
FI	1.55 ± 0.05^{NS}	1.57 ± 0.03^{NS}	1.65 ± 0.13^{NS}	0.812	0.497^{NS}

Small letters indicate significant differences (p<0.05) across rows

Table 4. - *Apparent digestibility coefficient of experimental diets*

Ingredients	**A (***mean±SE)*	**B (***mean±SE)*	**C (***mean±SE)*	ANOVA	
				F	p
Protein	60.02 ± 0.89^{a}	69.31 ± 4.20^{ab}	74.66 ± 3.18^{b}	5.856	**0.020***
Lipid	74.86 ± 2.86^{a}	80.26 ± 1.07^{ab}	84.76 ± 2.27^{b}	4.854	**0.033***
NFE	66.22 ± 1.70^{NS}	74.74 ± 5.14^{NS}	67.50 ± 9.07^{NS}	0.903	0.481^{NS}
Gross energy	64.86 ± 1.33^{NS}	73.22 ± 3.81^{NS}	74.50 ± 4.44^{NS}	2.929	0.100^{NS}
DE	14.37 ± 0.42^{NS}	14.82 ± 0.58^{NS}	13.30 ± 1.09^{NS}	0.934	0.468^{NS}

Small letters indicate significant differences ($p<0.05$) across rows

The results of feces analysis (Table 4) showed significantly higher ($p \geq 0.020$) values of apparent digestibility coefficient of proteins (74.66%) and lipids (84.76%) in fish fed with mixture C compared to fish fed with A. The digestibility of NFE-nitrogen free extracts (from 66.22% to 67.50%), energy (from 64.86% to 74.50%) and digested energy (from 14.37% to 13.30%), did not differ significantly ($p>0.05$) in fish fed mixtures with different protein content.

The average values of ADC of proteins from different mixtures increased with the increase of the protein content 60.02% (A), 69.31% (B) and 74.66% (C). This resulted in a significant difference ($p<0.01$) between protein ADC in feed C and A. The analysis of ADC of fat (74.86% in feed A, 80.26% in feed B and 84.76% in feed C) resulted in significant difference between feed A and C. The average value for ADC BEM was 66.22% (A), 74.74% (B) and 67.50% in feed C. ADC energy was on average 64.86% in feed A, 73.22% in feed B and 74.50% in feed C. Digestibility DE was 14.37% (A), 14.82% (B) to 13.30% (C). Analysis of variance for average values of ADC BEM, energy and DE showed no significant differences regardless of the level of proteins in different feed mixtures.

Salim et al. (2004) confirmed that the digestibility coefficient in feed of animal origin is higher that feed of plant origin. Additionally, Kumar et al. (2010) pointed out that higher utilization of proteins is provided by their higher availability. Having in mind that feed C had the highest level of proteins and highest content of FM, the best protein source with good palatability, the highest digestibility was achieved with this feed mixture. Hossain and Jauncey (2003) emphasize that the results of ADC of proteins are actually the effect of amino acid digestibility, thus the results of this study show the precision in feed formulation.

Conclusion

The results suggest that with the increase of proteins in mixtures, their digestibility increases. A higher content of fish meal in C mixture provided significantly higher digestibility of proteins compared to mixture A with higher content of plant proteins. It is known that the utilization, digestibility and availability of plant proteins are lower than in proteins of animal origin.

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References

1. Alanärä A, Kadri S, Paspatis M 2001. Feeding management. In: Food Intake in Fish (Houlihan D, Jobling M, Boujard T, eds.). Blackwell Science, Oxford, UK, pp. 332–353.
2. AOAC, Association of Official Analytical Chemists 1990. Official Methods of Analysis. 15th edn., Washington, DC, USA.
3. Buyukcapar HM, Kamalak A 2006. Raw and heat–treated culban (*Vicia peregrina*) seed as protein source for mirror carp (*Cyprinus carpio)* fingerlings. South African Journal of Animal Science 36, 235–242.
4. Cho SH, Jo JY, Kim DS 2001. Effects of variable feed allowance with constant energy and ratio of energy to protein in a diet for constant protein input on the growth of common carp *Cyprinus carpio L*. Aquaculture Research 32, 349–356.
5. Dabrowski K, Murai T, Becker K 1986. Physiological and nutritional aspects of intensive feeding of carp. In: Billard R, Marcel J (eds) Aquaculture of cyprinids. INRA, Paris, pp 55–70.
6. Davies SJ, Gouveia A 2010. Response of common carp fry fed diets containing a pea seed meal (*Pisum sativum)* subjected to different thermal processing methods. Aquaculture 305(1–4):117–123.
7. Diler I, Tekinay AA, Guroy D, Guroy BK, Soyuturk M 2007. Effects of *Ulva rigida* on the Growth, Feed Intake and Body Composition of Common Carp, *Cyprinus carpio* L.. Journal of Biological Sciences, 7: 305-308.
8. Firas AMAJ, Ramadan SAS 2012. Evaluation of common carp *Cyprinus carpio* L. performance fed at three commercial diets. Mesoptamia J. of Agri. 4(4), 20–26.
9. Flajšhans M, Hulata G 2007. Genetic effects of domestication, culture and breeding of fish and shell fish, and their impacts on wild populations. Common carp – *Cyprinus carpio*. p 32–39, In: Svasand T, Crosetti D, Garcia-Vazquez E, Verspoor E (eds). Genetic impact of aquaculture activities on native populations. Genimpact final scientific report 2007 (EU contract n. RICA-CT-2005-022802).
10. Forster I 1999. A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. *Aquaculture Nutrition* 5:143–145.
11. Hossain MA, Jauncey K 2003. Studies on the protein, energy and amino acid digestibility of fish meal, mustard oilcake, linseed and sesame meal for commoncarp (*Cyprinus carpio* L.). Aquaculture 83, 59–72.
12. Hover RJ 1976. Vertical distribution of fishes in the central pool of Eufaula Reservoir, Oklahoma. MS. Thesis, Oklahoma State Univ. Stillwater. 72.
13. Kumar V, Makkar HPS, Becker B 2010. Dietary inclusion of detoxified *Jatropha curcas* kernel meal: effects on growth performance and metabolic efficiency in common carp, Cyprinus carpio L. Fish Physiology and Biochemistry 36, 1159–1170.
14. Marković Z 2010. Šaran, Gajenje u ribnjacima i kaveznim sistemima. Prof. dr Zoran Marković (2010), 152 str (In Serbian).
15. Maynard LA, Loosli JK 1969. Animal Nutrition, 6th edn. McGraw-Hill, New York, NY, 613 pp.

16. Ogunji JO, Nimptsch J, Wiegand C, Schulz C, Rennert B 2011. Effect of housefly maggot meal (magmeal) diets on catalase, and glutathione S–transferase in the liver and gills of carp *Cyprinus carpio* fingerling. International Aquatic Research 3, 11–20.

17. Salim M, Aziz I, Sultan JI, Mustafa I 2004. Evaluation of Apparent Digestibility of Fish Meal, Sunflower Meal and Rice Polishings for *Labeo rohita.* Pakistan Journal of Life and Social Sciences, 2(2): 139–144.

18. SOFIA 2007. The State of World Fisheries and Aquaculture 2006. FAO Fisheries and quaculture Department, Rome, pp. 1–180.

19. Wilson RP 2003. Amino acid requirements of finfish and crustaceans. In: D´Mello JPF (Ed.). Amino acid in farm animal nutrition. Wallingford: CAB International, 2003. p. 427–447.niloticus). Revista Brasileira de Zootecnia, v.34, n.5, pp.1433–1441, 2005.

Original paper

INSECTICIDAL ACTIVITY OF SAGE (*SALVIA OFFICINALIS*) ESSENTIAL OIL TO *VARROA DESTRUCTOR* (ACARI: VARROIDAE) AND *APIS MELLIFERA* (HYMENOPTERA: APIDAE)

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Abstract

The need to find alternative systems of the fight against *Varroa* mite without application of chemicals and provide healthy bee products resulted in investigation of application of different plant essences to arthropod control. In order to perceive the sage essential oil (*Salvia officinalis*) bioactivity, contact residual toxicity of mites and bees was examined in the laboratory conditions. The chemical composition of essential oil was determined by standard GC and GC/MS methods. Different doses of the sage essential oil dissolved in acetone (0.1–10 µl/Petri dish) were applied in Petri dishes and left to dry for 20 minute at a room temperature. Following this period of time, ten honey bees and five adult female mites were added in each Petri dish and they were all maintained in controlled conditions (T = 30°C, Relative humidity = 60%). Survival of examined honey bees and *Varroa* mites was recorded two times, after 24 h and 48 h. The most prominent toxic effect on the examined *Varroa* mites was observed after 24 h and 48 h, with application of 10 µl of sage oil (the average values for dead mite individuals were 3.25 and 3.50, respectively). Recorded biological activities of the oil tested in different doses on both honey bee and *Varroa* mite revealed opportunity to proceed with further investigation by selecting the most appropriate variants and combinations of the most prominent individual components of the examined sage oil.

Key words: *Apis mellifera, essential oil, Varroa mite*

Introduction

In modern agriculture presence of harmful residues in food as a result of use of pesticides in control of harmful organisms appeared as a great problem. Beekeeping suffers damages caused mainly by ectoparasite *Varroa destructor*, but also due to application of acaricides used to protect honey bees from this mite. Following use of synthetic chemical agents against mites, harmful metabolites use to be cumulatively deposited in wax, and from there they migrate to the honey and other bee products (Kochansky et al., 2001; Anne-Claire et al., 2007; Karazafiris et al., 2008). Solution to the problem is to be found in application of so-called "green pesticides", since their basis should be of plant origin, and they have to be “environmental friendly” (Gashout HA and Guzmán-Novoa E 2009; Umpiérrez et al., 2011). Preparations composed of plant essential oils seem to be promising for replacement of the synthetic substances used in combating *Varroa* mites. This way, it can also lower the

risk of harmful residues in bee products and their appearance in the food chain. So far, over 150 different essential oils have been tested, but only few of them have showed acceptable tolerability by bees and good efficacy in controlling *Varroa* mites (Lindberg et al., 2000; Imdorf et al., 2006). The complex composition of essential oils and a very low tolerability of honey bees in laboratory studies make difficult discovering the oil that would be safe for the bees and effective against the mites (Nedić et al., 2012).

The objective of this work was to examine in laboratory conditions the miticidal effect of the commercial *Salvia officinalis* essential oil against *Varroa destructor* and tolerance of bees to contact residual toxicity of the oil.

Materials and methods

The sage oil (*Salvia officinalis*) used in experiment, was purchased as commercial samples from "Elmar d.o.o.", Trebinje, the Republic of Srpska. Analytical gas chromatography (GC-FID) was performed on the GC HP-5890 Series II apparatus, equipped with autosampler (ALS), split-splitless injector, attached to HP-5 fused silica capillary column (25 mm × 0.32 mm, 0.52 μm film thickness) and fitted to flame-ionization detector (FID). Identification of individual essential oils constituents were accomplished by comparison of their mass spectra with those from available MS libraries (NIST/Wiley) and by comparison of their experimentally determined retention indices (calibrated AMDIS) with data from the literature (Adams, 2001).

Testing of bees tolerance to contact residual toxicity of essential oils, was conducted in Petri dishes, in four replications, under controlled laboratory conditions (T = 30° C; Relative humidity = 60%). Different essential oils doses dissolved in acetone (0.1–10 μL/Petri dish) were applied in Petri dishes (11 cm in diameter) and left to dry for 20 minute at a room temperature, and then the dishes were supplemented with 10 newly emerged adult bees (0 to 3 days old) and five adult female mites. The bees were fed with 3 g of candy and watered with water from a plastic micro tube (1.5 mL). Acetone was used as control. Survival of bees and mites in each Petri dish was recorded after 24 h and 48 h. The obtained results were processed by analysis of variance (LSD test at the significance level of 5 and 1%). Means were separated by the Duncan test at $P < 0.05$. Probit analysis (E in %) was used to calculate the contact residual toxicity of the essential oil for adult bee and *Varroa* mites.

Results and discussion

Chemical composition of *Salvia officinalis* essential oil used in our experiment is presented in Table 1.

Table 1. *Chemical composition of the commercial Salvia officinalis essential oil used in the laboratory studies*

No.	Compounds	%
1.	Salvene<cis->	0.22
2.	Tricyclene	0.16
3.	Thujene<alpha->	0.18
4.	Pinene<alpha->	2.74
5.	**Camphene**	**5.46**
6.	Pinene<beta->	1.67
7.	Myrcene	0.84
8.	Terpinene<alpha->	0.15
9.	Cymene<para->	0.99
10.	Limonene	2.00
11.	**Cineole<1,8->**	**5.88**
12.	Terpinene<gamma->	0.22
13.	Mentha-2,8-dien-1-ol<trans-para->	0.30
14.	**Thujone<cis->**	**27.91**
15.	**Thujone<trans->**	**3.91**
16.	**Camphor**	**19.84**
17.	Pinocamphone<trans->	0.15
18.	**Borneol**	**3.14**
19.	Terpinen-4-ol	0.40
20.	Terpineol<alpha->	0.11
21.	Myrtenol	0.29
22.	Bornyl acetate	2.43
23.	Sabinyl acetate<trans->	0.25
24.	Myrtenyl acetate	0.21
25.	Copaene<alpha->	0.11
26.	**Caryophyllene<trans-beta->**	**4.05**
27.	Aromadendrene	0.36
28.	**Humulene<alpha->**	**7.85**
29.	Caryophyllene<9-epi-trans->	0.16
30.	Dauca-5,8-diene	0.14
31.	Viridiflorene	0.26
32.	Cadinene<delta->	0.20
33.	Caryophyllene oxide	0.63
34.	**Viridiflorol**	**3.94**
35.	Humulene epoxide II	1.46
36.	Manool<13-epi->	1.07
	Total compounds identified (%)	99.67

Total of 36 compounds was identified in the oil and the most dominant components were as follows: *cis*-thujone, camphor and 1.8-cineol (oxygenated monoterpenes), α-humulene (sesquiterpene hydrocarbon), and camphene (monoterpene hydrocarbon), all together covering 66.94% of the oil.

Results on impact of different doses of *S. officinalis* oil on honeybee (*A. mellifera*) and *Varroa* mite (*V. destructor*) are presented in tables 2 and 3.

Table 2. *Tolerability of A. mellifera to toxic effect of the commercial Salvia officinalis essential oil*

Nº of variants	Doses assayed (µL/Petri dish)	After 24h		After 48h	
		Mean *A. mellifera*	E in % (K=0)	Mean *A. mellifera*	E in % (K=0)
1.	0.1	0.00[a]	0.00	0.00[a]	0.00
2.	0.5	0.00[a]	0.00	0.00[a]	0.00
3.	1.0	0.00[a]	0.00	0.50[a]	5.00
4.	2.5	0.00[a]	0.00	0.25[a]	2.50
5.	5.0	0.50[a]	5.00	1.75[b]	17.50
6.	10.0	2.50[b]	25.00	3.25[c]	32.50
7.	0.0 (Control)	0.00[a]	0.00	0.0[a]	0.00
		$LSD_{0.05}$=0.49 $LSD_{0.01}$=0.68		$LSD_{0.05}$=0.77 $LSD_{0.01}$=1.06	

Following 24 h application, doses ranging from 0.1 to 5.0 µL *S. officinalis* oil per Petri dish had no statistically significant influence on bees mortality (Table 2). The highest tested oil dose (10 µL/Petri dish) caused bees mortality of 25%, significantly differing ($P<0.05$) from all the other doses assayed. In the same observing period (24h), between the oil doses ranging from 0.1 to 5.0 µL/Petri dish (Table 3) there were no significant differences in mortality of *Varroa* mites, and their efficacy amounted to 15.79 % of dead mites. Similarly to honeybees, the mortality of mites following application of the highest *S. officinalis* essential oil dose (10 µL/Petri dish) significantly differed from all the other oil doses tested and amounted 63.16 % of dead mites.

Table 3. *Toxic effect of the commercial Salvia officinalis essential oil on Varroa destructor*

Nº of variants	Doses assayed (µL/Petri dish)	After 24h		After 48h	
		Mean *V. destructor*	E in % (K=0)	Mean *V. destructor*	E in % (K=0)
1.	0.1	1.00[b]	15.79	1.79[b]	31.58
2.	0.5	1.00[b]	15.79	1.75[b]	31.58
3.	1.0	1.00[b]	15.79	2.75[ab]	52.63
4.	2.5	1.00[b]	15.79	2.25[ab]	42.11
5.	5.0	1.50[b]	26.39	2.25[ab]	42.11
6.	10.0	3.25[a]	63.16	3.50[a]	68.42
7.	0.0 (Control)	0.25[b]	0.00	0.25[c]	0.00
		$LSD_{0.05}$=1.19 $LSD_{0.01}$=1.64		$LSD_{0.05}$=1.21 $LSD_{0.01}$=1.66	

Following 48 h of application, there were no statistically significant differences in residual toxicity on bees in applied *S. officinalis* oil doses ranging from 0.1 to 2.5 µL per Petri dish. The applied oil doses of 5 and 10 µL/Petri dish resulted in significantly and very significantly different toxicity from all other tested doses, respectively, causing 17.50 % and 32.50% of dead bees, respectively. Toxic effect of *S. officinalis* oil used in our experiment following 48 h of application proved to be the most prominent when 10 µL/Petri dish was applied, which caused death of 68.42 % mites, significantly differing from all the other doses, while for doses ranging from 1 to 5 µL/Petri dish, mites mortality ranged from 42.11 to 52.63%.

The obtained results showed that the lower oil dose of 1 ml / Petri dish caused more dead mites in comparison to application of oil doses of 2.5 and 5 µL/Petri dish. This may be

explained by the fact that for the toxic activity of an essential oil, in addition to the age of the plant and the part of the plant used for oil extraction and method of oil extraction used, the age of the relevant harmful organism, in our case *Varroa destructor*, also plays an important role (Sampson et al., 2005). The complex composition of an essential oil, effect of its constituents, synergistic effects and variation of the content of even minor constituents can all lead to significant differences in residual toxic effect of tested essential oil.

If we stick to the criterion for selection of appropriate chemical for control of *Varroa* mite, as proposed by Lindberg et al., 2000 (i.e. it has to kill more than 70% of mites and less than 30% of bees), doses of our *S. officinalis* essential oil, ranging from 5 to 10 μL/Petri dish seem to be appropriate for further investigation on honeybees in the field conditions.

Lethal and sublethal effects of essential oils and their individual components on honeybees and *Varroa* mites have been already presented in several previous studies. Colin (1990) proved good efficacy of *Thymus vulgaris* essential oil (major oil constituents: p-cymene and thymol) and *Salvia officinalis* oil (major oil constituents: α-thujone, camphor and eucalyptol) on suppression of *Varroa* mites. Good bee tolerance to the sage oil (major oil contents: α-thujone, camphor, α-humulene, eucalyptol, caryophylene and β-thujone) in laboratory experiment of Imdorf et al. (2006) where the bees and *Varroa* mites were placed in Liebefeld cages and treated by the air enriched with sage oil (300-500 mg/L) what induced 100% mortality of the mites and 10-20% of the honey bees. In the same investigation the authors also proved that individual oil component, camphene, influenced an increase in mites mortality (from 60 to 100%) but also an increase in bees mortality (from 20 to 40%). Ruffinengo et al., (2007) presented great bees mortality due to activity of individual oil component, 1.8 cineol, while Imdorf et al. (1995) used the flow of air through the desiccator with bees and mites which contained 50-150 g / L of individual oil component, camphor, succeeded to achieve *Varroa* mites mortality without significant toxicity to the bees.

In our investigation, we have observed that following the initial toxicity of the *S. officinalis* essential oil applied in doses of 5 and 10 μL/ Petri dish, in the first 24 h the oil toxicity was pronounced and it was prolonged in the next 24 hours. Furhtermore, the oil activity was more pronounced on *Varroa* mites comparing to honeybees, which could the most probably attributed to specific composition of the *S. officinalis* essential oil used in our experiment (Table 1), particularly the monoterpene oil portion (oxigenated monoterpenes and monoterpene hydrocarbons).

Biological control is a very sensitive tool to combat harmful organisms and is considered as a safe alternative to chemical control (Onstad and McManus, 1996). However, it also carries the risk and possibility to cause adverse effects to non target organisms, thus imposing necessity to take in consideration interactions of a number of different factors and to try to quantify the final outcome. Until recently, there was little or no evidence that biological control has adverse effects on non-target species. As a result, it was considered that these measures are generally safe, and not likely to have significant effects on non-target organisms. However, as pointed out by Hovarth (1991) "the absence of proof is not a proof of the absence" of such effects. No-target effects can be direct (introduced biological agent attacks non target organisms) or indirect (through the effects on goal which is successfully controlled, the impact on the ecosystem, food changes, etc.). Fans of biological control have to accept that there are dangers and risks associated with the use of biological measures due to control of harmful agents and is therefore necessary to pay attention to the consequences of its non target impact and risk possibilities. The "impact" is what is actually happening when implementing biological measures, while the "risk" is a

priori evaluation of the possible impact of events. Therefore, the impact may be as follows: a high risk of minimal impact, or a low risk of enormous influence, or any combination of the two. In many cases, it is very difficult to evaluate a potential non-target impact or the risk of it. The combination of the overall risk and various potential impacts needs to be balanced in terms of benefits and likelihood of providing them (Cock, 2002). Therefore, our research conducted with the *S. officinalis* essential oil has been done in parallel to *A. mellifera* and *V. destructor*.

Conclusion

In order to overcome the consequences of using the synthetic preparations to protect honeybees from *Varroa* mites, such as the mite resistance and residues in honey and honey products, in the present study we have examined an alternative method with the use of essential oil of plant origin. In order to examine the oil toxic effects on the bees, we have applied the oil of *S. officinalis* at doses ranging from 0.1 to 10 μL per Petri dish.

The oil, applied at doses ranging from 1 to 5 μL, had an acceptable toxic efficacy to *A. mellifera* (from 2.50 to 17.50 %), and a somewhat satisfactory efficacy to *Varroa destructor* (from 31.58 to 52.63%). When applied at a dose of 10 μL per Petri dish, the oil caused toxicity to bees slightly above the eligibility threshold (32.50%), the same toxicity of the oil dose being more pronounced to *V. destructor* (68.42%).

The results indicate that the *Salvia officinalis* essential oil may be promising for the application against *V. destructor* which we set for our future research goals.

Acknowledgments

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References

1. Adams RP 2001. Identification of Essential Oil Component by Gas Chromatography / Quadrupole Mass Spectrometry. Carol Stream, Illinois, pp.1-456, Allured Publ. Corporation, USA.
2. Anne-Claire M, Sarah Z, Clément A, Patrick D, Jean-Paul F and Michel A. 2007. Acaricide residues in honey and wax after treatment of honey bee colonies with Apivar® or Asuntol® 50. Apidologie 6, 534-544.
3. Cock MJW 2002. Risks of non-target impact versus stakeholder benefits in classical biological control of arthropods: selected case studies from developing countries. 1st International Symposium on Biological Control of Arthropods, 25-33.
4. Colin ME 1990. Essential oils of Labiatae for controlling honeybee varroosis. J. App. Ent. 110, 19-25.
5. Gashout HA and Guzmán-Novoa E 2009. Acute toxicity of essential oils and other natural compounds to the parasitic mite, *Varroa destructor*, and to larval and adult worker honey bees (*Apis mellifera* L.). Journal of Apicultural Research 4, 263 – 269.
6. Howarth FG. 1991. Environmental impacts of classical biological control. Annual Review of Entomology 36, 485-509.

7. Imdorf A, Bogdanov S, Kilchenmann V and Berger T 2006. Toxic effects of essential oils and some of their components on *Varroa destructor* Oud. and *Apis mellifera* L. under laboratory conditions“, *ALP Science* 495, 3-18.
8. Imdorf A, Kilchemann V, Bogdanov S, Bachofen B and Beretta C 1995. Toxic effects of thymol, camphor, menthol and eucalyptol on *Varroa jacobsoni* Oud. and *Apis mellifera* L. in a laboratory test. Apidologie 26, 27-31.
9. Karazafiris E, Tananaki C, Menkissoglou-Spiroudi U and Tharsyvoulou A 2008. Residue distribution of the acaricide coumaphos in honey following application of a new slow-release formulation. Pest management science 2, 165-71.
10. Kochansky J, Wilzer K and Feldlaufer M 2001. Comparison of the transfer of coumaphos from beeswax into syrup and honey. Apidologie 32, 119-125.
11. Lindberg CH, Melathopoulos AP and Winston ML 2000. Laboratory evaluation of miticides to control *Varroa jacobsoni* (Acari: *Varroidae*), a honeybee (Hymenoptera: *Apidae*) parasite. J. Econ. Entomol. 93, 189–198.
12. Nedić N, Andrić G, Kostić M, Marković T, Kljajić P, Stanković S and Marković M, 2012. Tolerance of honey bees on three commercial essential oils. Proceedings of the Seventh Conference on Medicinal and Aromatic Plants of Southeast European Countries, Subotica, Serbia, pp. 362-369.
13. Onstad DW and McManus ML 1996. Risks of host range expansion by parasites of insects. BioScience.
14. Ruffinengo S, Maggi M, Faverin C, Garcia de la Rosa SB, Bailac P, Principal J and Eguaras M 2007. Essential oils toxicity related to *Varroa destructor* and *Apis mellifera* under laboratory conditions. Zootec. trop. 1, 63-69.
15. Sampson BJ, Tabanca N, Kirimer N, Demirci B, Hunsu Can Baser K, Khan IA, Spiers JM and Wedge DE 2005. Insecticidal activity of 23 essential oils and their major compounds against adult Lipaphis pseudobrassicae (Davis) (Aphidae: Homoptera). Pest Manag. Sci. 61, 1122-1128.
16. Umpierrez M, Santos E, Gonzalez A and Rossini C 2011. Plant essential oils as potential control agents of varroatosis. Phytochemistry Reviews 2, 227-244.

Original paper

MATHEMATICAL FORMULA OF A CONE MODEL USED FOR CALCULATION OF SNAIL SHELL VOLUME

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Abstract

Problem statement: There are many species of *Helix* snails, each with its own unique exoskeleton shape, or shell shape. Principal analysis of measurement data from snail shells occupied by *Helix* points out the importance of the description and quantification of the snail shells. The present paper is aimed to compare some of the formulas in the literature used to determine the volume of snail shape.
Hypothesis: The methods and formulae which exist do not reflect the real shell volume (at least not precisely enough) but rather use the external measurement and on that basis they draw conclusions for the biomass and the development stage.
Organisms: 142 species of the Turkish snail (*Helix lucorum*).
Approach: The purpose of this study is to improve and to offer a much better and accurate formula for calculations of the shell volume.
Conclusion: Our results support the usage of the formulae, and confirm some of these formulas while disproving others. As a result we developed a formula which takes into consideration the varying shape and thickness of the shell and reflects the real shell volume in most of the cases.

Keywords*: Helix lucorum, mathematical formula, snail shell*

Introduction

Scientific interest in land snails may have been caused by the fact that they are quite numerous species which inhabit different ecological niches and occupy different levels in food chains on land (Dedov, 2002). The growing interest in the technology of growing snails, the consumption of meat and their products cause massive interest in different types and their sizes (Georgiev and Atanasov, 2011). The research of specific types of features of the snail shells is essential for morphological characteristics and the relationship between volume and quantity of edible part of the snail meat. A number of models have been developed to describe the logarithmic form of coiled snail shells, some of which were also used to calculate the internal volume of the hull, surface area and material of shells (Graus and Raup, 1972; Stone, 1997; Williams and Price, 2008). Volume of the shells is rarely used in research, probably because it is not easy to use linear measurements. For the first time Moseley (1838) developed a series of equations to calculate volume, surface area and center of gravity of the spiral coiled shell. He intended these formulae to be applied in

functional morphological studies, but the difficulty in measuring the necessary parameters made their use impossible. Trueman (1941) developed another set of equations based on simplifying assumptions. Raup and Chamberlain (1967) recognized and converted the more accurate inadequacies of Trueman`s equations. When used in research, capacity is often determined based on linear dimensions of the shell (Solem and Climo, 1985; McClain and Nekola, 2008), but rarely measured directly (Kemp and Bertness, 1984; Örstan, 2011). Internal volume of the snail shell is an important indicator because it defines the space available for vital activity of the snail (Örstan, 2006). Besides this the shell volume of the shell is related to quantity of gametes produced (Heath, 1985), as well as a meat quantity.

Materials and methods

To fulfill the purpose of the study 142 shell snails of the genus *Helix lucorum* were examined. The used specimens were collected in the region of Stara Zagora, Bulgaria, during May and June 2011. Collected snails were treated initially with 20% solution of NaCl by the method described by Özogul et al. (2005). Afterwards, the internal organs were removed, and the shells were dried (Sarma, 2006).

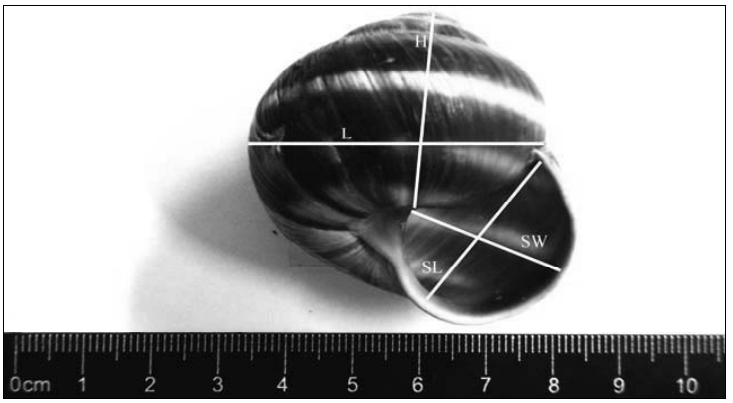
To measure the volume [V (cm^3)] of the shell, the method with calibrated sand (3 mm) of Sarma (2006) and Yamaguchi et al. (2009) was modified, according to the snail type. When filled with water, the shell was tightly closed with foil, ensuring the formation of a vacuum and was weighed on an analytical balance. The difference between the value of this empty shell and shell filled with distilled water presents values for the volume of the cavity.

For the greatest length [GL (mm)] the distance from the dorsal part - sutures to the caudal part - outer lip of the shell was measured.

For the greatest width [GW (mm)] longest distance between the two sides of the shell was measured.

Height [H (mm)] distance was measured from the top - sutures to the deep pit in the center of the shell – umbilicus (the entire length of solumella). The width [W-mm] distance was measured between the two vertical sides of the shell, just above the operculum.

Short length [SL (mm)] and width [SW (mm)] are perpendicular lines which represent the distance between the outer lip and inner lip of the shell. All the measurements were performed using a digital caliper with the accuracy of 0.1 mm (Mihaylov and Dimitrov, 2010).



In theory, a different 3D objects can be generated by simply rotating plane curves. But the forms and shapes that occur in nature are much more complex and intrinsic which make them extremely unsuitable for simulation. In such a way one can think of generating the snail shell by rotating the logarithmic curve (spiral) around one of the coordinate axis. Some researches on different snail shapes point out sections which are almost spirally arranged. Logarithmic spiral is a plane curve and in polar coordinates (r,θ) it can be written as

$$r = ae^{b\theta} \quad \text{or} \quad \theta = \frac{1}{b}\ln\left(\frac{r}{a}\right)$$

e = base of natural logarithms; $a,\ b$ = arbitrary positive real constants.

If $y = f(x)$ is a continuous non-negative function in the interval $[a,b]$ and consider the 3D object V which one can get by rotating $y = f(x)$ around Ox in the points $x = a$ and $x = b$ perpendicular to the Ox then the volume of V can be found by well-known formula in analysis:

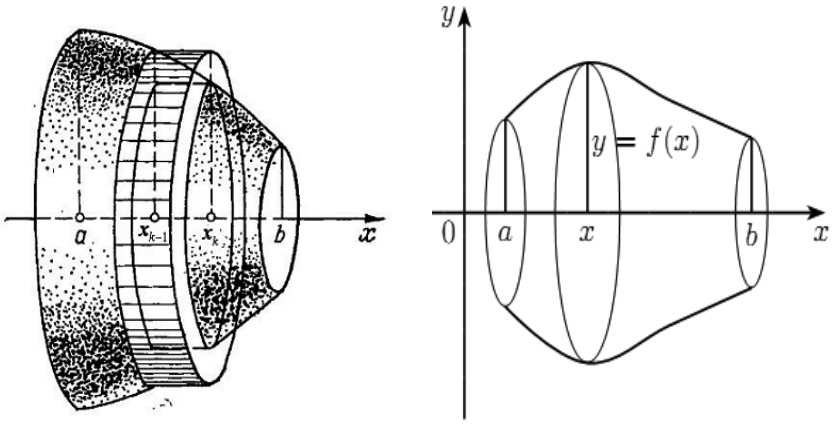


Figure 2. *Generation of a 3D object by rotating around the axis*

$$\mu(V) = \pi \int_a^b f^2(x)dx.$$

This classical approach, anyway, is quite inefficient for our purposes due to the irregularity of the snail shapes and the cavities inside the shells. Our approach is based on computation of the shell volumes using very basic measurements.

We measure four parameters of the snail shell, namely we measure *GL*, *GW, SL,* and *SW* (in *mm*) (see Figure 1), and we set the volume V_z of the snail shell as:

$$\mu(V_Z) = \frac{1}{3}\pi\left(\left(\frac{GW}{2}\right)^2 xGL + \left(\frac{SW}{2}\right)^2 xSL\right)$$

Our mathematical model is based on calculating the volumes of two cones.

Statistical methods. Data is reported as means ± Std.Dv. (N = number of snails), unless otherwise stated, and all statistical analyses were performed using STATISTICA version

6.0 (StatSoft Inc., 2002). Significant effects ($p<0.05$) were determined after applying t-test for dependent samples.

Results and discussion

V_A indicate the results of calculations by the formula:

$$V_A = \frac{1}{3}\pi\left(\frac{Shell\ width}{2}\right)^2 x(Shell\ height)$$

V_B are the results of calculations of the second formula for land snails (McClain and Nekola, 2008):

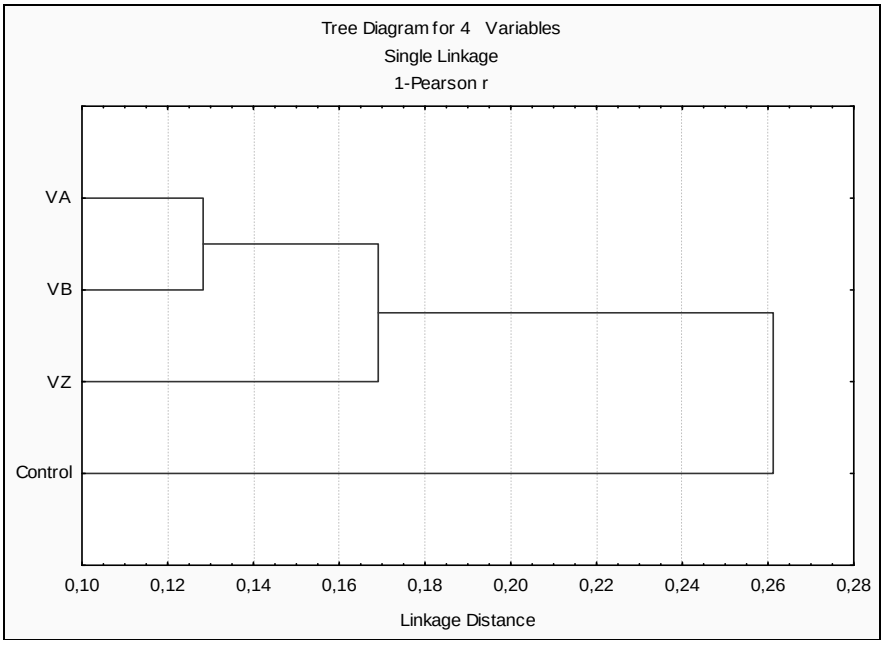
$$V_B = ((\pi r^2(1-t))h + \frac{\pi r^2 th}{3}.$$

The results of the calculations in both formulae are substantially different from actually determined volume of the shell with distiled water V (control group). Most probably these deviations are due to the various geometric shapes of the shell of this kind of land snail.
To determine whether the proposed formula provides similar results with practical application various parameters and their replacement in the formula have to be taken in consideration.

Table 1. *Statistical parameters for the groups*

	Mean	**Std.Dv.**	**N**	**Diff.**	**Std.Dv.**	**t**	**df**	**p**
$\mathbf{V_A}$	-4.07308	1.803816						
$\mathbf{V_Z}$	1.29597	1.528375	142	-5.36904	1.522663	-22.5780	40	< 0.05
$\mathbf{V_B}$	-4.70156	1.582109						
$\mathbf{V_Z}$	1.29597	1.528375	142	-5.99752	1.200752	-31.9824	40	< 0.05

At the end a Cluster analysis is presented (Dendogram 1). The results show that grouping a set of objects in such a way that objects in the V_Z group (called a cluster) are more similar V (0.17) comparing groups V_A and V_B (0.13). Correlation effects can be also seen in the dendogram.



Dendogram 1. *Correlation effect*

When comparing the results obtained from both formulae, very close values or minor deviations are present.

Conclusion

This investigation provides practical and useful information for calculation of snail shell volume. This study contributes to a description of the new mathematical formula of a cone model which could be used to extend existing information and improve calculation of the internal space of the shell available for vital activity. These results could be important for the snail producers and researchers for improving process of cultivation of land snails**.**

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References

1. Graus R, Raup D 1972. General Equations for Volume and Surface Area of a Logarithmically Coiled Shell. Mathematical Geology 4, 4, 307-316.
2. Heath D 1985. Whorl overlap and the economical construction of the gastropod shell. Biological Journal of the Linnean Society 24, 165-174.

3. Kemp P, Bertness M, 1984. Snail shape and growth rates: Evidence for plastic shell allometry in *Littorina littorea*. Proceedings of the National Academy of Sciences USA 81, 811–813 pp.
4. McClain C, Nekola J, 2008. The role of local-scale processes on terrestrial and deep-sea gastropod body size distributions across multiple scales. Evolutionary Ecology Research 10,129–146.
5. Mihaylov R, Dimitrov R 2010. Volume and size of the cranial cavity in some animals from *Felidae* family. Journal of Animal Science XLVII, 5, 67-76.
6. Moseley H 1838. On the geometrical forms of turbinated and discoid shells. Philosophical Transactions of the Royal Society of London 128, 351-370.
7. Örstan A 2006. Antipredatory function of the retractibility of *Batillaria minima* into its shell. Triton 14, 1-2.
8. Örstan A 2011. A method to measure snail shell volumes. Triton 23, 1-2.
9. Raup D, Chamberlain J 1967. Equations for volume and center of gravity in ammonoid shells. Journal of Paleontology 41, 566-574.
10. Rohlf F 2004. tpsDig, digitize landmarks and outlines, version 2.0. Department of Ecology and Evolution, State University of New York at Stony Brook.
11. Sarma K 2006. Morphological and craniometrical studies in the skull of Kagani goat (*Capra hircus*) of Jamu region. International Journal of Morphology 24, 3, 449-455.
12. Schmidt-Nielsen K 1984. Scaling: Why is animal size so important? Cambridge University Press, UK.
13. Solem A Climo F, 1985. Structure and habitat correlations of sympatric New Zealand land snail species. Malacologia 26,1-30.
14. Stone J 1997. Mathematical determination of coiled shell volumes and surface areas. Lethaia 30, 213-220.
15. Trueman A 1941. The ammonoid body-chamber with special reference to the buoyancy and mode of life of the living ammonoid. – Quaternary Journal of the Geological Society of London, 96, 339-383.
16. Williams R, Price R 2008. A mathematical model to determine the volume of snail shells.
17. Yamaguchi N, Driscoll C, Kitchener A, Glissen E, MacDonald D, 2009. Brian size of lion (*Pantera leo*) and tiger (*Pantera tigris*): implications for intragenic phylogeny, intraspecific differences and the effects of captivity. Biological Journal of Linneam Society 98, 85-93.

Original paper

ORGANIC LAMB MEAT PRODUCTION IN SERBIA BASED ON AUTOCHTHONOUS ZACKEL BREED: OPPORTUNITIES AND CHALLENGES

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Abstract

The global growing tendency of lamb and mutton meat production has been emphasized. The fastest growing meat production industry on a global level is lamb and mutton meat production. Lamb meat production increase is primarily based on genetic improvement of sheep breeds, reproduction management and disease reduction. The new Strategy for Agriculture and Rural Development in Serbia from 2014 to 2024 defines goals and priorities for further development of agriculture. One of priority topics is the promotion of organic production. Organic lamb production is a great opportunity for rural regions development. Botanical composition analyses, as well as plants nutrition value, have shown that natural resources of regions in Serbia have great potentials for organic lamb production.

Basic principles of organic production promote well adapted, autochthonous breeds. Various types of Zackel sheep are important genetic resources, evolutionary adapted on specific conditions and extreme climate. The study evaluated the value of various types of Zackel sheep populations in the regions where they are traditionally reared with special emphasis on some traits such as health status and robustness, important for organic production. Disease frequency analysis in different Zackel type populations has shown that gastrointestinal parasitic infections represent the main health problem in sheep farming. The introduction of veterinary medicine standards in organic sheep production is a great challenge. Considering that conventional prevention and medication are forbidden in organic production, veterinary control of parasitic infections is not satisfying. Our investigations are focused on preventive measure and genetic resistance/tolerance to parasitic infections of various types of Zackel sheep as very important for organic lamb production.

Key words: *organic sheep production, veterinary control, Zackel breed*

Introduction

The global growing tendency of lamb and mutton meat production has been evidenced. Lamb meat production increase is primarily based on genetic improvement of sheep breeds,

reproduction management and disease reduction (Deblitz, 2013). Increasing interest for lamb meat production has positive effect on development of organic sheep production. The organic ovine production is dominated by three Member States: the United Kingdom (1 161 717 heads), Italy (705 785 heads) and Spain (614 413 heads), representing together 62.7% of the entire EU organic herd (3.9 million heads), (EC 2013). The support to this production system provides scientific information that highlight differences between conventional and organic systems. Organic products are considered to be safer for human consumption, since organic production must adhere to strict regulatory measures, regarding acceptable levels of compounds harmful to human health such as synthetic fertilizers, pesticides, pharmaceutical products, or other medications. The majority of studies indicate that, in various management systems, feeding regimens have the greatest impact on the composition of nutritionally relevant components in milk and meat (Schmid et al., 2006). Animal products with a high content of polyunsaturated fatty acids, conjugated linolenic acid (CLA) and antioxidants, such as carotenoids and vitamin E, are more preferable from a nutritional standpoint. The particular consideration was given to examining fatty acid composition and CLA content in milk and meat from organic and conventional systems (Nudda et al., 2011). In many ways, these fatty acids are beneficial to human health, since they play an important role in protecting the organism from cardiovascular diseases, as well as from certain types of malignant and autoimmune diseases, such as lupus, rheumatoid arthritis, etc. (Connor, 2009). In recent years, the demand for organically produced meat has been increasing. Current studies report higher levels of nutritionally desirable compounds in meat from organic production systems compared to meat from conventional systems. Other studies have either reported no significant difference in composition between organic and conventional meat, or the results have been inconsistent. Considering that lamb is one of the most sought after animal products in the marketplace today, an extensive study was undertaken in Great Britain to determine if there were any differences in the quality of meat raised under either organic or conventional production systems (Angood et al., 2008). Results from this study recorded the nutritional quality, fatty acid composition and a sensory panel; data indicated that organic lamb had better eating quality than conventional lamb in terms of juiciness, flavor and overall liking, thus providing some evidence for the perception among consumers that organic products "taste better". Differences in flavor were attributed to differences in fatty acid composition, in particular, the higher level of linolenic acid and total n-3 PUFA in organic meat. Bearing in mind that suckling lamb, raised exclusively on maternal milk from birth to slaughter, is an important commodity raised typically in Mediterranean areas and that it yields a high-quality product of great economic significance for this region, a comparison of the sensory characteristics of suckling lamb meat from organic and conventional production systems has been conducted and the data evaluated (Revilla et al., 2009). Results obtained show that the appearance of organic meat was more fibrous, darker, and with a lower aroma intensity than the conventional counterpart, but with no differences in homogeneity or juiciness. In grilled meat, the organic samples had less subcutaneous fat, were less marbleized, had a less fibrous texture and less aroma intensity, and were less juicy as well. When ranking overall consumer satisfaction, higher scores were given to the organically produced samples.

The new Strategy for Agriculture and Rural Development in Serbia from 2014 to 2024 defines goals and priorities for further development of agriculture. One of the priority topics is the promotion of organic production. Organic lamb production is a great opportunity for rural regions development.

Botanical composition analyses, as well as plants nutrition value, have shown that natural resources of hilly-mountainous regions in Serbia have great potentials for organic lamb production. Grasslands in Serbia represent natural resource and great production potential in development and improvement of livestock production, especially in hilly-mountainous regions which have not been exploited to the full extent so far (Lazarević et al., 2010; Vučković et al., 2010; Simić et al., 2011).

In the field of organic farming the knowledge transfer is particularly important since this type of farming is an example of an innovation system (Niggli et al., 2008). The specific characteristics of organic production models based on the use of local resources can have a significant impact on promoting development of individual regions themselves.

Materials and methods

The study was performed on a total of 100 sheep (aged 12 to 18 months), on strains of autochthonous Zackel sheep breed (Sjenica and Lipe sheep) in regions where they are traditionally reared (Sjenica-Pešter plateau and in the region of Lipe village in lower Morava valley). Investigations were performed on the farms included in the program of conversion from conventional to organic meat production, according to the standards regulated by the Law on organic production (the Official Gazette of the Republic of Serbia 30/10).

Health status and robustness, important for organic production, were examined by clinical and laboratory investigations. Body condition score (BCS) has proved to be an important practical tool in assessing sheep body condition. Scoring was performed in sheep using a BCS ranging from 1.0 to 5.0.

Sampling of feces for parasitological tests was performed during the course of three days, in the morning and in the evening. The distribution of parasites in sheep was evaluated using the native smear coprological technique, by sedimentation and flotation methods. The degree of infection intensity per sheep was quantitatively established by the method of McMaster (modified by Zajiček). The results were statistically processed using the program GraphPad Prism.

Results and discussion

Basic principles of organic production promote well adapted, autochthonous breeds. They are evolutionary adapted to specific conditions and have a higher tolerance to bacterial pathogens and viral and parasitic infections, which is very important for organic production (Karrow et al., 2014). Various types of Zackel sheep are valuable genetic resources (Savić et al., 2011, and 2012; Savić et al., 2013a and 2013b). Most of them are included in the process of conservation of animal genetic resources (Food and Agriculture Organization, FAO 2010).

According to the preliminary results, opportunities for organic lamb production are in the favorable fatty acid profile and sensory characteristics obtained in some types of Zakel sheep, included in the process of conversion from conventional to organic meat production in traditional habitat.

Body condition score (BCS) is a simple indicator of available fat reserves used by animals in periods of high energy demands, stress, or suboptimal nutrition. In tested populations of Sjenica and Lipe sheep the average BSC was determined.

Health control in Sjenica sheep population has shown a contagious bacterial footrot disease and infectious keratoconjunctivitis in summer period.

Parasitic infections are of great significance for animal welfare and for economics. Economical harms due to parasitic infections in sheep include emaciation, reduced fertility and low milk and meat yield.

Coprological examinations revealed various extensities and intensities of parasitic infections in strains of autochthonous Zackel sheep in the studied farms. Parasitic infections in the Sjenica sheep population were detected as follows: *Trichostongylidae*, *Nematodirinae*, *Protostrongylidae*, *Trichuris* spp*, Anoplocephalidae* and *Eimeria* spp*.* A high percent of sheep (60%) was simultaneously infected by three parasitic infections *Trichostongylidae*, *Protostrongylidae* and *Anoplocephalidae*, while 15% of sheep were simultaneously infected by four parasitic infections *Trichostongylidae*, *Protostrongylidae*, *Trichuris* spp and *Anoplocephalidae*. In the examined population 20% percent of sheep were simultaneously infected by two parasitic infections *Trichostongylidae*, *Protostrongylidae*.

The results of testing the intensity of parasitic infection have shown the mild intensity of *Trichostongylidae* (438±304) and *Trichuris spp* (28±9), moderate intensity of *Protostrongylidae* (101±34), moderate (81±19) and high intensity (156±25) of *Anoplocephalida* and mild (67±32), moderate (432±154) and high intensity (640±33) of *Nematodirinae*.

Parasitic infections in the Lipe sheep population were detected as follows: *Trichostongylidae*, *Trichuris* spp and *Strongyloides papillosus.* Analyses of extensivity of parasitic infections in the Lipe sheep population have shown that 70% of sheep were negative for the presence of parasitic infections. The results of testing the intensivity of parasitic infections have shown a mild intensivity of *Trichostongylidae* (55±46), *Trichuris* spp (11±3) and *Strongyloides papillosus* (41±2) infections.

The disease frequency analysis in autochthonous Zackel types (Sjenica and Lipe sheep) has shown the presence of infections by parasites *Trichostongylidae*, *Nematodirinae*, *Protostrongylidae*, *Trichuris* spp*, Anoplocephalidae* and *Eimeria* spp*.*, the fact that requires intensive treatment (Dimitrijević et al., 2013).

Conclusion

From the veterinary aspect of animal health there are many problems in organizing organic sheep production. Considering that conventional prevention and medication are forbidden in organic production, veterinary control of parasitic infections is not satisfying. New methods of nematode control include several methods, such as biological agents, biological antihelminths and strategic grazing management, the methods that have not yet been efficient enough. Our investigations showed that, in our conditions, preventive measures and strategic grazing management must be focused on in the process of conversion. The selection of breed of choice for organic production is a very important decision. The attention in organic breeding must be based on BCS-traits, considering that sheep with fat score 3 are less susceptible to worm

infections. Genetic improvement based on genetic resistance/tolerance to parasitic infections is of great importance for animal health and organic lamb production. Therefore, our investigations are directed towards using SOD 1 as a biomarker of parasitic infections intensity in sheep (Dimitrijević et al., 2013).

Acknowledgements

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References

1. Angood KM, Wooda JD, Nute GR, Whittington, FM, Hughes SI and Sheard PR 2008. A comparison of organic and conventionally-produced lamb purchased from three major UK supermarkets: Price, eating quality and fatty acid composition. Meat Science 78(3), 176-184.
2. Connor WE 2009. Importance of n-3 fatty acids in health and disease. The American Journal of Clinical Nutrition 71(1), 171-175.
3. Deblitz C 2013. Global meat production, drivers and challenges, Beef and Sheep Conference, June 13-20, York , England, 10 pp.
4. Dimitrijević B, Borozan S, Jović S, Bacić D, Katić Radivojević S, Stojanović S and Savić M 2013. The effect of the intensity of parasitic infection with *Strongyloides papillosus and albendazole* therapy on biochemical parametars in sheep blood. Acta veterinaria 63,581-601.
5. European Commission 2013. DG Agriculture and Rural Development, Facts and figures on organic agriculture in the European Union. pp 1/46
6. FAO 2010 Breeding strategies for sustainable management of animal genetic resources. FAO Animal Production and Health Guidelines No.3.
7. Karrow N, Goliboski K,Stonos N, Schenkel F and Peregrine A 2014. Review: Genetics of helminth resistance in sheep. Canadian Journal of Animal Science 94,1, 1-9.
8. Lazarević D, Dajić Z. Vučković S and Terzić D 2010. Production of livestock food on natural and sown grasslands, Biotechnology in Animal Husbandry 26, 219-234, 2010.
9. Niggli U, Slabe A, Schmid O, Halberg N. and Schluter M 2008. Vision for an organic food and farming research agenda to 2025 organic knowledge for the future, IFOAM EU Group.
10. Nudda A, McGuire MK, Battacone G, Manca MG, Boe R and Pulina G 2011. Documentation of fatty acid profiles in lamb meat and lamb-based infant foods. Jurnal of Food Sciences 76, 43-47.
11. Ramírez-Retamal J, Molares R, Martinez E and de la Barra R 2014. Effect of the type of pasture on the meat characteristics of chilote lambs. Food and Nutrition Sciences 5, 635-644.
12. Revilla I, Luruena-Martinez MA, Blanco-Loprz MA, Vivarquintana AM, Palacios C and Severiano-Perez P 2009. Comparison of the sensory characteristics of suckling lamb meat: organic *vs* conventional production. Czech Jurnal of Food Sciences, 267-270.
13. Savić M, Dimitrijević B, Borozan S, Katić-Radivojević S and Jovanović S. 2012. The determination of the preferable sheep breed for organic production in the hilly mountainous

region of Sjenica. 27 th World Buiatrics Congress, Lisbon, 3 - 8 June 2012. Abstract book, pp 252-53.

14. Savić M, Vegara M, Vučković S, Prodanović S and Dimitrijević B 2013b. Importance of Zackel sheep for conservation of biodiversity at Sjenica-Pester plateau, 64th EAAP Annual Meeting, Nantes, France, 26-30 August 2013 b Abstract book, pp 384.
15. Savić M, Jovanović S, Trailović R, Dimitrijević V and Dimitrijević B 2011. Autochtonous breeds as livestock potential in sustainable farming in Serbia. In: Proceedings of the 19th International Congress of Mediterranean Federation of health and production of ruminants, 25-28 May, Belgrade, pp 16-22.
16. Savić M, Aleksić S and Živković D 2013a. Breeds of choice in organic production systems. Proceedings of the 10th International Symposium Modern Trends in Livestock Production 298-306, Invited paper, 2-4. October, Belgrade
17. Schmid A, Collomb M, Sieber R and Bee G 2006. Conjugated linoleic acid in meat and meat products: A review, Meat Science 73, 29–41.
18. Simić A, Vasiljević S, Vučković S, Tomić Z, Bjelić Z and Mandić V 2011. Herbage yield and botanical composition of grass-legume mixture at different time of establishment. 3rd International congress "New Perspectives and Challenges of Sustainable Livestock Production", 5-7th October 2011, Belgrade.
19. Zajiček D 1978. Comparison of the efficiency of two quantitative ovoskopic methods.Veterinarni Med. 23, 275-280.
20. Vučković S, Simić A, Ćupina B, Krstić Đ and Duronić G 2010. Effect of mineral fertilization on yield of Agrostidetum vulgaris – type meadows in mountainous grasslands in Serbia. Biotechnology in Animal Husbandry 26, 389-394.

Review paper

LIVESTOCK FARMING IN RURAL AREAS

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Abstract

Serbia has favorable natural conditions for the development of livestock despite the third decade of recording negative trends. Autochthonous breeds are closely connected to geographical and cultural diversity in Serbian rural areas. Unfortunately, number of autochthonous breeds significantly decreased during the last 5 - 6 decades. This is the potential threat for further decrease in production and loss of agro-biodiversity in Serbia. There are 400 - 500 companies in Serbia which are involved in the breeding of rare, autochthonous, domestic animal breeds. According to the 2012 Agricultural Census, total number of cattle in the Republic of Serbia on the family farms had a share of 91.7%. In the last ten years, poultry and pig production is 20% of total number of pigs and 37% of poultry is grown on the farms of legal entities. The paper aims to point out the need for defining measures that should be adopted in the near future (special programs and developing legal, administrative and technical instruments) for preservation and support of the animal resources of Serbia.

Key words*: livestock, measure, rural areas*

Introduction

There is almost no official symposium where it is not underlined that livestock production in Serbia is facing with obstacles, which are mainly results of insufficient attention (on all levels) paid to this agricultural sector (Tomić et al., 2011; Bogdanov and Petronijević, 2009; Subić et al., 2009). In globalisation era, development and industrial growth in many countries, as well as prevention and control of biological catastrophes, depend on agricultural and food policy and economy. Responsible ministry must become ally with livestock breeders, by precise agricultural support measures aiming to preserve diversity of animal breeds in accordance to geographical diversity.

In the last decades local animal breeds are in disappearance, which makes threat for decreasing or even loss of some bio-diversity in Serbia. The main reasons for this are:

1. Loss of important genetic characteristics (general resistance, resistance to illness), which is caused by loss of genetic heterogeneity (specific for domestic- indigenous breeds). These

characteristics can be very important in genetic selection between animal breeds and improve quality of other breeds;
2. Local-indigenous animal breeds (cattle, sheep, and goats) are highly adapted to the breeding in poor/mountainous conditions and very important for general agro-eco system biodiversity maintenance, by traditional pasturing in high value pastures (in hilly and mountainous regions).

Rural areas description

The Republic of Serbia has no officially defined rural areas, since harmonization with EU geo-codes has not been done yet (*NUTS*[1] *regionalisation*; Bogdanov and Stojanović, 2006). OECD[2] defines rural areas-regions where 15 - 50% of population and more than 50% live in rural areas. By the Law on Territorial Organization of the Republic of Serbia the status of the city is defined for 24 towns, so that all of the rest of the territory can be considered as rural areas in Serbia. In accordance to abovementioned classification *(OECD)* some municipalities of city of Belgrade and Niš are classified as rural, where agricultural production is performed[3]. Apart from restructuring processes in agricultural sector, EU rural development policy defines ecological legal acts and very diverse rural population needs (Pejanović, 2009).

Based on the 2012 Agricultural Census data can be distracted objective pictures on regional development, what will be the starting point for eligibility for implementing the regional EU policy support as well as defining a rural development program activities in Serbia. Based on the 2012 Agricultural Census in Serbia animal farming takes place in the territory of Serbia with a higher share in Southern Serbia - region Šumadija and Western Serbia, and Southern and Eastern Serbia (Chart 1. - *Share of livestock production by regions, based on the* 2012 *Agricultural Census in Serbia).*

Livestock production by regions in Serbia

According to the 2012 Agricultural Census about 77% of total agricultural holdings are registered for livestock production. Livestock breeding is most dominant in the region of Šumadija and West Serbia and Mačva region. Leskovac municipality is the richest in the animal herd number. Regarding the herd size (in charts 2 - 4) the largest number of cattle, pigs and sheep is registered in households with 3 - 9 animals. Poultry is a dominant breed in Vojvodina region (45%).

[1] *NUTS* – Statistic nomenclature on territorial units is set of developing and harmonized statistical data on EU region level, which is used for socio-economical analyses.

[2] According to OECD definition there are two levels of territorial units: local and regional. *OECD defines rural areas by the population density below and above 150 citizens/km^2. On regional level OECD reports functional and administrative units, depending on ratio of population living in rural area in total population in particular region:*

1. Regions with more than 50% of population living in rural areas - dominant rural regions;
2. Regions with 15-50% population living in rural areas – mainly rural regions/transition regions;
3. Regions with less than 15% population living in rural communities - predominantly urban.

[3] Rural areas are municipalities *Barajevo, Sopot* and *Surčin* of thecity of Belgrade and *Niška Banja* municipality of the city of *Niš*.

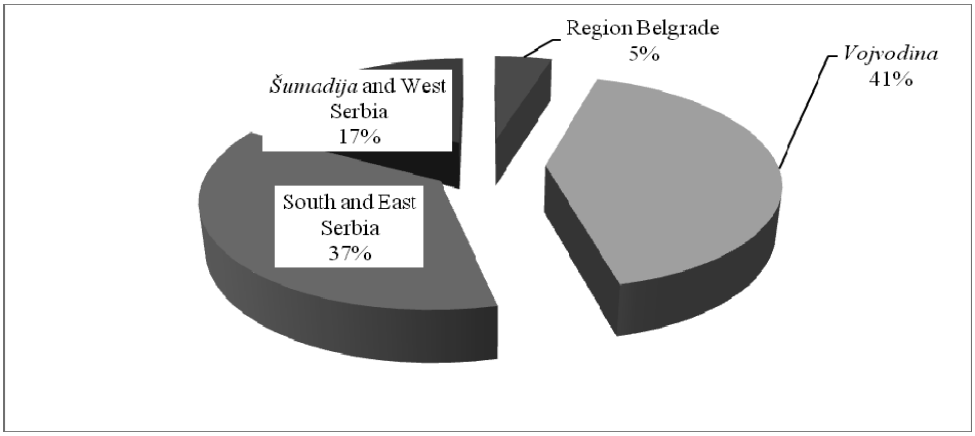

Chart 1. *Share of livestock production by regions, based on the 2012 Agricultural Census of Serbia*
Source: Statistical Office of the Republic of Serbia

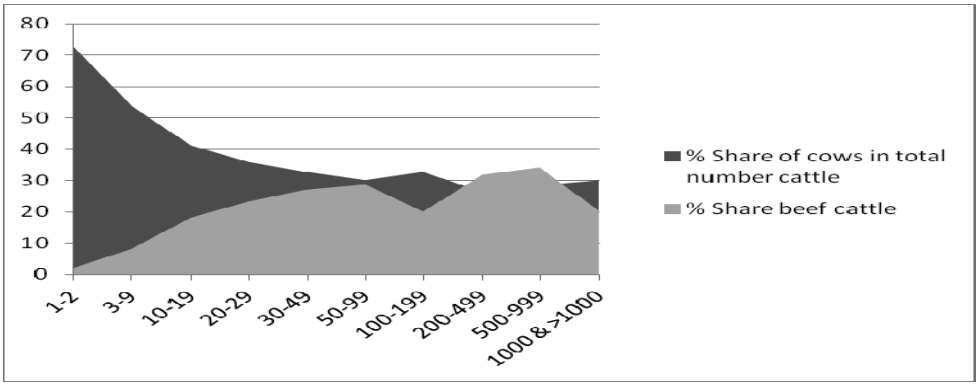

Chart 2. *Share of milk production and fattening in total cattle population*
Source: Statistical Office of the Republic of Serbia

C**attle breeding** is the most important livestock sector in Serbia. According to the Report (PO12) of the Statistical Office of the Republic of Serbia, on 1/12/2013 balance, total number of cattle is 912,147 (Chart 2). According to 2012 Agricultural Census the biggest share of milk production (72.5% approx.) is organized on small households with 1 - 2 heads, while cattle fattening is predominant in households with 200 heads, i.e. 500 - 1000 heads. In the last 30 years a decreasing trend in number of cattle and calf heifers was recorded. Number of breeding cows in Serbia, in 2012 was 155,356 (MAEP, source). Total number of cows in Serbia is 445,365. Observing cow's breeding and milk production chain it can be assumed that 88% of total number of households owns 1 - 9 heads, and that structure makes 75.8% of households in the north of Serbia, but almost 90% (89.9%) in the south of Serbia. Generally speaking households with less than 3ha, breeds one cow, but this number makes almost 25% of total number of cows. According to the 2012 Agricultural Census, 9.5% of animals are

breed on pastures. Average pasture size is about 1.1ha (little more in central and south Serbia than in Vojvodina). Based on Veterinary Department data in 2012 it was registered 1,414 holdings for cattle fattening, from which 80 farms are of 500 heads capacities. Middle sized farms breed 100 - 499 heads and their number is 204 in Serbia (Table 1).

Sheep breeding in Serbia has a long tradition and very significant regional component. According to the Report (PO12) of the Statistical Office of the Republic of Serbia, on 1/12/2013 balance, total number of sheep is 1,616,220. Sheep breeding is organized on extensive pastures which are not suitable for other agricultural activities. Based on the 2012 Agricultural Census about 24.3% of sheep is on pasturing. Sheep production is organized on small holdings (with 3-9 heads) and their share is 51% in total sheep number in Serbia (Chart 3). From the total sheep number in Serbia, 79.8% is bred in the south of Serbia, while only 20-2% is on the holdings in the north of Serbia. Sheep production is dominant in Šumadija and west Serbia region. Total number registered holdings for sheep fattening is 190 (Veterinary Department, 2012; MAEP). There are 10 farms with the capacities of 2,000-4,000 heads recorded, followed by 9 farms with 500 – 1,999 heads (Table 1).

Table 1. – *Number of registered facilities for livestock breeding (for fattening)*

	Total registered facilities for fattening	Capacities for fattening		Other/unidentified for fattening capacities
Cattle (number of heads)		100-499	≥500	
Number of registered facilities	1,414	204	80	1,130
Pigs (number of heads)		10,000-19,999	20,000-50,000	
Number of registered facilities	959	13	17	929
Sheep (number of heads)		500-1,999	2,000- 4,000	
Number of registered facilities	190	9	10	171
Goats (number of heads)		200-500	600-1,500	
Number of registered facilities	24	7	5	12
Poultry (number)		20,000-100,000	>100,000	
Number of registered facilities	1,081	70	28	983

Source: Veterinary Department (2012)

According to the Report (PO12) of the Statistical Office of the Republic of Serbia, on 1/12/2013 balance, total number of **goats** in Serbia is 225,077. Goat breeding is mainly extensive in hilly and mountainous areas, although there are some tendencies for intensive production. The 2012 Agricultural Census reports about 24.8% goats on pastures. Total number of registered farms for goat fattening is 24 (Veterinary Department, MAEP; 2012). There are 5 farms with the capacity of 600 – 1,500 heads and 7 farms with the capacity of 200 - 500 goats (Table 1).

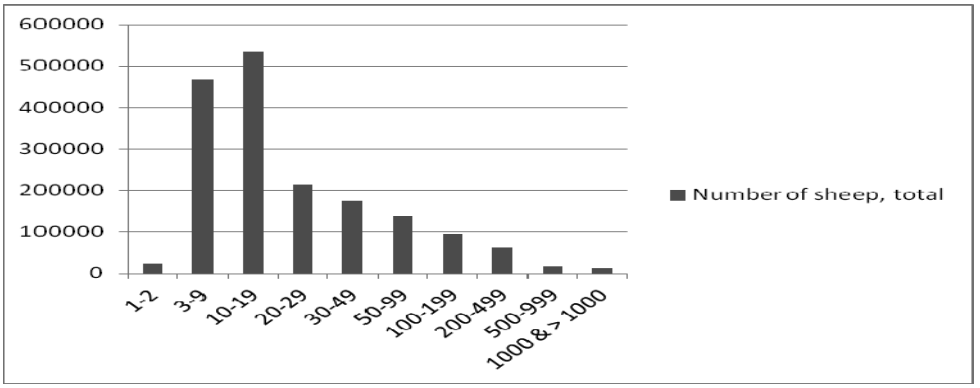

Chart 3. *Structure of deployment of sheep, by herd size*
Source: Statistical Office of the Republic of Serbia

Pig number reported in the Report (PO12) of the Statistical Office of the Republic of Serbia on 1/12/2013 is 3,144,207. Southern Serbia is the region where more than 53% of pigs are bred (Šumadija region - 34%, South and East Serbia - 19%). In Vojvodina 41% of total pig number is recorded and 6% are bred in Belgrade region. The most important area for pig production is Mačva region. Total number of breeding pigs in Serbia, in 2012, was 33,000 (27,000 in Vojvodina and 6,000 in central and south Serbia - MAEP source). On a 01/12/2013 date total number of fattening pigs was 2,339 thousand, which makes share of 74% in total pig number in Serbia. Share of sows and gilts in 2013 was 12% of total pig number. Veterinary Department (MAEP) registered in 2012, 959 farms for pig fattening (Table 1). There are 17 farms with the capacities of 20,000 – 50,000 pigs in one turn. There are 13 middle sized farms with 10,000 – 19,999 pigs. From the total number of registered agricultural holdings in Serbia, 90% are registered for pig production (2012 Agricultural Census). Agricultural holdings with less than 10ha share 92% of total pig number, with the average pig number of 3.8 (Table 2*)*.

Table 2. *Agricultural holdings and number of animals according to agricultural land use in 2012*

	total	**no land**	**0-2 ha**	**2-5 ha**	**5-10 ha**	**10-20 ha**	**20-50 ha**	**50-100 ha**	**> 100ha**
Agricultural holdings, No	632,062	10,763	294,421	184,936	89,930	32,675	13,102	4,382	1,853
used agricultural land, ha	3,360,346	-	265,026	594,681	617,060	436,843	388,858	310,255	747,623
Cattle, No	908,990	10,95	75,565	184,844	233,318	184,070	108,824	32,409	78,965
Pigs, No	3,403,288	200,048	585,158	755,724	677,162	387,431	229,457	122,131	446,177
Sheep, No	1,729,278	15,334	293,104	524,355	485,729	249,87	108,674	27,524	24,971
Poultry, No	26,27,315	7,182,671	5,957,274	4,489,861	3,106,968	1,560,153	1,230,741	822,258	2,277,389

Source: Statistical Office of the Republic of Serbia

According to the Report (PO12) of the Serbian Statistics Office, with the 1/12/2013 balance, total number of **poultry** in Serbia is 23,450,387. Number of farms registered for broilers breeding is 1,081 (Vet. Dept., 2012 - Table 1) and there are 28 farms with capacity of over 100,000 heads. There are 70 middle capacity farms with 20,000 – 100,000 heads. Poultry production is characterized by the fast technology transfer. Broiler production is concentrated in few big installments, with modern slaughterhouses, and numerous small farms, which makes significant share in total production. This production, by its organization and high level of industrialization increase share in total production and consumation. Many small farms produce poultry for individual use and for selling through non registered trade chains.

The 2012 Agricultural Census reports 673,000 **beehives**, in Serbia, mainly in the south part of Serbia (South and East Serbia - 41%, Šumadija and Central Serbia - 35%). Potentials for bee pastures are used only 5%, but in this sector of livestock production, increase in number of registered holdings, as well as in number of beehives is recorded. Total number of bee flocks in Serbia is 665,022.

In total livestock production in Serbia, a cattle breeding is on the first place, which with the milk and meat production in 2012 shared about 43% of total value of livestock production, followed by the pig production (33%), poultry production (15%), goat production (7%) and beekeeping (1%). The data in Table 3 on the number of animals and poultry, which are reported on the slaughter line, observed in the total number of slaughtered animals, are important in terms of the level of compliance with international standards set by the slaughter line (Table 3).

Table 3. *Number of slaughtered animals/birds and the number reported on the slaughter*

Year	Cattle		Pigs		Sheep		Poultry	
	slaughtered	slaughtered in slaughterhouses	slaughtered	slaughtered in slaughterhouses	slaughtered	slaughtered in slaughterhouses	slaughtered	slaughtered in slaughterhouses
2011	368	153	5795	1819	1172	56	51,026	34,333
2012	387	143	5453	1714	1108	42	46,229	35,174
2013	318	147	5684	1483	1537	52	64,552	35,424

Source: Statistical Office of the Republic of Serbia

Organic livestock production in Serbia is organized dominantly for “small” and “big” animals with the share of 68% of total production, beehives shares 18% and poultry 14% (Chart 5). Serbia has a great natural potentials and favorable climatic conditions for organic livestock production. This production method can assure significant profit on small farms, specific for Serbia (with the 2 - 4ha sizes). Table 4 shows the number of animals in the organic production in Serbia.

Table 4. *Number of animals in the organic production in Serbia (January - December 2013)*

Category	Conversion Period-number of livestock, poultry birds, beehives	Organic status-number of livestock, poultry birds, beehives	Total
Large throat (cattle, buffaloes, horses, donkeys)	481	2,972	3,453
Small animals (sheep, goats, pigs)	3,473	708	4,181
Poultry (chickens, geese, ducks, turkeys, guinea fowls)	1,432	183	1,615
Beehives	764	1,273	2,037

Source: MAEP and SCCI

Livestock production in rural areas

Encouraging the sustainable systems of livestock production in Serbia was always a challenge for the representatives of science. (Jovanović et al., 2009; Jovanović et al., 2011; Savić et al., 2009; Todorović et al., 2009). In the cattle meat production in Serbia, Simmental breed is dominant, which had dual characteristics. Low share of fattening, of 31%-34%, which is organized on households of legal entities and entrepreneurs, shows dominant fragmented production. Rural characteristics of this production (70%) are additionally supported by the self-sufficiency on the farms. Genetic potentials and breeds composition, as a basis for livestock production, have been on the decrease for many years. Furthermore, a producer's economical motivation is not on a satisfactory level as well as the meat-input prices parities. Low purchase price cannot cover production costs. Livestock sector is directly influenced by products which are consumed in the household (farm) or placed on the market through illegal trade channels (in 2013 about 54% of total production). Quantities of beef and veal meat in Serbian market are neither sufficient to cover domestic consumption nor for export. Table 3 reports small increasing of number of cattle on slaughter line (5%) in relation to total number of slaughtered animals for period 2011 - 2013. Although, small increasing, it is important parameter for adjusting the market flows and international standards for slaughter line.

Milk production is strategically the most important livestock sector and it is considered as an axle of agricultural and rural development in Serbia. This sector reports the highest value of primary production (300 million euro/year, 7.6% of total agricultural GDP and with its regional structure can significantly contribute to the increasing of rural development in the Republic of Serbia. Annual milk production is stable at about 1.5 billion litres (Jovanović, S. et al., 2009). Milk production in Serbia implies production of raw milk in 98% of total production. In some quantities and some season's parts goat and sheep milk is present on the market, as well as its products. Of total milk production in Serbia, 98% is the share of cow milk and 1.6% goes on goat milk. Goat milk production in 2012 was 24 million litres (15

million litres in 2011). The highest milk production is in Central Serbia (42%) and Vojvodina (36%). In southern parts of Serbia, despite big number of households and milking cows, production share is only 22% of the total milk production.

Sheep production is important from the point of herd renewal, i.e. production of young animals, as a base for fattening and reproduction, revitalisation of traditional breeding systems in hilly-mountainous areas and biodiversity preservation. Self-sufficiency, which includes trade and food in sheep production was almost 97% in 2013.

Pig production in Serbia is mainly organized on small and middle farms (41% of total number of farms) in breeding herd (Chart 4). In the '90s in pig production in Serbia a period of intensive breeding started and the farms were the leaders in production and supplying of market with the surpluses of pig meat. In the recent years about 20% of total pig number is bred in the households of legal entities and entrepreneurs. The number of sows and gilts is biggest on the farms with 3 - 19 heads (share of 22% - 27%). Typical traditional pig breeding is dominant in Serbia and deviates from the most EU standards (bio safety measures, animal welfare on farms). Pig production sector expects important reforms in the near future.

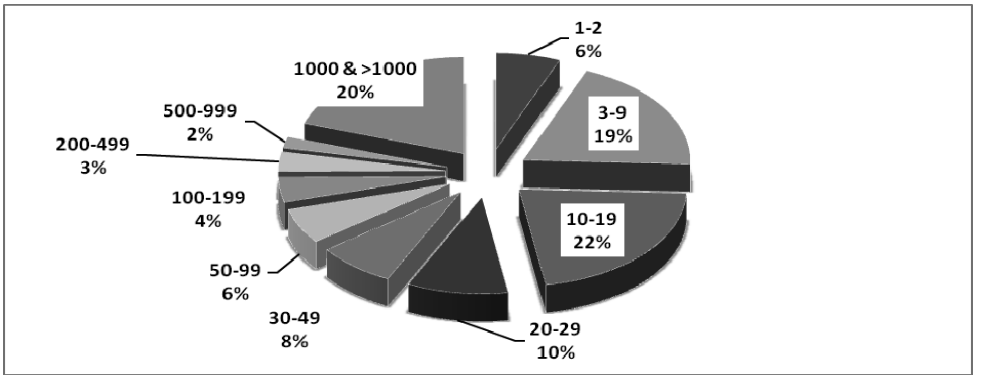

Chart 4. *Structure of deployment of pigs, by herd size*
Source: Statistical Office of the Republic of Serbia

Organic livestock is becoming more and more important although there are not official data on animal number with the "organic" status. Share of these animals in the total livestock production is still low but potentials for organic animal breeding are big, particularly in hilly-mountainous areas (Chart 5). Specific (economic) importance in the organic livestock production is cattle and sheep breeding. The results of numerous studies show the big potential of Sjeničko-Pester plateau natural resources in organization of sheep organic production. Some studies were performed speciality in organization of sustainable production of organic growth of lambs as a support of rural development (Savić et al., 2013).

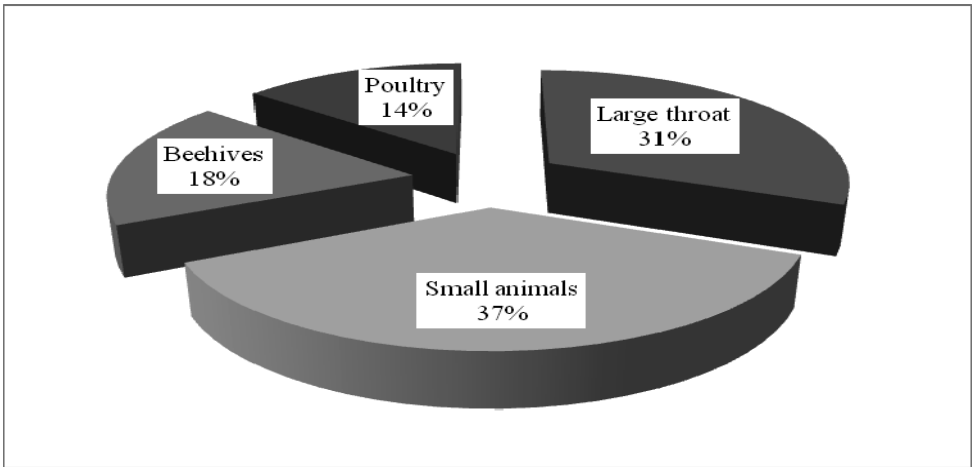

Chart 5. *Share of organic livestock production in Serbia in 2012*
Source: Statistical Office of the Republic of Serbia

Possibilities for development

In the beef meat production there is recorded decreasing in the number of breeding animals, which leads to smaller number of fattening cows as well. Further facing with these problems may be expected in the near future, because increasing of the milking per cow and decreasing number of animals for milk production is expected. Therefore, it is necessary to establish specialized systems aimed for beef meat production. Beef meat export quota (under preferential status) for EU market is 8,880 tons/year, but only about 5% is used. There are potentials for beef and veal meat export on the Russian Federation market and Asian countries as well. Low percentage of cattle on pastures (9.5%) is the result of introduction of new technological solutions where milking heads are in closed systems during whole year, with silage-concentrated feeding and easier milk collection (milking). Previously arranged pastures are excluded from technological turnover and stay unused what brings bigger economical problem because grass is much cheaper (2 - 3 times) than any other feed for milking animals (ruminants in general). Pastures offer important advantages in a livestock production because of the possibilities of decreasing the milk production price and, for a high productive pastures, can reach 9,000 – 12,000 kg/ha of green mass for feed (1kg of dry matter has more than 100gr of digestible proteins, 3 - 70gr of sugar and 10 - 12 MJ of energy).

Sheep meat production can be improved by increasing the herd number and improvement of genetic characteristics, particularly by the feed and welfare conditions which can lead to better fattening and higher lamb meat production.

As goats are resistant to certain diseases the experts are pointing to a high value of goat cheeses. Serbia was not an important producer of goat cheeses which are well known as high value food, particularly for dietetic human consumption. Importance of goat breeding is closely connected to touristic potentials of development of national industry in the EU countries (France, Swiss, Italy, Belgium, Holland), so it can be important resource for rural development in Serbia as well.

Goat cheeses are prepared in the same way as bovine milk cheeses. Specificity of milk drops of goat milk is that they are smaller, casein crud is softer than from cow milk (or sheep). Soft white or hard goat cheeses are mostly present on the market today. During the recent several years goat cheeses imported from France, Swiss or Greece have been very popular on a domestic market.

Serbian farmers are not burdened by the legal request for quality assuring, i.e. domestic market is for them completely opened. However, EU market puts some additional demands for them, so apart from harmonisation with animal welfare regulation it must be accomplished traceability and "from farm to fork" control system too. Currently in Serbia there is not present classification of carcasses in the slaughter line, therefore the payment to the farmers is not based on the quality of carcasses. In many cases payment is based on a net mass of the animal and/or on the animal's age at the time of selling (trade). Carcass classification on the slaughter line must be introduced in the near future. Pig meat production can be improved by the increasing of number of piglets per sow, and decreasing share of piglets in slaughtered animals. In order to achieve this goal, it is necessary to change farmer's awareness on importance of fulfilling the animal welfare standards, improvement of genetic characteristics, animal housing and appropriate feed. This can accomplish domestic market needs with an export increase (particularly of traditional products such as ham, "kulen", etc.).

Conclusions

Agricultural policy measures do not include animal feeding nor direct producers to the possibility of significant use of pastures and production based on the combined animal feeding. For this reason significant Serbian territory under the meadows and pastures should be used for feed production, which is more economical and typical for local breeding areas (cattle, sheep, and goat breeding). Serbian farmers must be informed about animal conditions in stable, so it is important to educate farmers on capability to perform individual animal's body conditions. If the animal's health and body condition is not stable on a daily basis it could reflect on a productivity and production on the farm. One of the most important segments of the assessment of body condition is animal nutrition and daily feed intake.

Traditional products, which are connected to the cultural heritage of Serbia, are typical for old rural population (pig breeding, beekeeping, dairy cows, sheep and goat breeding). It will be very important to define by the national standards and with the legal background, those traditional products and place them on a world market as "made in Serbia" products.

Republic of Serbia has many regions with favourable conditions for organic livestock production, but it is not supported by the legal background, so the conditions and advantages therein are not well known. All natural, economical, cultural and other elements of geographical units can be considered as characteristic for rural development in Serbia.

For the final conclusions it could be pointed out that un-competitiveness of small production units, unfavourable industrial structure and specialization of livestock production is followed by farmer's level of education about livestock production and running business in rural areas. Taking into account the reduction in natural resources worldwide, the goal is to preserve territorial diversity and quality of the environment in the Republic of Serbia.

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References

1. Bogdanov N, Petronijević M 2009. Structural Changes of Agricultural Sector - experience after two years of transition; Chapter: Agriculture and Rural Areas of Serbia, Publisher: DAES, University of Belgrade Faculty of Agriculture, pp.1-24
2. Bogdanov N, Stojanović Ž 2006. Metodologija utvrđivanja ruralnosti i identifikacija ruralne Srbije, Chapter: Agriculture and Rural Areas of Serbia, DAES i Institut za agroekonomiju Poljoprivredni fakultet u Beogradu, str. 47-70.
3. Census of Agriculture 2012 AGRICULTURE IN THE REPUBLIC OF SERBIA I&II (2013)
4. Council Regulation (EC) No 1698/20-05 of 20 September 2005 on support for rural development by the European agricultural fund for rural development (EAFRD). *OJ*, L277, 21. 10.,1-40
5. Jovanović S, Savić M, Živković D 2009. Genetička varijabilnost otpornosti farmskih životinja na bolesti. Biotechnology in Animal Husbandry, vol. 25, br. 5-6-1, str. 339-347
6. Jovanović S, Savić M, Aleksić S, Živković D 2011. Production standards and the quality of milk and meat products from cattle and sheep raised in sustainable production systems. Biotechnology in Animal Husbandry, vol. 27, br. 3, str. 397-404
7. Law on Agriculture and Rural development ("Off. Gazette of RS", no. 41/2009 and 10/2013 - dr. Law)
8. Law on Territorial Organization of Republic of Serbia, 29 December 2007;
9. Pejanović R 2009. Koncept integralnog ruralnog razvoja Evropske unije, Poslovna politika, vol. 38, br. 3-4, str. 16-22
10. Regulation (EC) 1059/2003 (Annex II) (www.europarl.europa.eu)
11. Savić Mila, Vučković Savo, Jovanović Slobodan 2013. "Prilog sagledavanju potencijala prirodnih resursa Sjeničko-pešterske visoravni za organizovanje organske ovčarske proizvodnje" Veterinarski glasnik 2013, vol. 67, br. 1-2, str. 97-104.
12. Savić M, Jovanović S, Popović VA 2009. Sustainable livestock production in Serbia mountain regions in: Proceeding of the th Annual Meeting of the European Association for Animal Production. Barcelona, Španija, 60(15): 24-28.
13. Subić J, Vasiljević Z, Ivanović S 2009. Education of agricultural producers intended to improve farm operations and management, 113th Seminar of the European Association of Agricultural Economists: «The role of knowledge, innovation and human capital in multifunctional agriculture and territorial rural development», Thematic Proceedings, 9th –11th December 2009, Belgrade, Serbia, p.p. 257 – 265.
14. Tomić D, Ševarlić M, Zekić S 2011. Agrarna i ruralna politika u Srbiji nužnost ubrzanja reformi, DAES - Društvo agrarnih ekonomista Srbije Beograd; ISBN 978-86-86087-24-9, str. 171 Novi Sad
15. Todorović S, Munćan M, Miljković M 2009. The growing importance of activities diversification for enhancing family farms competitiveness. In Tomić D., Vasiljević Z., Cvijanović D. (Eds.), "The role of knowledge, innovation and human capital in multifunctional agriculture and territorial rural development" Belgrade: European Association of Agricultural Economists, pp. 331-336.

Original paper

OVERVIEW OF THE IPARD PROGRAM FUNDS USAGE IN THE REPUBLIC OF CROATIA

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Abstract

The Instrument of Pre-Accession Assistance – IPA was offered as an aid to the Republic of Croatia for implementation of required reforms within its preparation for full membership in the European Union. The assistance is aimed to support the set-up of a system for efficient management of European Union funds. The IPA program was established by the EU Council Regulation no. 1085/2006, which defines general principles and assistance framework. Detailed provisions are defined in the Regulation no. 718/2007, made up of five components: IPA-TAIB, IPA-CBC, IPA-RD, IPA-HRD and IPARD. Financial framework of the Program is determined by the Multiannual Financial Framework of the European Commission - MFF for each participating country in the IPA components. The main objective of the IPARD program is to improve the agricultural sector and its market efficiency according to the EU standards, to implement preparatory activities for incorporation of agricultural and environmental measures and the LEADER, as well as to develop rural economy. The IPARD program in the Republic of Croatia is carried out through the Measure 101 "Investments in agricultural holdings to restructure and upgrade to the EU standards", Measure 103 "Investments in processing and marketing of agriculture and fishery products to restructure and upgrade to the EU standards", Measure 301 "Improvement and development of rural infrastructure", Measure 302 "Development and diversification of rural economic activities" and Measure 202 "Preparation and implementation of local rural development strategies". The objective of presented paper is to overview the usage of IPARD funds in the Republic of Croatia in the period 2010-2013.

Key words: *European Union, funds, IPARD program, Republic of Croatia*

Introduction

The Republic of Croatia was officially granted a status of a candidate country for membership in the European Union on June 18, 2004, and thus gained the possibility to use pre-accession assistance in order to facilitate adjustment to EU standards and to prepare itself for EU membership. Pre-accession aid is a part of the European Union strategy, which is focused on specific needs of each candidate country within its process of preparing for membership in the European Union. The Republic of Croatia, as a candidate country for accession to the European Union, was approved the pre-accession funds for the PHARE, ISPA and SAPARD programs in the period of 2005-2006, as well as the IPA program, which was available from 2007-2013.

The Instrument for Pre-accession Assistance – IPA is a program of preparatory measures for Croatian joining to the EU in the period 2007-2013. The IPA program was established by the EU Council Regulation no. 1085/2006. Financial value in the stated period 2007-2013 was 11.468 bill. €. Indicative financial framework for IPA in 2007-2012 is presented in theTable 1.

Table 1. *Indicative financial framework for IPA 2007-2012 (in mil. €)*

Multi-annual indicative financial framework for IPA in Croatia		**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**Total**
I.	Transition Assistance and Institution Building	49.61	45.37	45.60	39.48	39.96	40.87	260,89
II.	Cross-border Cooperation	9.69	14.73	15.90	16.22	16.54	16.87	89,95
III.	Regional Development	45.05	47.60	49.70	56.80	58.20	59.35	316,70
IV.	Human Resources Development	11.38	12.70	14.20	15.70	16.00	16.04	86,02
V.	Rural Development	25.50	25.60	25.80	26.00	26.50	27.27	156,67
Total		**141.23**	**146.00**	**151.20**	**154.20**	**157.20**	**160.40**	**910.23**

Source: European funds for Croatian projects (2009), SDURF

The Croatian Parliament passed the Law on Ratification of the Framework Agreement between the Croatian Government and the European Commission on rules for cooperation concerning the European financial assistance for the Republic of Croatia and the implementation of assistance under the Instrument for Pre-Accession Assistance-IPA (Official Journal – OJ 10/07). The main components of the IPA program are Transition Assistance and Institution Building (35%), Cross-border Cooperation (7%), Regional Development (32%), Human Resources Development (8%) and Rural Development (18%).

In the period 2007-2012, the Component 5 through IPA –IPARD program provided a total of 156.67 million € (Table 1). Based on the Strategy of Agriculture and Fisheries of the Republic of Croatia (OJ 89/02), the Ministry of Agriculture, Forestry and Water Management defined long-term strategic objectives, such as follows: improved competitiveness and efficiency of primary agricultural, forestry and fishery production, improved competitiveness and efficiency of processing and marketing of agricultural, forestry and fishery production, improved quality and hygiene standards, as well as standards related to environment and animal welfare, additional employment opportunities and income for farmers and all who live in rural areas, which shall lead to improved living and working conditions, achievement of sustainable rural development through optimized and balanced economic development, environmental protection and social cohesion. As a continuation of the SAPARD program and in order to fulfill all objectives within the national strategy, the European Commission prepared the new IPARD program - Agriculture and Rural Development Plan 2007-2013. The program was evaluated positively by the Committee for Rural Development in Brussels on December 19, 2007, and on February 25, 2008 it was accepted by the European Commission. The program was implemented through three strategic priorities with specific technical assistance. Within Priority 1 beneficiaries are agricultural farms (family farms, small entrepreneurs and legal entities) as defined by the Law on Agriculture (OJ66/01, 83/02) and registered in the Registry of agricultural farms that are subject toVAT.

Within Priority 2 there are investments for landscaping and environment (Measure 1), as well as preparatory activities for implementation of rural development strategies (Measure 2). Beneficiaries within the Measure 1 are craftsmen and companies that are 100% privately owned and those that are up to 25% owned by the state and are in the VAT system. They classify as micro, small and medium enterprises as referring to the Act on subsidizing small enterprises (OJ29/02). Beneficiaries within the Measure 2 are local authorities, agricultural farms and farmers united in the Local Action Group (LAG). Within the Priority 3, there are investments planned for development of rural economy through the following measures: development of local infrastructure (unclassified roads, sewer systems, etc.) and support for rural tourism, construction of processing plants for dairy products and meat, traditional crafts, the use of renewable energy sources. Beneficiaries are local self-government units, municipalities and cities with population of up to 10.000 (Census 2001). The IPARD program funds are aimed to increase the competitiveness of sustainable producers by improving market efficiency and by meeting EU standards. This financial assistance is focused on development of rural economy, on increase of income and on increased employment in rural areas, by reducing at the same time gap between rural and urban areas". The objective of presented paper is to overview the usage of IPARD funds in the Republic of Croatia in the period 2010-2013.

Materials and methods

The most of performed analyses in the paper were made on the basis of the data published in the statistical publications of the Croatian Bureau of Statistics, Eurostat Bruxelles, annual reports on agriculture published by the European Commission, as well as publications of the Croatian Government and the Ministry of Agriculture, Forestry and Water Management of the Republic of Croatia. The data were processed and analyzed by the methods of descriptive statistics, and presented in tables and figures. The analysis results were processed with the MS Office - Excel 2010.

Results and discussion

According to the Regulations on the scope and content of responsibilities and authorizations of entities responsible for management of the Instrument of Pre-Accession Assistance – IPA (OJ 34/08), the IPARD program is managed by the Directorate for Rural Development-Managing Authority for SAPARD /IPARD program, and carried out by the Paying Agency for Agriculture, Fisheries and Rural Development in Croatia. The IPARD Program in Croatia has been carried since beginning of 2010, for measures 101 "Investments in agricultural holdings to restructure and upgrade to the EU standards" and 103 "Investments in processing and marketing of agriculture and fishery products to restructure and upgrade to the EU standards". Since 2011, the measures 301"Improvement and development of rural infrastructure" and 302 "Development and diversification of rural economic activities" have been implemented. Certain amount of funds (25.3 mil. €) from 2007 and 2008 is irreversibly lost due to the n+3rule[1]. Since the beginning of IPARD program implementation, there has been a total of 18 calls announced for measures 101, 103, 301, 302 and 202. The overview of calls is presented in the Figure 1.

[1]financial means within European Union structural funds for certain beneficiary country have limited time for utilization, and after that time limit, funds are returned to the European Union budget

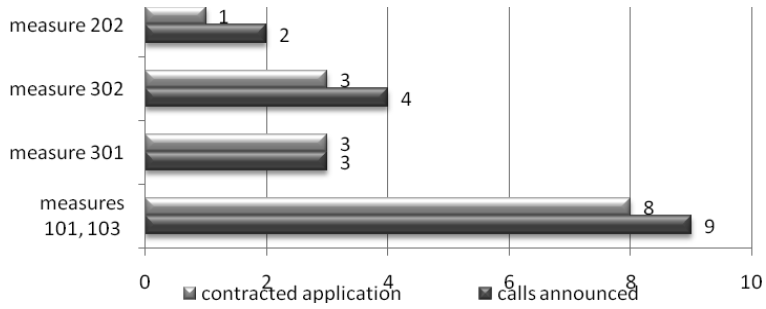

Figure 1. *Number of calls announced for specific measures*
Source: Report on the use of pre-accession assistance programs of the European Union

By the end of the reporting period, the Paying Agency for Agriculture, Fisheries and Rural Development in Croatia received 1.143applications in total, which requested a total of 285.54 mil. € (Report on the use of pre-accession assistance programs of the European Union). Number of applications according to specific measure is shown in Figure 2.

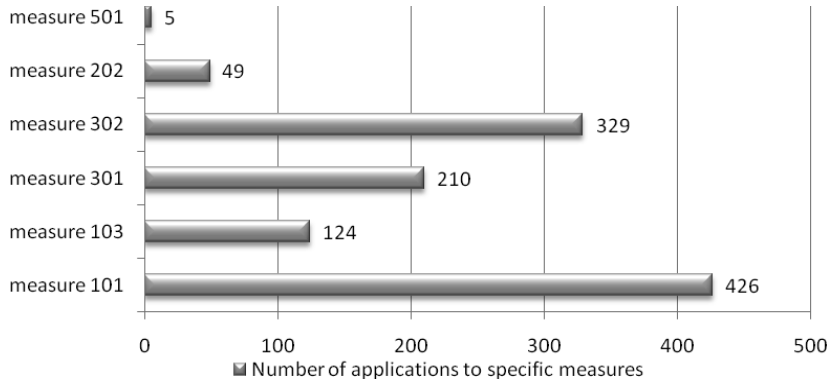

Figure 2. *Number of applications to specific measures*
Source: Report on the use of pre-accession assistance programs of the European Union

In reference to the Report on the use of pre-accession assistance programs of the European Union, the most project contracts within the Measure 101 were signed in the fruit and vegetables sector (85 contracts), then meat sector (27 contracts), followed by investments in the milk production (20 contracts) and egg production (9 contracts), and sector of cereals and oil seeds (6 contracts). Within the Measure 103, the most project contracts were signed in the fruit and vegetable sector (18 contracts), the fishery sector (13 contracts), the meat sector (11 contracts), winemaking (7 contracts) and dairy (4 contracts). As of the Measure 302, the most contracted projects were referring to rural tourism sector (46 projects), traditional crafts and processing at agricultural farms (3 projects,

respectively) and one project for the sector of freshwater fishery and renewable energy sources, respectively.

For the Measure 301, there were 34 contracted projects. The highest number of contracts was signed in the sector of sewerage and wastewater management (18 contracts), local unclassified roads (16 contracts). Referring to the Report on the use of pre-accession assistance programs of the European Union, out of the total number of contracted projects since the beginning of the Program period (292), the most of them were contracted for the County of Istria (44 projects, 15.07%) and the Osijek-Baranja County (42 projects; 14.38%), while the least projects were contracted for the Šibenik-Knin County (1; 0.003%) and Krapina-Zagorje County and the City of Zagreb (3 projects, 0.01%, respectively).

Since the beginning of IPARD implementation, Croatia has been allocated financial funds in the amount of 129.4mil. €. A total of 292projects were contracted in the value of 65.06 mil. €. In the reporting period, Croatia has used 50.28% of funds from the IPARD program (Table 2).

Table 2. *Financial indicators of IPARD program implementation from 2007- 30th June 2013 (in mil. €)*

	101	**103**	**301**	**302**	**501**	**Total**
Total allocated funds			129.4			**129.4**
Total contracted funds	24.49	26.45	11.11	2.99	0.02	**65.06**
Total contracted / Total allocated						**50.28%**
Total funds received from EC	7.83	7.80	0.25	0.42	0	**39.79***
Total paid to beneficiaries	7.89	9.13	1.05	0.42	0	**18.48**
Total paid/ Total contracted funds	32.22%	34.50%	9.41%	14.07%	0.00%	**28.40%**
Total paid / Total allocated funds						14.28%
Total paid / Total allocated						30.75%
Total validated	7.83	7.80	0.25	0.42	0	**39.79***
Total validated / Total contracted	31.97%	29.49%	2.25%	14.07%	0.00%	**25.06%**
Total validated /Total allocated						**12.60%**

*advance payment of 23.49 mil. € included
Source: Report on the use of pre-accession assistance programs of the European Union

Presentation of the IPARD Program according to the production sectors

If considering their objectives, the Measure 101 and Measure 103 were mostly recognized by potential beneficiaries of IPARD, whose primary business activity refers to animal husbandry. The relative portions of representation per production sectors and years for the Measure 101 are shown in Figure 3.

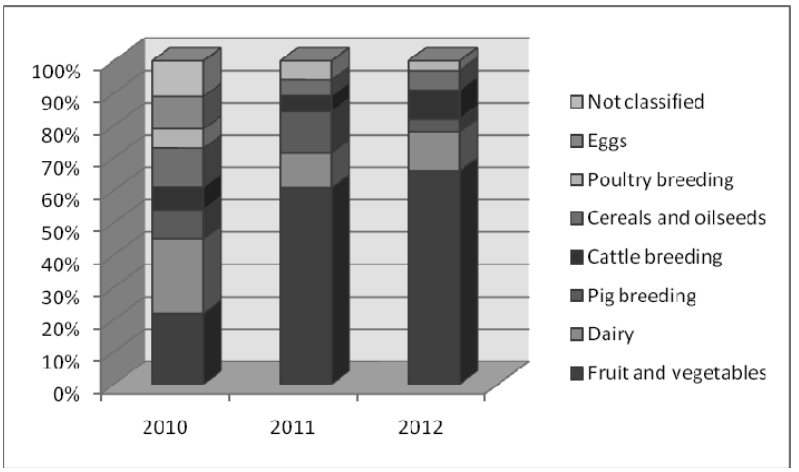

Figure 3. *Relative portions of representation per production sectors and years for the Measure 101,* Source: Annual Report on the implementation of IPARD in Croatia

The figure showing a three-year representation per production sectors and years for the Measure 101 indicates that there were 126 applications within the sector of fruit and vegetables, 32 applications for dairy sector, 20 applications within the pig breeding sector, 18 within cattle breeding sector, 11 for the poultry breeding sector and sector of cereals and oilseeds and 9 applications within the egg production sector (Annual Report on the implementation of IPARD in Croatia). The analysis by sectors for the year 2010 indicates that there was a greater portion of applications within the sector of animal husbandry (54%) if compared to 2011 and 2012 (36% and 37%, respectively). In Croatia, the sector of animal husbandry takes up about 36% of the total value of agricultural production, which reflects in a balanced trend of applications in 2011 and 2012 compared to the total value of livestock production in the Republic of Croatia (the ratio of the number of applications within the sector of animal husbandry and total value of livestock production is 1:1).

Referring to the Annual Report on the implementation of IPARD in Croatia for the Measure 103, the largest portion of applications (31%) was marked in the sector of fruit and vegetable processing, followed by the fishery sector and meat and viticulture sector (Figure 4.). There were significantly less received applications in the meat processing sector than in the fruit and vegetable sector, but it took up larger portion in the total amount of grants received. The largest amount of requested grants in 2012 was in the meat sector (~ 45%) and in the sector of fruit and vegetables (30%). In 2010, relative portions in number of applications to the Measure 103 in sectors of animal husbandry were within milk and dairy 15%, meat 12%, and fishery 44%. In the first year of implementing the Measure 103, there were no applications for the fruit and vegetable sector. In 2011 and 2012, relative portion of applications in the meat sector amounted to 24% and 27%, respectively, while portions in the number of applications in the milk and dairy sector reduced to 5% and 7%, respectively.

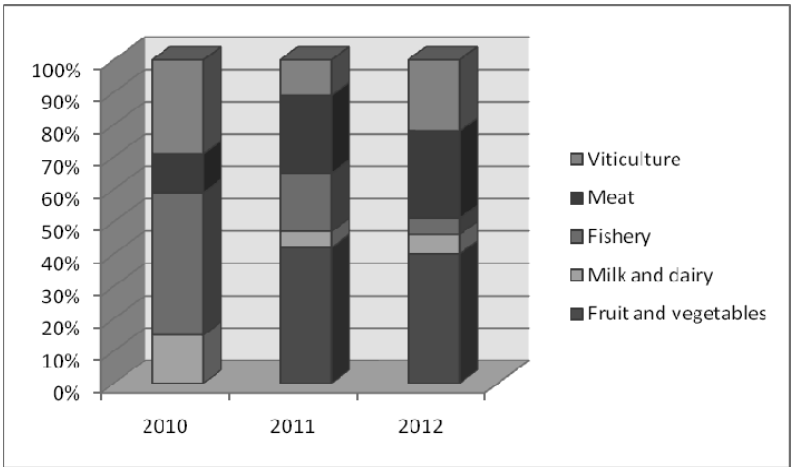

Figure 4. *Relative portions of representation per production sectors and years for the Measure 103*
Source: Annual Report on the implementation of IPARD in Croatia

IPARD program achieved much better results in its implementation than the SAPARD program. For example, in one call within the Measures 101and 103, there were more grants awarded than in the entire SAPARD program. Portion of small–scale projects within the Measure 101 was significantly increased, so that there was 2.75 times more contracted projects within the Measure 101 in comparison with the Measure 103, while within the SAPARD program number of contracted projects in these two measures was equal. Referring to the grant support, the ratio increased from 0.35:1 to 0.88:1. The project success rate rose from 42% in the SAPARD program to 58% in the IPARD program, and if excluding the Measure 301, the average project success rate is70%.

Conclusion

If considering previous experiences with the implementation of the SAPARD program, the Republic of Croatia was better prepared to use funds from the IPARD program. In the reported period the group of potential beneficiaries of the European Union funds has developed, project calls within specific measures were more often announced, project success rate within individual calls increased, and amounts of awarded grants also increased. Since the Republic of Croatia was awarded full membership status in the EU on July 1, 2013, it has become eligible to apply for grants from much wealthier European Union structural funds for agriculture, food industry, fishery and rural development, such as the European Agricultural Fund for Rural Development – EAFRD, as well as the European Maritime and Fisheries Fund - EMF. Within these structural funds there are grants awarded for development projects that contribute to promotion of European society and economy competitiveness and help to reduce gaps between developed and less developed parts of the EU.

References

1. Act on subsidizing small enterprises (OJ29/02)
2. Annual Report on the implementation of IPARD in Croatia 2013, Government of Republic of Croatia, Zagreb

3. Census 2001. Croatian Bureau of Statistics, Zagreb
4. EU Council Regulation No. 1085/2006
5. EU Council Regulation No. 718/2007
6. European funds for Croatian projects 2009. , SDURF Zagreb
7. IPARD program - Agriculture and Rural Development Plan 2007-2013, Ministry of Agriculture, Zagreb.
8. Law onAgriculture (OJ66/01, 83/02)
9. Law on Ratification of the Framework Agreement between the Croatian Government and the European Commission on rules for cooperation concerning the European financial assistance for the Republic of Croatia and the implementation of assistance under the Instrument for Pre-Accession Assistance-IPA (OJ 10/07).
10. Regulations on the scope and content of responsibilities and authorizations of entities responsible for management of the Instrument of Pre-Accession Assistance – IPA(OJ 34/08)
11. Report on the use of pre-accession assistance programs of the European Union 2013. Government of Republic of Croatia, Zagreb
12. Strategy of Agriculture and Fisheries of the Republic of Croatia (OJ 89/02)

Original paper

STRUCTURE AND DYNAMICS OF SHEEP SYSTEMS IN BOSNIA AND HERZEGOVINA

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Abstract

The paper presents the analysis of dynamics and structure of the sheep systems in Bosnia and Herzegovina assuming that they suffered a decrease of animal and farms consistency in the last 6 decades. Since 1991 neither a general nor agricultural censuses were made to provide information about the present state of sheep farming in the country. An analysis of the available statistical records of agricultural trends related to the sheep sector was performed. In addition, a depth questionnaire by consulting national experts was performed in order to obtain relevant information on the spatial distribution, consistency, feeding management, production and environmental impact on the present structure of sheep production systems. A decrease in sheep number was observed over the last six decades, but less than in other species. Six main sheep systems in three biogeographical regions were identified. Differences in animal spatial distribution, production purpose and other characteristics of the systems indicate that the environmental and socio-economic factors throughout the country strongly influence the choice of breeding methods and management. All consulted experts indicated the lack of support for sheep systems in relation to agro environmental management, landscape conservation and biodiversity preservation.

Key words: *Bosnia and Herzegovina, sheep systems, system characteristics, trends*

Introduction

With a centuries-long tradition sheep breeding has played a very important role in the livestock production in Bosnia and Herzegovina (B&H). According to official statistical data from the Agency of Statistics of B&H it is estimated that the total number of sheep in the country is around 1,005,000 heads. More detailed information on the number of sheep and sheep farms is unknown, especially for small private farms, because neither a general nor agricultural censuses have been made since 1991, which could provide a real picture of post-war farm structures (Karlogan-Todorović, 2012). Before the First World War there were round 2,000,000 sheep; thereafter their number decreased especially during the recent war (1992-1995) (Alibegović-Grbić, 2009). It is believed that overall sheep population has decreased by 30% after 1991 (Erbez and Rogić, 2010).

The lack of farm animals, especially small ruminants, led to the abandonment of grassland and scrubland utilised by pastoral activities. This decrease negatively affected the biodiversity and open surfaces became overgrown and non-usable for agricultural purposes (Sedić et al., 2014). The decrease of farm animals, on the other hand, allowed the existing farmers to extend over more fertile land which was abandoned, losing the need to depend on less favoured areas. In the past all wars led to a temporary hold of transhumance throughout the Balkan area. Excluding the wars, a key role in the reduction of farm animals was the reform of agriculture law denying the use and ownership of mountain pastures by peasants and farmers (Kazakova and Stefanova, 2010).

This study aims to analyse the dynamics of sheep production systems and the present state of sheep farming through biogeographical regions. The obtained results are expected to be used as starting point for assessing the relationship of sheep farming with biodiversity, landscape conservation and improvement of site conditions.

Materials and methods

For the purpose of this study two types of information were used. The quantitative data used for the trend analysis were obtained from statistical records listed in Table 1. General changes that occurred in the agricultural trends related to sheep production systems were analysed with a paired t-test (Garcia-Martinez et al., 2009) to compare differences between decades. Statistical analysis was performed in SAS 9.1.

Table 1. *Source of data for statistical analysis*

Source	Reference period
National First Release and annual reports for agriculture and livestock of Bosnia and Herzegovina	2005 to 2013
Statistical Yearbook of Yugoslavia	1950 to 1991
FAO stat online database	1950 to 2012
World Bank online database	1950 to 2012

A collaborative effort was requested to a group of 8 experts for collecting information for the sheep systems analysis according to their representative biogeographical region (2 Alpine; 4 Continental; 3 Mediterranean). The survey tools including a depth questionnaire (fact sheet), a detailed survey guideline and a geophysical map of the study area provided information on the sheep systems which remained after the Independence Declaration in 1992. The questionnaire was structured in six main sections (Caballero et al., 2009). The first section (expert's identification, biophysical conditions and organisation of agriculture land) was aimed at obtaining an overview of the analysed study area according to its environmental features. The second section (breeds used, breeding purpose and method, number of farms, number of animals, specific products, animals and land ownership) provided information about the scale and relevance of the addressed systems. The third section (annual feeding regime including feeding resources used and their yield, annual grazing cycle, grazing method, stocking density, herding, mobility, ownership of animals and facilities, grassland management) provided information about the resource base for feeding and management details during the grazing period. The fourth section (housing, technology, labour) was aimed at obtaining information about the level of innovations and technological advances applied in the system. The fifth section (consulting, co-operative aspect) provided information about the dependency of the farmers on other stakeholders.

The sixth section (management comparison, large scale and biodiversity aspect) was aimed at obtaining information about competitiveness, environmental and biodiversity impacts related to the management practices. The sections contained from 4 to 25 questions being either qualitative with multiple choice answers, or quantitative, requesting a single answer. Section one contained 2 qualitative and 8 quantitative variables; section two, 7 qualitative and 5 quantitative variables; section three, 7 quantitative and 3 qualitative variables, section four, 5 qualitative and 1 quantitative variable; section five, 3 qualitative and 1 quantitative variable; section six, 24 qualitative and 1 quantitative variable.

The systems identified by the experts have been analysed according to their location in the main biogeographical regions of B&H (EEA 2002a,b,c): Alpine, Continental and Mediterranean.

Results and discussion

The analysis of agricultural and livestock trends related to sheep production systems resulted in values with statistically significant differences between the averages of the observed decades (Table 2).

Table 2. *Agricultural trends in Bosnia and Herzegovina*

Variable	50s	% difference between decades					Total
		50-60s	60-70s	70-80s	80-90s	90-2000s	50-2000s
Sheep	1,882,585	13.6**	-26.8**	-11.9*	-31.6**	4.5	-52.3**
Ewes	1,216,030	18.4**	-21.8**	-9.4*	-8.6	20.4**	-41.8**
% LU sheep/LU total	20.5	11.0**	-16.3**	-1.4	3.2	40.9**	37.4**
TAA	2,575,344	1.7	-1.3	-1.2	-12.1	-4.7	-17.6**
Arable land	1,692,610	-0.8	-3.8	-0.6	-27.9**	-1.8	-34.9**
Grassland	1,321,873	1.7	3.0	2.4	-17.4**	-13.0**	-23.3**
% Grassland/TAA	51.3	0.1	4.2**	3.5**	-2.9	-10.9**	-6.2**
% Forage Crops/Grassland	2.7	3.5**	4.8**	2.7**	-0.1	-0.2	10.8**
LU sheep/Grassland	0.26	11.0*	-26.8**	-12.1*	-20.4**	13.6*	34.8**
LU total/TAA	0.62	0.3	-7.9	-9.1*	-43.5**	9.0*	-51.1**
Population	2,847,790	24.1**	12.7**	15.1**	-16.6**	0.5	35.7**
Agricultural Population	2,721,216[1]	NA	-8.0	-6.4	-3.6	-4.9	-22.9**

LU-Livestock unit; TAA-Total agriculture area; *P<0.05; **P<0.001
[1]Average value for 60s used as base

A decrease of most observed variables occurred over the last six decades. Four of the twelve chosen variables showed increasing trends, with increases up to 35%. An increase of sheep LU in proportion to LU from other farm animals (cattle, sheep, goats, horses) is present, yet the decrease of the total number of sheep and ewes hints to an even bigger decrease of LU from other farm animal species, resulting in the proportional increase of sheep LU in the total LU. The relationship of forage crops and LU of sheep in relation to available grassland increased as well (10.8 and 34.8%, respectively).The total population increased by 35.7%, leading to a bigger need for food supply. Agricultural population did not decrease significantly per decade, but the total difference has significant importance comparing the present state to the 60s. Various authors indicate that the socio-economic

changes during the past led to emigration towards central Europe and other neighbouring countries and partly related to this is de-ruralisation which occurred during economic crisis in the 90s.

After consulting the available statistical records and literature, present sheep production system was analysed according to Caballero et al. (2009). Experts were able to locate the present sheep production systems in the corresponding biogeographical regions (Figure 2). Even if potential and actual area affected by sheep production systems may differ greatly, hot spots in the corresponding national map indicate locations where identified sheep systems are most concentrated.

The **Alpine biogeographical region** of B&H makes 5% of Europe Alpine biogeographical region (EAA 2002a, b, c), and according to Čengić and Cabaravdic (2005), 51% of the country is mountainous. In this region 70% of the total surface is cowered by forests, only 25% is agriculture area, and up to 75% of the agriculture area is grassland. Around two thirds of the available agricultural area in the region is being utilised.

Two main sheep systems were identified by the consulted experts in the Alpine region: a dairy (**1a**) and a meat (**1b**) dominant oriented system (Fig. 1 and Table 3).

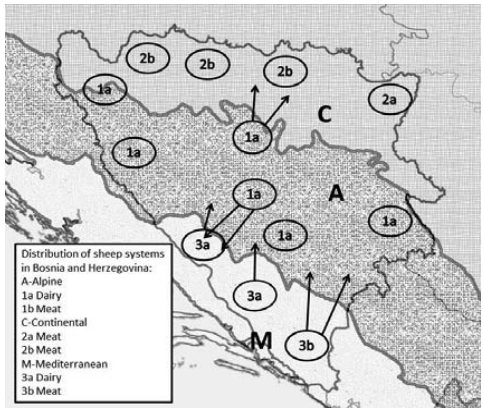

Figure 1. *Distribution of sheep production systems in the biogeographical regions of Bosnia and Herzegovina (arrows highlight mobility areas of the flocks).*

1a - The Alpine dairy sheep system involves approximately 350,000 out of the 750,000 animals expected to be present in this region (Table 3). Both sedentary and transhumant flocks are spread over various altitudes over the region. From the movements performed in the past, just few remained, partly because of the decreased animal number, partly due to the banning of transhumance starting from the '40s to prevent diseases spreading and damages on winter cereals in plain areas. Remaining movements are local and the distances are reduced to few kilometres from the farmstead. Present movements include migrations from the Kupres area towards Livno County and from Vlašić Mountain towards the continental area in the north of B&H. Grazing periods and the use of forage crops through farms in this system are provided in Figure 2. Milking of animals is performed after weaning of lambs, and the most common way of utilising obtained milk is conversion into various types of cheese labelled according to the place of origin (e.g. Vlašićki cheese).

1b - The Alpine meat system is rather dispersed and spatial distribution is nearly impossible to define, but about 40% of the sheep of the region are located in this system (Table 3). The feeding is adapted to the available local resources, while the low stocking

Table 3. *Characteristics of the sheep systems in Bosnia and Herzegovina.*

System identification code	Production purpose[1]	Mobility[2]	Number of farms	Animals in the region (%)	Animals in the system (% of animals in the region)	Breeds used [3]	Animals per farm	Self-sufficiency-feeding (%)	Self-owned land (%)	Grazing length (days)	Stocking density (LU/ha)	Ownership of the grazing facilities[4]	Flock size during grazing	Labour dependence (animals per worker)
1a	D	S, V	4500	75	60	DP, KP, PP, XP	80	>75	40	240-365	1.0	M	80	>100
1b	M	S, V	2500		40	DP, KP, HP, XP	80	>75	40	240-365	1.0	M	200	>100
2a	M	S, H	100	15	20	XP, TX, R, IF	50	>75	40	270	1.5	P	40	30-50
2b	M	S, H	900		80	XP, JSO, IF, WB	25	>75	40	365	1.5	P	25	10-30
3a	D	S	150	10	30	XP, DP, KP, HP	60	<75	50	240	0.5	P	60	50-100
3a	D	V	30		20	XP, DP, KP, HP	60	>75	50	365	0.5	M	400	>100
3b	M	S	150		30	XP, DP, KP, HP	60	<75	50	240	0.5	P	60	50-100
3b	M	V	20		20	XP, DP, KP, HP	60	>75	50	365	0.5	M	400	>100

[1]Production purpose: D-Dairy; M-Meat.
[2]Mobility: S-Sedentary; V-Vertical; H-Horizontal.
[3]Breeds: XP-Pramenka crosses; DP-Dubska Pramenka; KP-Kupreška Pramenka; HP-Hercegovačka Pramenka; PP-Privorska Pramenka; R-Romanov sheep; WB-Wirtenberg; IF-Il de France; JSO-Jezersko Solčavska sheep; TX-Texel.
[4]Ownership of the grazing facilities: P-Private; M-Mixed includes private and public.

densities of the region allow for a large source of feedstuff during the year (Fig. 2). Flock sizes and breeding methods are adapted mostly to the capacities of the farmers themselves, with lambs being the main production output. Young animals are sold mostly during April and May and during the tourist season with desirable live weights of around 25 kg. Adult animals are mostly sold and slaughtered during the Kurban Bajram holiday whose date varies by year.

The **Continental biogeographical region**, including Prijedorsko field, Semberija, Posavina and Lijevče field covers around 30% of the national surface. More than 75% of the total surface is an agricultural area, 40% of the TAA is utilised and 25% is grassland.

An intensive (2a) and extensive (2b), mainly meat-oriented systems, were identified by the consulting experts in this region (Fig. 1 and Table 3).

2a – The Continental intensive meat system is located in the north-eastern part of B&H, with 100 farms, being one of the smaller systems inside the country (Table 3). A particular feature of this system is the breed structure which includes, except Pramenka strains, high productive meat breeds and crosses. The grazing period is shortened compared to other systems, at the same time including silage during the feeding period (Fig. 2).

2b – The Continental extensive meat system (Table 3) is located in three main plain areas of the continental area of north B&H (Prijedorsko field, Semberija and Lijevče field). Due to being an area suitable for cereal and forage production this system has a short grazing period (Fig. 2), with rare to none movements either vertical or horizontal. Single farms extend the grazing period throughout the whole year if the climate conditions allow it. The area in which this system is present is being used as destination for flocks from the Alpine region during winter (1a dairy systems).
The **Mediterranean biogeographical region** in Bosnia and Herzegovina covers an area of approximately 10,000 km², which corresponds to 20% of total country surface. The region is mostly located in Herzegovina and south-west Bosnia. Less than 75% of the TAA is covered by agricultural area. Of the total agricultural area, less than 25% is utilized, and up to 75% of the TAA is cowered by grassland.

Two main sheep systems were identified by the consulting experts in the Mediterranean region: a dairy (**3a**) and a meat (**3b**) dominant oriented system (Fig. 1 and Table 3).

3a - The Mediterranean dairy system involves approximately 50% of the total 80,000 animals expected to be present in this region (Table 3). It includes both sedentary farms with vertical and horizontal movements on a daily base, and transhumant farms with various flock sizes; during transhumance around 10% of total farms mobilize 30-40% of the total sheep population present in the system. Flocks during transhumance extend from 300 up to 1,000 animals. Trans-border movements from Dalmatia towards Herzegovina ceased to exist after the recent independence of the former Yugoslavia countries; the remaining transhumant movements involve migrations from lower areas (lower Herzegovina) to Blidinje Park, flocks from Livno towards Cincar Mountain and flocks from Stolac, Čapljina, Ravne and Rudine are migrating towards Treskavica Mountain.

Transhumant movements in this region are a result of environmental constrains, the drought period forces farmers to migrate towards higher altitude levels to satisfy the demand of the animals for feed (Fig. 2). The production of traditional sheep cheese (Kupreški cheese, Livanjski cheese, 'sir iz mješine') is commonly present inside this system.

3b - The Mediterranean meat system (Table 3) is similar to the Alpine meat system dispersed throughout the region, having an epicentre in the area of Ljubinje and Stolac.

The harsh environmental conditions and karst topographic features resulted in smaller animals being better adapted to the poor feeding supply and drought period. The feeding is fully adapted to the environment, and grazing on scrublands and grasslands serves as the main source of feedstuffs for the animals (Fig. 2). In this system the smallest Pramenka strain is present.

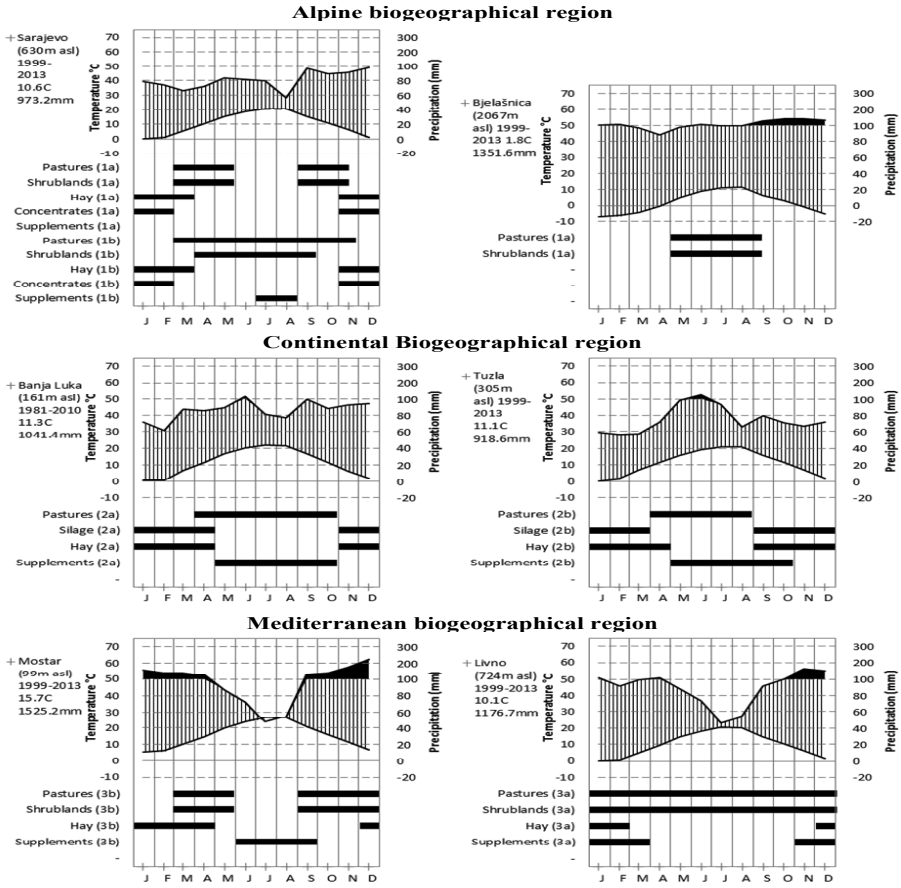

Figure 2. *Feeding resources used in different sheep systems (between parenthesis the system identification code is indicated) according to the climate of the biogeographical regions (Walter-Lieth climate diagrams were derived by the data of the Federal and Republic Metrological Institutes of B&H).*

Common features of the whole sheep sector, including all systems, are dominant presence of Pramenka breed strains, dominant adoption of continuous stocking and absence of rotational stocking. Regardless the biogeographical or administrative region they belong to, all systems receive two main types of support, namely financial, through subsidies per head of registered adult animal, and consulting, from various administrative bodies. Marketing and organisation types of support are absent in the sheep sector, on which all consulted experts agreed. Unions of farmers exist mainly to influence strategic planning and measures to be applied in the agriculture sector. Marketing, selling and distribution of

sheep products is pulverised; dairies and small household processing plants convert milk to various types of cheese and sell it either from the doorstep (household made) or in the shops and supermarket chains. Market for adult animals is secured through processing by traditional drying and smoking of sheep carcasses, resulting in a nationally recognised product whose name varies from 'sheep stelja', 'sheep pastrma' to 'sheep dried meat'. Sheep systems depends heavily on labour in terms of herding during the grazing season, especially in cases in which the sheep breeding is a part time activity rather than the full time occupation of the farmer.

The sixth section of the survey tool revealed that sheep production systems are not properly considered as a tool for maintaining the landscape and biodiversity. Despite the present rules which allow transhumance and mobility of farm animals by meeting particular conditions (e.g., use of lorries, renting grazing land, etc.), expectations are that the remaining transhumant flocks are not sufficient to prevent landscape changes and biodiversity loss in areas which were a common destination for pastoralists.

Conclusion

Because of the decrease in sheep number over the last six decades and the increase of population, an increasing demand of sheep products is present. Considering the features of the biogeographical regions present in the study area all collaborators pointed out that a big potential for the development of sheep systems exists.

Differences in animal spatial distribution, production purpose and other characteristics of the present state of sheep systems indicate that the environmental and socio-economic factors throughout the country strongly influence the choice of breeding methods and management.

The lack of support for sheep systems in relation to environment, landscape and biodiversity conservation, as indicated by all the consulted experts, underlines the need of policies to address these gaps.

References

1. Alibegović-Grbić S 2009. Bosnia and Herzegovina, Country Pasture/Forage Resource. www.fao.org.
2. Caballero R, Fernandez-GonzàlezF, Perez-Badia R, Molle G, Roggero PP, Bagella S, D'Ottavio P, Papanastasis VA, Fotiadis G, Sidiropoulou A and Ispikoudis I 2009. Grazing systems and Biodiversity in Mediterranean areas: Spain, Italy and Greece. In: Pastos, 39, (1), 154.
3. Čengić I and Cabaravdic A 2002. Watershed management in mountain regions in Bosnia and Herzegovina – a general overview. In: Europe (eds Achouri M, Tennyson L) Megeve, France, 113-123.
4. Dixon J, Gulliver A and Gibbon D 2001. Global Farming Systems Study: Challenges and Priorities to 2030. Food and Agriculture Organization of the United Nations, Rome, Italy, 90.
5. EEA (European Environment Agency) 2002a. The Alpine region – mountains of Europe. In: European Environment Agency, Europe's biodiversity - biogeographical regions and seas. EEA, Copenhagen, 52.

6. EEA (European Environment Agency) 2002b. The Continental biogeographical region – agriculture, fragmentation and big rivers In: European Environment Agency, Europe's biodiversity - biogeographical regions and seas. EEA, Copenhagen, 52.
7. EEA (European Environment Agency) 2002c. The Mediterranean biogeographical region - long influence from cultivation, high pressure from tourists, species rich, warm and drying. In: European Environment Agency, Europe's biodiversity - biogeographical regions and seas. EEA, Copenhagen, 54.
8. Erbez M and Rogić B 2010. Ovčarstvo u BiH. Retrieved on 09 April 2014 fromwww.stocarstvo.rs.ba.
9. Garcia-Martinez A, Olaizola A and Bernues A 2009. Trajectories of evolution and drivers of change in European mountain cattle farming systems. Animal 3(1), 152-165.
10. Karlogan-Todorović S 2012. Bosna and Hercegovina. In: High Nature Value Farming in Europe (edsOppermann R, Beaufoy G, Jones G), pp. 138-145. Verlagregionalkultur, Ubstadt-Wiehner.
11. Kazakova Y and Stefanova V 2010. High Nature Value Farming in the Western Balkans: Current Status and key challenges – a Scoping Document. European Forum on Nature Conservation and Pastoralism. Bulgaria, 34.
12. Sedić E, Brka M and D'Ottavio P 2014. Trends and drivers of change of cattle systems in the Western Balkans. In: Forage resources and ecosystem services provided by Mountain and Mediterranean grasslands and rangelands (edsBaumont R, Carrère P, Jouven G, Lombardi G, Lòpez-Francos A, Martin B, Peeters A, Porqueddu C), Options Méditerranéennes (109), Clermont-Ferrand, France, 735-739.

Original paper

THE POSSIBILITY OF ORGANIZING AN ORGANIC SHEEP BREEDING IN THE MUNICIPALITY OF ŽAGUBICA

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Abstract

Žagubica Municipality, in Eastern Serbia, is one of the most undeveloped municipalities in Serbia. It accounts for only 0.067% of the total GDP (Gross Domestic Product) of the Republic of Serbia. A large number of people migrated from this area to big cities or countries of the EU, mainly because of the economic underdevelopment and the lack of production and processing capacities. Nowadays agriculture has a major role in the economy of Žagubica, contributing to the total value created with 59.33%, which clearly speaks of its dominance, while far behind it is the manufacturing industry with 8.23% and commerce with 6.93%. Geographical position of the Žagubica Municipality, the Homolje territory, is an important natural resource of great potential, with specific characteristics of HNV (High Nature Value) region. Region of Homolje has a long tradition of sheep breeding and the quality of products has become well-known. However, depopulation of villages caused a decline in the production and closure of a number of processing facilities. The development of organic farming is one of the possible ways to revitalize the village and the development of this region rich in natural resources. This paper analyzes the strengths, weaknesses, opportunities, and threats (SWOT analysis) in the organization of organic sheep production in order to apply the necessary measures on the field for the organization of a new production system. The significant "advantages" of Žagubica Municipality are its herbal resources and the fact that the arable agricultural land makes 48.5% of the municipality area (42.9% of meadows and 26% of pastures) which is a good basis for the organization of the production. The issue of animal genetic resources is particularly analyzed and discussed. Under the "possibility" the attention is focused on various aspects of education in this field and its significance.

Key words: *Homolje region, organic production, rural development*

Introduction

Livestock in the Žagubica Municipality has drastically decreased with the collapse of cooperatives in Serbia during the nineties. This is primarily due to the loss of opportunities for organized purchase of animals provided by the cooperatives. Another problem was an unstable market and sanctions which the community faced in the years after the collapse of the state.

The problems that the country faces today regarding the employment of people force the community to find alternative opportunities to make money. The independent production is emphasized in all fields of economy, including agriculture and animal husbandry. Special emphasis is placed on the production of healthy and organic food of plant and animal origin. The Access Fund of the Republic of Serbia in the field of agriculture facilitates an easier commencement of such production. In addition, the possibility of eventual export and sale of products to the European Union provides a secure economic benefit to producers and the state.

Serbia is faced with many challenges when it comes to marketing of products. The possible entry of Serbia into the EU would lead to reorganization of the existing production and adjustment to the criteria required by the EU for the selected production. This is certainly possible to achieve with a planned and systematic analysis of all steps in the production cycle. One of the goals is to create branded products with protected geographical origin and to encourage rural development.

Materials and methods

Analysis was done regarding all opportunities that Žagubica Municipality provides and the threats which could hamper the organization of organic sheep production in Homolje region. Furthermore, all resources (strengths) and weaknesses that could make the production unprofitable were overviewed.

SWOT analysis was performed based on the results of work in the field of organization of organic production and data related to natural and economic potential of Žagubica Municipality.

Results and discussion

The strength for developing sheep organic production are natural and human resources of Žagubica Municipality. Agriculture has the major role in the economy of Žagubica Municipality and it participates with 59.33%, which clearly speaks of its dominance. Economic underdevelopment of Homolje resulted in the preservation of a healthy environment and non-infringement of the natural beauty of this area. Water of Homolje region is a versatile potential, which is of great importance for the development of organic farming.

Flora and fauna of Homolje is particularly rich and abundant in many plant and animal species, some of them being relict. Agricultural land covers 36,880 ha or 48.5% of the municipality area. The agriculture land consists of 42.9% meadows, 26% pasture and 31.1% arable land. Large pastures and climatic conditions in the region of Homolje are favorable for efficient sheep production.

The interaction of environmental factors and genotypes of autochthonous Zackel sheep in this region came to the forefront in the evolutionary adaptation of strains of sheep which constitutes one of the pillars in the selection of suitable breeds for organic production (Trailović R. et al., 2006; Savić M. et al., 2011 and 2013). Svrljig type of Zackel sheep is the most common indigenous sheep breed in the territory of Municipality of Žagubica, based on the data registry office. Besides this sheep the Sjenica strain gradually appears, as well as cross-breds by crossing with Merino breeds. The process of crossing with Merino, Wuerttemberg and Ile de France breeds is in progress now in order to improve meatiness characteristics and Romanowski breed and to increase the fertility of indigenous breed.

Well organized selection service provides the ability to monitor, preserve and improve the livestock as well as giving direct access to the manufacturer's fund of the Ministry of Agriculture for farming of animals and improvement of livestock. Producers have expressed willingness to work in sheep breeding and for continuous education about breeding animals and monitoring of new technologies, such as the introduction of organic production standards. A well-organized work of the veterinary service in the field is of great importance for the successful organization of organic production and implementation of veterinary supervision in accordance with the law of organic production.

The number of sheep did not differ significantly in the Municipality of Žagubica in the past three years, based on data from the central database for flagging domestic animals, which are based on the commission of the programme of animal health protection measures. Based on these data, the number of sheep was 10228 (2011); 10279 (2012) and 10275 (2013). This number refers only to the animals in the reproduction cycle. Homolje region has a long tradition in the production of sheep and dairy products, which are well known for quality what makes them highly competitive on the market.

Weaknesses in organizing the organic farming, despite the availability of significant natural resources, still exist. The climate of this area is characterized by a variability of weather conditions, the summers are rather stable, with occasional drought and uneven rainfall, while winters are quite long and cold. There is also improper use of pastures and meadows, which resulted in weed growth and soil degradation.

The Municipality of Žagubica is one of the lowest developed municipalities in Serbia. It accounts for only 0.067% of the total GDP (Gross Domestic Product) of the Republic of Serbia. Municipality of Žagubica, with other municipalities of Eastern Serbia, is demographically an old municipality, with an average age of population 45 years, indicating an unfavorable age structure of farmers. A large number of people migrated from the area to big cities or countries of the EU, mainly because of the poor economic development and lack of production and processing capacities. There is a quite small number of young, working-age population in the Žagubica Municipality.

This municipality is far behind in the development of transportation infrastructure compared to the national average. All villages are connected by the local road network that is in rather poor condition. Significant processing facilities, slaughterhouses, dairies are not functional since the time of collapse of cooperatives in Serbia during the nineties of the last century.

Long-term stagnation in technological development and the lack of farmer motivation joined into functional associations in cooperatives also hamper the proper product placement. Small possessions, as well as non updated machinery for preparing animal food and for milking, further complicate production. Sales of products such as milk, meat and wool are not organized. Nonetheless, purchasing power, low standard and poor purchasing habits play an important role (Živković, 2012) therein.

Opportunities for sheep organic development are based on better management of natural resources and on farmers' education. Geographical position of the Žagubica Municipality is between 44° 05'and 44° 22' north latitude and between 21° 31' and 21° 50' east longitude. Homolje territory looks like a rectangle, with east-southeast-west-northwest position, length of 35 km and width of 26 km. The border with neighbouring areas generally extends over the highest peaks of the mountain ranges. The length of Municipality of Žagubica boundaries is 136 km, of which 129 km is terrestrial and 7 km aquatic border (Miljković, 1992). The Homolje territory is an important natural resource of great potential, with specific characteristics of HNV (High Nature Value) region (Cooper et

al., 2010). The region of Homolje has a long tradition of sheep breeding and quality of product has become well-known.

Providing high quality, healthy and safe products of animal origin is an imperative in favoring methods of organic production with the aim of protecting specifics and taste of the indigenous animal products and diversification of products of animal origin. The introduction of standardized measures in sheep breeding, preparation and processing of animal products, the improvement of veterinary-sanitary protection in order to reduce the risk for the hygiene of product, can contribute significantly to the turnover of standardized and controlled animal products.

Special emphasis should be given to the creation of distinctive regional products and their organized participation and promotion on the market. Increasing the competitiveness can be achieved by improving the quality of agricultural products with value added products, such as organic products.

Development of the organic livestock production through continuous education of farmers, modernization of livestock production through involvement in trade of high-quality breeding animals would increase employment of young people in the animal husbandry and foster the sustainable rural development in the Žagubica Municipality. Timely applications and applying for funding, scholarships and training of staff is a planned approach to the organization and management of long-term good livestock production. Joining of the producers would allow easier access to the development funds, the implementation of rural development programs, strengthening regional connectivity and cooperation.

The threats for organizing the organic sheep production are mainly based on insufficient and inadequate exploitation of natural resources. There is a great need for the modernization of technology, effective production and greater market orientation, in the Municipality of Žagubica. The current process of depopulation of rural areas leads to insufficient exploitation of natural resources, reducing the population of sheep and leaving pastures and meadows unused. In regard to the quality of the pastures, the major problem is their degradation, the presence of shrub that can eventually lead to the reduction in the area for grazing animals when their growth and spread is not controlled.

Current production systems of livestock production in the Municipality of Žagubica generally do not provide a cost-effective production, so it is necessary to approach their rapid change. Solving property issues and fragmentation of possession pose a significant threat to the organization of organic production.

Conclusion

Organic production provides an opportunity to foster the proper protection of animal health and welfare and to promote maximum environmental protection.

Education of producers, association of manufacturers and credit for production are the engine of development of livestock production in the region. Enlarging the possessions with the gradual construction of animal food processing facilities would lead to the implementation of sustainable livestock production.

Education of farmers and encouraging family farms engaged in sheep breeding to begin with process of conversion to organic production system is one way to revitalize the region. Production of organic products can lead to contribution of rural development and to the promotion of a whole region.

Acknowledgements

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References

1. Cooper T, Pezold T, Keenleyside C, Đorđević-Milošević S, Hart K and Ivanov S 2010. Developing a National Agri-Environment Programme for Serbia. Gland, Switzerland and Belgrade, Serbia: IUCN Programme Office for South-Eastern Europe. 1-88 pp.
2. Miljković Lj 1992. Homolje. Geografska monografija, Univerzitet u Novom Sadu, Prirodno-matematički fakultet-Institut za geografiju, Novi Sad.
3. Savić M, Aleksić S and Živković D 2013. Breeds of choice in organic production systems. Proceedings of the 10 th International Symposium Modern Trends in Livestock Production Invited paper, Belgrade, Serbia, 298-306 pp.
4. Savić M, Jovanović S, Trailović R, Dimitrijević V and Dimitrijević B 2011. Autochtonous breeds as livestock potential in sustainable farming in Serbia. Proccedings of the 19th Internationale Congress of Mediterranean Federation of health and production of ruminants, Belgrade, Serbia 16-22 pp.
5. Trailović R, Djedović R, Bogdanović V, Jovanović S and Ćinkulov M 2006. Pramenka Breed Types of Serbia. In Catalogue of West Balkan Pramenka Sheep Breed Types [eds Porcu K and Marković B], pp. 74-88. Faculty of Agricultural Sciences and Food, Skopje.
6. Živković B 2012. Perspektive održivog razvoja turizma opštine Žagubica. Kulturno-prosvetni centar Jovan Šerbanović. Žagubica.

Original paper

REGIONAL DISTRIBUTION OF GHG EMISSIONS FROM LIVESTOCK ENTERIC FERMENTATION AND MANURE MANAGEMENT IN THE REPUBLIC OF MACEDONIA IN THE PERIOD 2007-2012

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Abstract

The estimation of GHG emission from domestic livestock in the Republic of Macedonia during the period from 2007-2012 is performed according to the IPCC Good Practice Guidance and Uncertainty Management in National Greenhouse Gas Inventories applying the Tier 1 method for calculation of emissions. This article has accepted the following gasses: Methane (CH_4), Nitrous oxide and CO_2-eq from the domestic livestock sub sector using data sources from the official Statistical Yearbooks of the Republic of Macedonia. According to available data for livestock species, distribution of total annual methane emission and nitrogen excretion was calculated in each planning region for the period 2007-2012. In the analyzed period two regions showed continuously highest (Southeast region) and lowest (Vardar region) CH_4 emission and nitrogen excretion. In the period 2007-2012 CH_4 emission ranged between 26.43 Gg (2007) and 23.78 Gg (2012), with the lowest value in 2012 (23.78 Gg). Depending on the animal waste management system the highest value for nitrogen excretion (solid storage and drylot) was observed in 2010 (kt/N/yr). Almost similar value for nitrogen excretion from pasture and paddock was observed in 2007 and 2008, 9.54 and 9.53 kt/N/yr respectively, with the lowest value for analyzed period noted in 2009 (8.82 kt/N/yr). In this period the highest value (3.32 kt/N/yr) for other type of AWMS was recorded in 2007. Separation of certain regions in the country regarding GHG emission can be clearly noted. Data for the period 2007-2012 show a downward trend in GHG emission. Applications of modern breeding technology, balanced feed as well as better feed quality in the future are the main objectives in order to reduce GHGs emission from this subsector.

Key words: *livestock, methane, nitrous oxide and CO_2-eq*

Introduction

Livestock production can be noted as a major contributor to the environmental pollution, because this sector is contributing in emission of GHG in the form of CH_4 from enteric fermentation, and CH_4 and N_2O from manure management and deposition of animal manures on pastures. According to FAO (2006) livestock contributes 18% of global GHG emissions although emission is highly variable in different regions. The global dairy sector contributes from 3.0% to 5.1% of the total anthropogenic GHG emissions (FAO, 2010). Livestock GHG emissions by IPCC inventory system equals to 10.8% of global emissions according to FAO

(2006) and this sector constitute nearly 80% of agricultural emissions. Denman et al. (2007) and EPA (2006) calculate values of 8 and 9.6%, respectively, of livestock's contribution to global agriculture emissions. Comparing data mentioned above livestock's contribution to global GHG emissions is in the range of 8 up to 10.8%.

Greenhouse gas emissions from livestock are closely related to the number of each animal species and different categories inside the species. Main impact on GHG emission is from ruminants, especially cattle. Besides the number of animals, general factors that have their influence on emission levels are: size of animals, productivity level of animals, system of manure management, type of diet, type of production system and the climate conditions in the region.

Methane emissions in the period 2003–2009 account for 12.38% of total GHG emissions in Macedonia. One third of emissions is generated by enteric fermentation and manure management, and as a consequence these emissions are directly proportional to livestock numbers. High amount of CH_4 emissions (89%) are generated by enteric fermentation from domestic livestock, and these emissions have been continuously decreasing as a result of reduction of livestock populations. Manure management emissions account for 8% of GHGs, while the remaining emissions come from rice fields and the burning of residues. N_2O emissions from the management of agricultural soils are 89%, including the use of fertilizers, the amount and type of manure applied, leaching, nitrogen-fixing crops and atmospheric deposition, while remaining emissions are generated by manure management and to a less significant extent, from the burning of crop residues (Zdraveva et al., 2013).

This paper shows the greenhouse gas emissions from the livestock production sector in the Republic of Macedonia during the period 2007-2012. To obtain more precise information on GHG, emissions were calculated for each state statistical region separately in order to determine the influence of each region in the total annual emission.

Materials and methods

Official data from the State Statistical Office (SSO 2007, SSO 2008, SSO 2009, SSO 2010, SSO 2011 and SSO 2012) were used as entrance data in order to calculate GHGs emission. Data were processed according to the Tier 1 method (IPCC, 1997), which is actually a simplified method of calculation of greenhouse gas emissions. Tier 1 method includes the following data: number of each animal species regarding breeding category and data for climate conditions in the analyzed region which in turn define emission factors/coefficients. The application of the sophisticated Tier 2 method requires detailed information about the livestock sector. The study covers the main animal species in the country: cattle (dairy and non dairy), small ruminants (sheep and goats), pigs, poultry and horses.

Results and discussion

If we compare total annual CH_4 emissions from enteric fermentation (Figure 1) during the analyzed period (2007-2012), highest emission values are present in the Southwest region. From total CH_4 emission in the country 21% of total CH_4 emissions from enteric fermentation in 2011 and up to 24% in 2009 belong to this region. Second region in the country, regarding GH_4 emission is the Pelagonia region. Contribution of this region to total annual emissions ranges between 16% and 22% of total CH_4 emissions from enteric fermentation. Same values (22%) were noted in both regions in 2009 and 2012. Emissions from other regions in the country were lower compared with Southwest and Pelagonia

regions. Contribution to total annual CH_4 emission with less than 15% is present in Vardar region, with a value of 5 to 7% of total annual CH_4 emission.

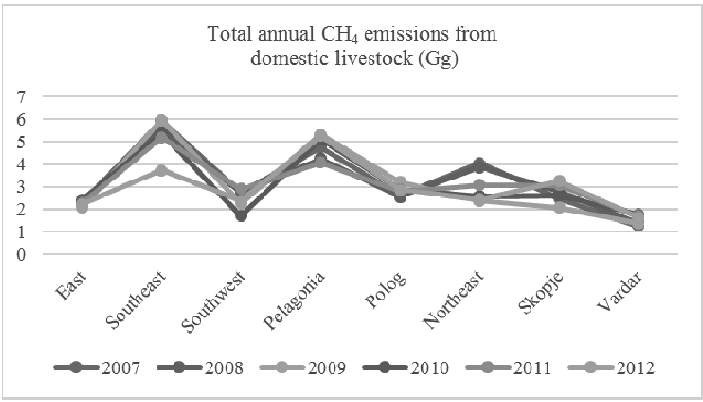

Figure 1. *Methane emission from eight regions in the country*

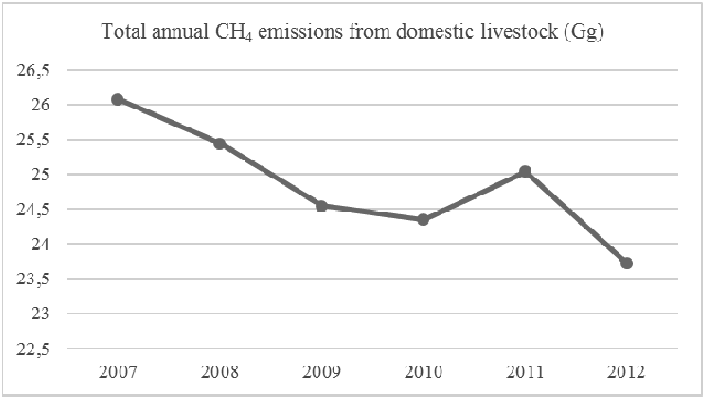

Figure 2. *Total methane emission in the country*

Total annual emission of CH_4 (Figure 2) from enteric fermentation at country level during the analyzed period showed a decreasing tendency. Highest value was noted in 2007 (26.43 Gg) and lowest value in 2012 (23.78 Gg). Management system, composition of manure, the type of bacteria responsible for manure decomposition, presence of oxygen and fluid in the manure management systems significantly affect production of N_2O during the process of manure management. Manure aerobic decomposition is characterized with increased emissions of N_2O, transformation of N_2O in NO results in a reduction of ozone. Lowest values for nitrogen excretion from solid storage and drylot for non-dairy cattle during the analyzed period (2007-2012) were noted in the Vardar region, while highest nitrogen excretion was present in the Southeast region (Figure 3).

Opposite to nitrogen excretion from solid storage and drylot for non-dairy in dairy cattle breeding, highest values were continuously noted in the Pelagonia region with the exception

of 2011, when the highest part of total emission was present in the Southeast region (Figure 4).

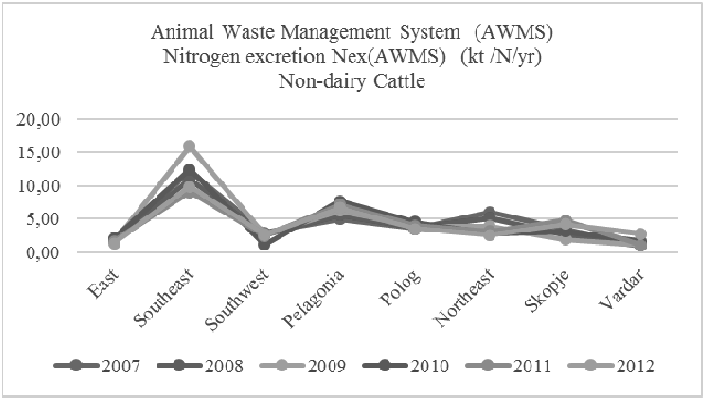

Figure 3. *Total annual $Nex_{(AWMS)}$ excretion, non - dairy cattle*

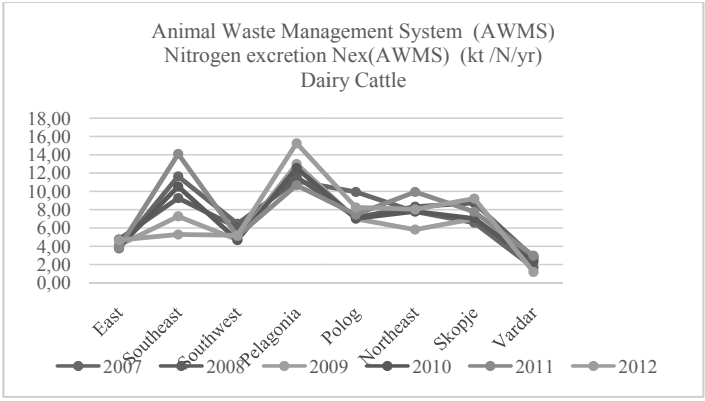

Figure 4. *Total annual $Nex_{(AWMS)}$ excretion, dairy cattle*

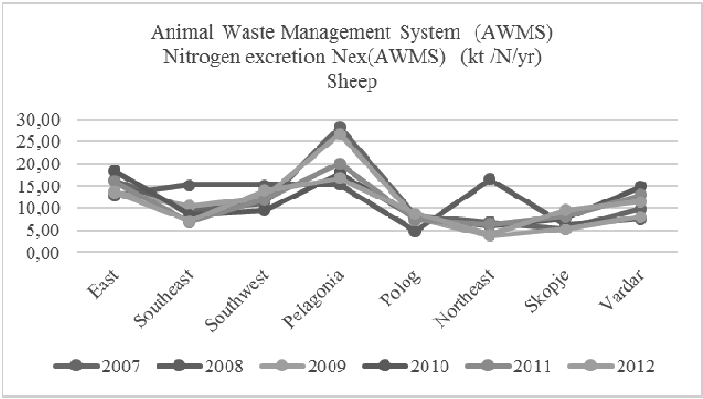

Figure 5. *Total annual $Nex_{(AWMS)}$ excretion, sheep*

Reducing the sheep number in Macedonia directly affects nitrogen excretion from pastures and paddock. Two regions (East region and Pelagonia) in the country continuously had the highest nitrogen excretion during the analyzed period, while the Northeast region has the lowest contribution in the analyzed period (Figure 5). Nitrogen excretions from poultry (Figure 6) were highest in the Southeast region until 2009, but at present leading regions are Pelagonia and Polog. Lowest excretion was present in the East region of the country.

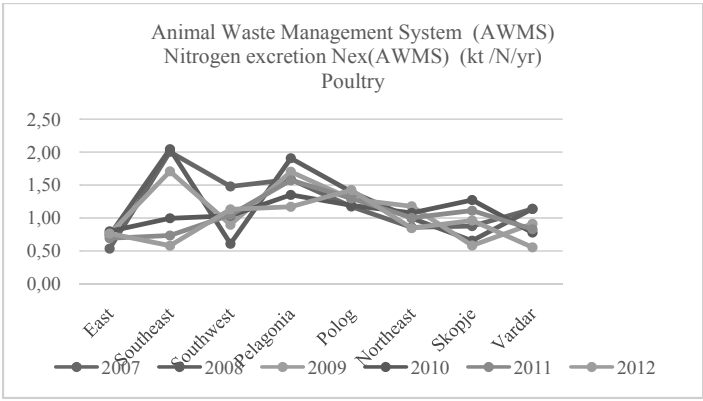

Figure 6. *Total annual $Nex_{(AWMS)}$ excretion, poultry*

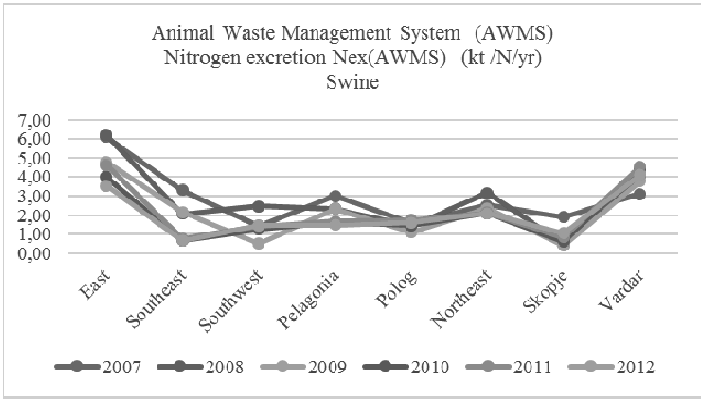

Figure 7. *Total annual $Nex_{(AWMS)}$ excretion, swine*

Due to the location of swine breeding in the East and Vardar regions of the country, nitrogen excretion in the analyzed period was higher in those two regions, while lowest values were noted in the Polog and Southeast regions (Figure 7).

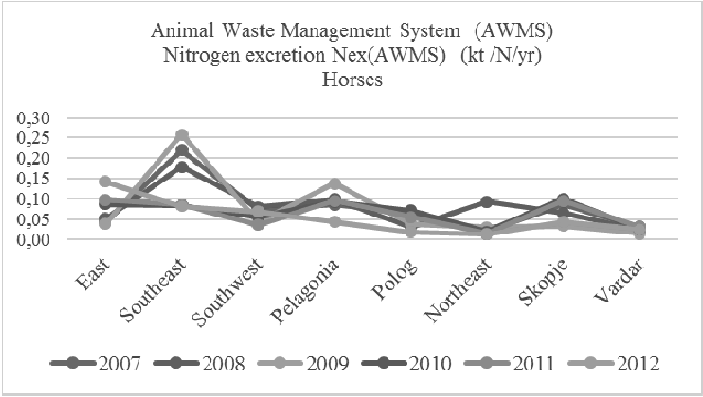

Figure 8. *Total annual $Nex_{(AWMS)}$ excretion, horses*

Highest share from overall nitrogen excretion from horses AWMS in the period 2007-2009 was noted in the Southeast region, in the Pelagonia region in the period 2010 and 2011 and in the East region in 2012, while the Vardar region had the lowest share (Figure 8).

Data for annual emission of N_2O from solid storage and drylot are shown in Figure 9, from other type of AWMS in Figure 10 and from pasture range and paddock in Figure 11. Similar to obtained results for CH_4 emission and nitrogen excretion, high levels of annual emissions of N_2O are mainly located in the Southeast and Pelagonia region for solid storage and drylot (Figure 9). For other type of AWMS (Figure 10) highest levels of annual emissions of N_2O were recorded in the East and Vardar region. The Pelagonia region was the leading region in the country regarding annual emission of N_2O from pasture range and paddock AWMS (Figure 11).

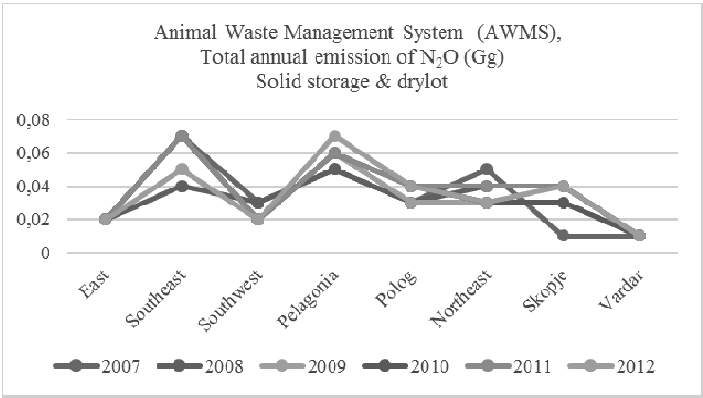

Figure 9. *Total annual emissions of N_2O from dairy and non-dairy cattle*

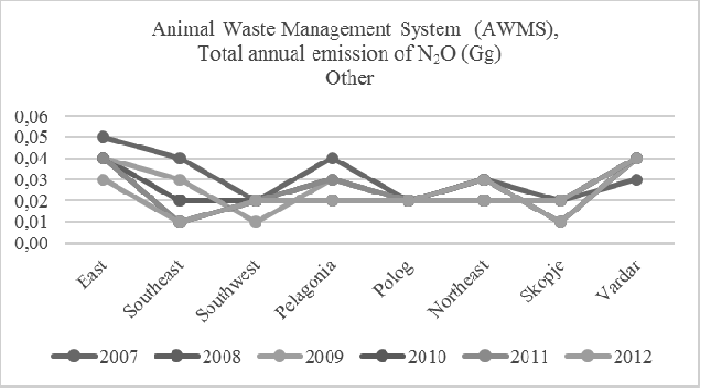

Figure 10. *Total annual emissions of N_2O from other types*

Carbon dioxide equivalent (CO_2-eq) is a metric measure used to compare the emissions from various greenhouse gases based upon their global warming potential (GWP). In the analyzed period Southeast region had highest emission level for CO_2-eq from enteric fermentation followed by Pelagonia region, while the lowest value was recorded in Vardar region (Figure 12). CO_2-eq emission from all types of AWMS during the period 2007-2012 was highest in the Pelagonia region.

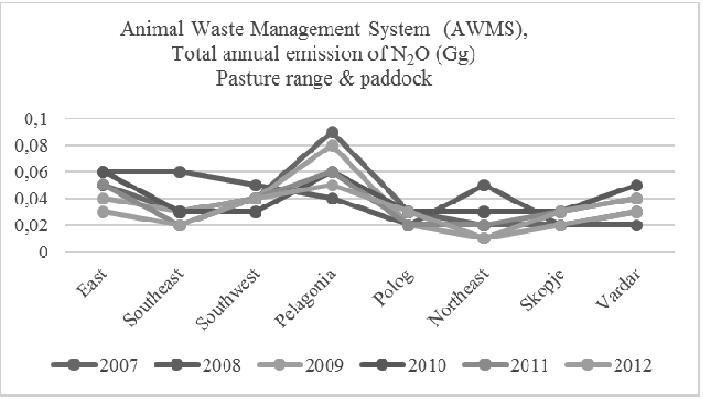

Figure 11. *Total annual emissions of N_2O from sheep*

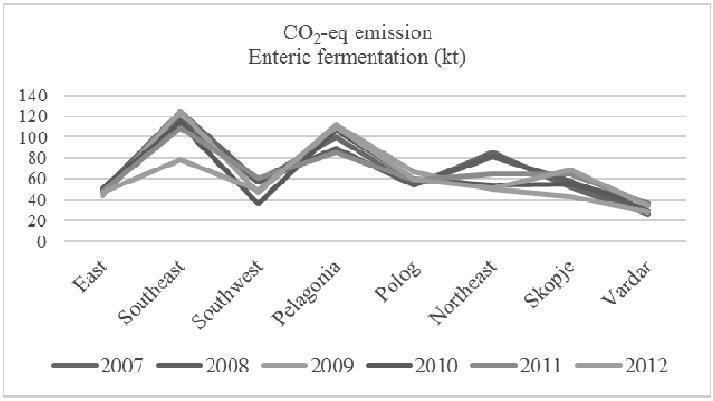

Figure 12. *Annual CO_2-eq emission, enteric fermentation (kt)*

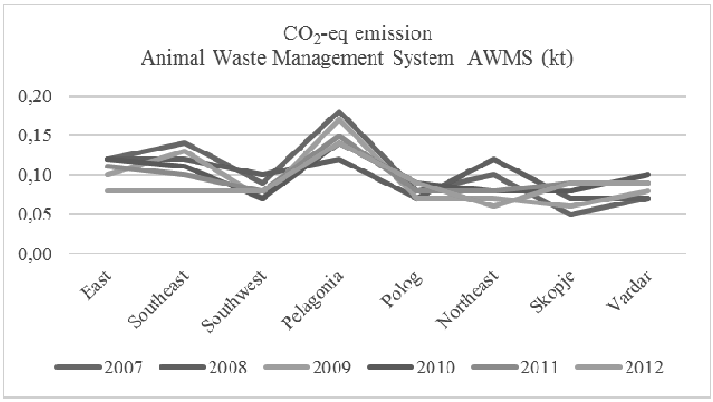

Figure 13. *Annual CO_2-eq emission, Animal Waste Management System AWMS*

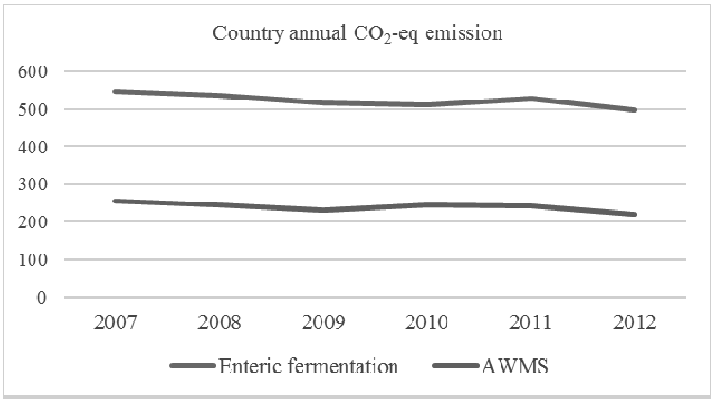

Figure 14. *CO_2-eq emission, per year*

Integral component of the whole process of digestion of plant material in ruminants is the formation of methane. High proportion of CH_4 emission (87%) during the enteric fermentation is a result of the activity of the rumen and the lower part of small intestine (13%) (Murray et al., 1976). Animal type, age, size, quantity of feed, fodder and fodder intake and the lactation period have a significant impact on methane emission in animals (Jungbluth et al., 2001). During the manure management CH_4 emissions are also present, although still significantly lower than emissions from enteric fermentation. Main component of the manure is the organic matter which under the influence of methanogenic bacteria is digested to methane. Methane emission calculation between different manure management systems is primarily based on the amount of manure (depending on the type, category and number of animals) as well as the fraction of manure that is anaerobically decomposed (connected to the climate conditions in the region) (Džabirski et al., 2008).

Regarding CH_4 emissions from enteric fermentation from different regions in the country it is obvious that all regions do not have an equal contribution in the overall CH_4 emission from enteric fermentation. Differences in emission levels are connected with the climate conditions in each region and the level of development of agriculture. These factors are connected and they have influence on the development of the livestock sector and the presence of certain domestic animal species in the region. In Southwest and Pelagonia regions that have a higher contribution in CH_4 the emissions from enteric fermentation come from different breeding categories of the same species (cattle). In the Southwest region non – dairy cattle is more dominant, while in the Pelagonia region the dairy cattle is dominant. There are also differences in species distribution throughout the country, with the number of sheep during the analyzed period continuously increasing in the Pelagonia region, while the number of animals in the other regions only slightly increasing (East region). Poultry from the Southeast region is transferred to Polog and Pelagonia region and swine breeding is dominantly present in the East and Vardar regions.

Variations in the number of domestic animals, unchanged breeding technology but also composition of the diet of animals directly affect the volume of CH_4 emissions from enteric fermentation. CH_4 emission had a decreasing tendency during the analyzed period with the lowest value in 2012 (23.78 Gg). Slight increasing of CH_4 emission in 2011 compared to 2010 is a result of light increase in the number of cattle in the country.

More factors (management system, composition of manure, the type of bacteria responsible for manure decomposition, presence of oxygen and fluid in the manure management systems) significantly affect N_2O production during the manure management process. High levels of nitrogen excretion in the Southeast region are connected with the highest number of bovine in this region.

The Pelagonia region is the leading region in nitrogen excretion from dairy cattle followed by Southeast region. Continuously low contribution was present in the Vardar region (less than 5 %).

Due to highest concentration of poultry breeding in the Pelagonia and Polog regions, nitrogen excretion is higher in those two regions compared to other regions in the country. The East and Vardar regions are the leading regions in nitrogen excretion from swine AWMS. In the first three years of the analyzed period more than 29 % of the overall nitrogen excretion from horses AWMS was present in the Southeast region of the country, with the highest value recorded in 2009 when this region was responsible for the excretion of 44% of the overall nitrogen excretion from horse AWMS.

Highest amount of annual emission of N_2O in the analyzed period was recorded in Southeast and Pelagonia regions. Annual emission of N_2O from solid storage and drylot is mainly connected to number of animals and the presence of any variation has a direct influence on emission levels. Bovine breeding in these two regions in the country is responsible for more than 40% of total annual emission of N_2O, with lowest values for the analyzed period recorded in the Vardar region.

More than one third of total annual emission of N_2O from other type of AWMS was present in East and Vardar regions. Main reason for the predominant emission of N_2O in these two regions is the high concentration of poultry and swine. The dominance of the Pelagonia region in the total annual emission of N_2O over the other regions is connected with the number of sheep in this region.

Different gases have different global warming capacity, and their capacity can be defined as the effect of a gas on climate change. Universal standard unit of measurement by which the various gases can be assessed is CO_2-eq. That enables converting greenhouse gases into a common unit of measurement.

Cattle, at a global level, are the largest contributor to total sector emissions covering approximately 65 % of total livestock sector emissions or 4.6 gigatonnes of CO_2-eq. Depending on type of cattle production, average emissions can be between: 2.8 kg CO_2-eq per kg of fat and protein corrected milk in dairy production and 46.2 kg CO_2-eq per kg of carcass weight for meat production. Globally, pork production is estimated to emit 9 % of the livestock sector emissions or approximately about 668 million tonnes of CO_2-eq. Chicken supply chains emit GHG emissions of 606 million tonnes of CO_2-eq (Gerber, et al., 2013).

Southeast region was the leading region in CO_2-eq emission from enteric fermentation in the first three years of the analyzed period due to the highest number of breeding cattle in all categories. In the next three years the leading region in annual emission of CO_2-eq from enteric fermentation was the Pelagonia region as a result of increased sheep number in the region. These two regions are responsible for more than one third of the total annual emission of CO_2-eq from enteric fermentation in the country.

Small ruminants at a global level represent close to 6.5% of the sector's global emissions, or 475 million tonnes of CO_2-eq, of which 62.94% are allocated to meat production, 27.36% to milk production and 9.68% to other goods and services. Average emission intensity for small ruminant meat is 23.8 kg CO_2-eq/kg, with no large differences between sheep and goat meat (Gerber et al., 2013). Due to highest concentration of ruminants in the Pelagonia region, as well as others species present in this region, first of all poultry, annual emissions of CO_2-eq from AWMS in this region are in the range of 15% of the total annual emission of CO_2-eq from AWMS in 2008 up to 23% in 2009.

At country level in the analyzed period emission of CO_2-eq form enteric fermentation and AWMS are decreasing as a result of overall reduction of livestock.

Reduction in the formation of CH_4 during the enteric fermentation can be achieved through the use of granular foods while improperly balanced feed and the appearance of a lack of protein or minerals would also result in an increased level of CH_4 production. Breeding technology and animal waste management directly affects the amount of produced CH_4 and it has particular impact on cattle breeding, where the formation and emission of CH_4 is lower at fixed breeding system (using balanced diet and a higher level of grains) in respect to pasture breeding (Ominski and Wittenberg, 2006).

Conclusion

Main influence of GHGs emission levels from livestock has the annual livestock number, breeding categories in each species as well as breeding technology. Separation of certain regions in the country regarding GHG emission can be clearly noted. Pelagonia and Southeast regions are connected to cattle breeding and the highest emission of GHG emission (CH_4) from enteric fermentation is present in those two regions. Main sheep breeding regions in the country are East and Pelagonia regions, therefore CH_4 and nitrogen excretion from this specific AWMS are mainly located in these regions. The Eastern and Vardar region of the country are main regions of nitrogen excretion from swine breeding.

Overall GHG emission presented as CO_2-eq emphasize two main regions in the country, Pelagonia and Southeast regions, responsible for emission of more than one third of overall CO_2-eq emission in the country.

Data for the period 2007-2012 show a downward trend in greenhouse gas emissions. Applications of modern breeding technology, balanced feed as well as better feed quality in the future are the main objectives in order to reduce GHGs emission from this subsector. Application of further more sophisticated methods for estimation of GHGs is tightly connected with the application of a system for integrated administration as well as with possession and application of sophisticated equipment.

References

1. Denman KL, Brasseur G, Chidthaisong A, Ciais P, Cox PM, Dickinson RE, Hauglustaine D, Heinze C, Holland E, Jacob D, Lohmann U, Ramachandran S, da Silva Dias PL, Wofsy SC, Zhang X 2007. Couplings between changes in the climate system and biogeochemistry. In: Solomon, S., Qin.
2. EPA, 2006. Global Anthropogenic Non-CO2 Greenhouse Gas Emissions: 19902020. United States Environmental Protection Agency, EPA 430-R-06-003, June 2006. Washington, DC, USA. www.epa.gov/nonco2/econ-inv/dow.
3. FAO, 2006. Livestock's Long Shadow: Environmental Issues and Options. Food and Agriculture Organization, Rome, Italy.
4. FAO, 2010. Greenhouse Gas Emissions from the Dairy Sector: A Life Cycle Assessment. Food and Agriculture Organization, Rome, Italy.
5. Gerber PJ, Steinfeld H, Henderson B, Mottet A, Opio C, Dijkman J, Falcucci A & Tempio G 2013. *Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities.* Food and Agriculture Organization of the United Nations (FAO), Rome.
6. IPCC, 1997. Revised 1996 IPCC guidelines for national greenhouse gas inventories.
7. Jungbluth T, Hartung E & Brose G 2001. Greenhouse gas emissions from animal houses and manure stores. Nutrient Cycling in Agroecosystems 60:133-145.
8. Murray RM, Bryant AM & Leng RA 1976. Rates of production of methane in the rumen and large intestine of sheep. Br. J. Nutr. 36:1-14.
9. Ominski Kim and Karen Wittenberg 2006. Strategies for Reducing Enteric Methane Emissions, in Climate Change and managed Ecosystems. pp. 1-23. New York.
10. Zdraveva P, Grncarovska TO, Markovska N, Poposka E, Gavrilova E, Ristovski I 2013. Preparation of the GHG Inventory for the Third National Communication to the UNFCCC National Inventory Summary Report.

11. State Statistical Office Yearly Book. (2007).
12. State Statistical Office Yearly Book. (2008).
13. State Statistical Office Yearly Book. (2009).
14. State Statistical Office Yearly Book. (2010).
15. State Statistical Office Yearly Book. (2011).
16. State Statistical Office Yearly Book. (2012).
17. Џабирски В, Порчу К, Андонов С, Чукалиев О, Мукаетов Д, Србиноска С 2008. Емисија на стакленѝчки гасови од домашните животни во Република Македонија ентерична ферментација и управување со арското ѓубриво. Зборник на трудови, Трет Конгрес на Еколози на Македонија, 407-413.

Original paper

MORPHOLOGICAL CHARACTERISTICS OF BREAST AND THIGH MUSCLES OF AUTOCHTHONOUS BREEDS OF CHICKENS

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Abstract

Morphological characteristics of skeletal muscles of autochthonous breeds of chickens are very important for meat quality and comparison with current hybrids for intensive production. The autochthonous breeds used in the experiment were Sombor crested and Banat naked neck, both sexes. For the purposes of morphological examination, tissue samples were taken from the thigh muscle (*m. biceps femoris*) and muscles of the breast (*m. pectoralis profundus*) of 5 male and female animals of each breed. After a standard histological procedure for conventional light microscopy, samples were stained with hematoxylin - eosin. After the processing of the samples for the histochemical analysis, samples were stained with the enzyme succinate - dehydrogenase (SDH) with the aim of determining the presence of different muscle cell types (red, white and intermediate). Morphological parameters, in this study, were diameter of muscle cells, nucleocytoplasmic ratio of muscle cells, volume density of connective tissue within the muscle and the presence of red, white and intermediate muscle cell types. Comparison of diameters of muscle cells thigh and breast muscles between Sombor crested and Banat naked neck have showed that kind of muscle, race or gender have no significant effect on the differences in this parameter. There were no statistically significant differences in the nucleo-cytoplasmic ratio of the volume density of the connective tissue of muscles. Red muscle cells were, in both autochthonous breeds, significantly more represented in *m. biceps femoris* than *m. pectoralis profundus*. The results of this study indicate that no differences were observed between autochthonous breeds in morphological parameters for examined breast and thigh muscle.

Key words: *autochthonous breeds, morphological parameters, muscle*

Introduction

The interest of consumers in products from alternative systems (organic, free-range) is increasing mainly because these systems can be environmentally friendly, sustaining animals in good health with high welfare standards and resulting in higher quality products (Sundrum, 2001).

Alternative systems for poultry meat production could be organized by using commercial fast-growing broiler hybrids or middle and slow-growing genotypes. Bogosavljević-Bošković et al. (2007) showed that there are differences in production parameters of commercial broiler hybrids reared in poultry house or free-range. The appropriate choice of genotype for meat production in free-range system is very important because final body weights are different according to genotype (Blagojević et al., 2009). In addition, different broiler genetic potential for growth has significant influence on production results as well as on carcass quality (Škrbić et al., 2013). In the process of changing commercial rearing system with a traditional one different autochthonous breeds have very important role for poultry meat production (Pavlovski et al., 2009). Franco et al. (2013) point out that meat quality was significantly different between autochthonous breeds and commercial broiler hybrids.

The aim of this study was to evaluate the morphological parameters of breast and thigh muscles of autochthonous breeds of chickens in free-range rearing system.

Material and methods

Morphological characteristics of breast and thigh muscles of autochthonous breeds of chickens were examined on 10 chickens (5 males and 5 females) of Banat naked neck and 10 chickens (5 males and 5 females) of Sombor crested housed in free-range system. Chickens were sacrificed at the age of 12 week. Samples of tissue were removed from the *m. biceps femoris* of the leg and the *m. pectoralis profundus* of the breast. The muscle tissues were initially fixed in a 10% buffered formalin solution, followed by a sequence of dehydration and clearing. The samples were then embedded in paraffin then cut into serial 5 μm thick sections using a microtome. Histological preparations for determining diameter and nucleocytoplasmic ratio of muscle cells were stained with hematoxylin-eosin (H&E), while the Mallory method was used for showing connective tissue (Disbrey and Rack, 1970). For hystochemical analysis the samples of muscles, size of 1 cm^3, were taken from each bird, and after that, the samples were frozen by liquid nitrogen at temperature of -196˚C. In laboratory, they were cut on Cryo-cut (-20˚C, sections of 10μm). These sections were fixed on microscopic plates and stained with standard methods for succinate-dehydrogenase (SDH) (Gerebtzoff, 1970).

The following parameters were observed in the analysis of histological preparations of the muscle samples: the diameter of muscle cells, the volume density of connective tissue of muscle, the nucleocytoplasmic ratio of muscle cells and identification of different muscle cells type (red, white and intermediate). Microscopy analysis was performed using a light microscope Leica DMLS with a Leica DC 300 digital camera, and the software package IM 1000 (Leica Imaging Systems Ltd, Cambridge, UK). The diameter of muscle cells was measured as the average of the longest lines drawn across the length and width of their cross-sections. For stereological analyses of the volume density of connective tissue, the nucleocytoplasmic ratio of muscle cells and percentage of certain types of muscle fibers, measurements were performed using the M42 testing system described elsewhere (Weibel, 1979; Pissinatti et al., 2003; Burity et al., 2004; Drabekova et al., 2005).

The statistical significance of differences obtained in measurements was determined using factorial ANOVA and the *post hoc* Tukey test for each of the parameters measured. The statistical significance of differences was expressed as significant at $P \leq 0.05$. Statistical

processing of data was carried out using the software package Statistica for Windows ver. 12.0 (Statsoft, 2012).

Results and discussion

The results of our trial show that the diameter of the muscle cells in the thigh was ranged from 48.83μm to 53.38 μm (Table 1). Diameters of muscle cells in the breast observed in our experiment were from 50.63 μm to 55.39 μm. Statistical analysis showed that there was no statistically significant difference in the diameter of muscle cells between genotype, muscles and sex.

Table 1. *Effect of genotype, sex and muscle on the diameter of muscle cells (μm), values are means ± SEM*

	Banat naked neck		Sombor crested	
	Male	Female	Male	Female
Breast muscle	50.63±5.04	52.32±5.80	55.39±3.63	54.88±6.29
Thigh muscle	48.83±5.76	53.38±4.25	53.37±4.85	53.04±7.67
Source	*P* value			
Genotype (G)	n.s.			
Sex (S)	n.s			
Muscle (M)	n.s			
GxSxM	n.s			

In accordance with our results of the impact of sex Mobini and Khoshoii Asadi (2013) and Mobini (2013a) suggests that sex did not significantly affect the difference in diameter of muscle fibers. But some papers show a statistically significant effect of sex on the diameter of muscle cells, *m. pectoralis profundus,* in domestic poultry (Mobini, 2013b). The results of this study indicate that the diameters of deep pectoral muscle in male individuals were significantly higher than in female ones. The comparison of our and Mobini (2013b) results show the difference in diameter and muscle cells. Comparing the results of these two studies could not be complete because in the paper of Mobini (2013b) they could not determine the age, genotype and poultry breeding system as the factors which have significant influence on muscle fiber characteristics. Branciari et al. (2009) investigated the effect of different genotypes and poultry rearing systems on characteristics of muscle cells. They observed three genotypes, slow-growing chickens (Leghorn), medium-growing (Kabir) and fast-growing chickens (ROSS) and found that genotype significantly affects the surface of the muscle fibers in *m. pectoralis superficialis*, *m. ileotibialis lateralis* and *m. semimembranosus*. Furthermore, they found that the rearing system (with respect to the conventional organic) significantly affects characteristics of muscle cells only at Leghorn genotype which is adapted for growing in organic rearing systems.

In order to determine the dynamics of development and activity of skeletal muscle cells of Sombor crested and Banat naked neck chickens, in the experiment was compared nucleo-cytoplasmic ratio of muscle cells (Table 2).

Table 2. *Effect of genotype, sex and muscle on the nucleocytoplasmic ratio of muscle cells, values are means ± SEM.*

	Banat naked neck		Sombor crested	
	Male	Female	Male	Female
Breast muscle	0.015±0.002	0.016±0.003	0.014±0.005	0.014±0.002
Thigh muscle	0.015±0.004	0.017±0.002	0.018±0.002	0.017±0.003
Source	*P* value			
Genotype (G)	n.s			
Sex (S)	n.s			
Muscle (M)	<0.05			
GxSxM	n.s			

The results of this study indicate that nucleocytoplasmic ratio ranged from 0.014 to 0.018 and that only muscle type hads significant influence on this parameter. However, Stojanović et al. (2013) show that the value of the nucleocytoplasmic ratio in ROSS broiler chicken hybrids, at the age of 42 days was 0.15. In the same study, it was shown that the age has a significant influence on this parameter. Comparing the age of individuals in our experiment we can conclude that the difference is a consequence of age, but we should not ignore the possibility that the difference is a consequence of hybrids relative to autochthonous breeds.

The trial results of the volume density of the connective tissue in the muscle directly indicate the degree and the speed of development of the skeletal muscle tissue as well as the organization and arrangement of muscle cells in muscle bundles. Stereological analysis used in our study shows the percentage between the connection tissue and muscle cells.

Table 3. *Effect of genotype, sex and muscle on the volume density of connective tissue of muscle (%), values are means ± SEM*

	Banat naked neck		Sombor crested	
	Male	Female	Male	Female
Breast muscle	21.62±6.82	25.82±1.19	23.52±8.05	29.66±5.94
Thigh muscle	27.67±4.84	20.62±4.13	19.80±6.18	22.57±5.53
Source	*P* value			
Genotype (G)	n.s			
Sex (S)	n.s			
Muscle (M)	n.s			
GxSxM	n.s			

The results of our study indicate that neither the types of muscles or sex, nor genotype can affect the volume density of the connective tissue within muscle. Mobini (2013c), comparing the amount of intramuscular connective tissue of *m. pectoralis profundus,* found no differences between domestic poultry and ROSS broilers hybrids. The same author also points out that sex did not affect the percentage of intramuscular connective tissue. However, Mobini (2013c) observed significant histological differences in epimysium. The number of collagen connective fibers was higher in epimysium in broiler chickens compared to domestic poultry. The softness of the meat depends on the amount of collagen in the connective fibers epimysium and perimysium and in accordance with the results of the authors we can conclude

that the autochthonous breeds have better meat quality than commercial hybrids. In order to compare the volume density of connective tissue within the muscle of broiler and autochthonous breeds, we compared the results of our studies and the results from earlier research done on broiler chickens (Stojanović et al., 2013). The results of the study on autochthonous breeds show that the volume density of connective tissue was from 19.80 to 29% while in broiler chickens average value was 17.97 % in the pectoral muscle of ROSS 308 broiler chickens at 42 days (Stojanović et al., 2013). The same paper points out that with the increase of age of broilers there occurs reduction in volume density of the connective tissue. Due to the volume density in autochthonous breeds in our study it was measured in the 12th week of life, while the dynamics changes depending on the age were not determined, therefore a complete comparison is not possible.

Examination of the percentage of different types of muscle cells which were determined on the basis of the activity of the succinate - dehydrogenase (SDH) enzyme, in our study, suggests that the type of muscle significantly affects the observed parameter. The results show that in the thigh muscle of both genotype the intermediate muscle cells are dominant type, followed by red and white muscle cells, whereas in the breast muscle in all groups dominant are the intermediate muscle cells, followed by white muscle cells, whereas the red-muscle cells are extremely minor.

Table 4. *Effect of genotype, sex and muscle on the muscle fiber type percentage (%), values are means ± SEM*

		Banat naked neck		Sombor crested	
		Male	Female	Male	Female
Breast muscle	Red	0.82±0.27	1.19±1.09	0.49±0.26	1.36±0.73
	White	27.05±4.46	28.35±1.42	29.88±3.25	29.75±1.37
	Intermediate	71.67±4.67	70.58±1.87	69.62±3.35	68.88±1.30
Thigh muscle	Red	23.93±2.70	20.65±2.24	24.29±1.21	24.56±4.15
	White	15.78±8.75	17.36±3.82	14.97±4.32	17.12±3.53
	Intermediate	60.24±10.11	61.98±2.11	60.74±4.27	58.32±2.20
Source		*P* value			
Genotype (G)		n.s			
Sex (S)		n.s			
Muscle (M)		<0.01			
GxSxM		n.s			

Our results of the activities of the succinate - dehydrogenase enzyme are consistent with the results obtained in the experiments of other authors. Šijački et al. (1986) studied the percentage of red, white and intermediate muscle cells in two breeds of poultry in the post-natal period and also concluded that the skeletal muscles have all three types of cells, and that the red muscle cells are much more present in the leg muscle than in the breast. Uščebrka et al. (1999) examined the percentage in the red, white and intermediate muscle cells in skeletal muscle of the partridges. It has been shown the existence of three types of muscle cells in the muscles of the breast and the legs. It is noted the dominance of intermediate muscle cells and in the breast and leg muscles, while red muscle cells were significantly more presented in the leg muscles compared with breast muscle.

Conclusion

The results from this study indicate that there are no significant differences in morphometric parameters of breast and thigh muscles of autochthonous breeds reared under the same environmental conditions. Because the morphometric characteristics significantly affect the quality of meat, studies like this are important to provide answers about usage of autochthonous breeds in alternative rearing systems.

References

1. Blagojević M, Pavlovski Z, Škrbić Z, Lukić M, Milošević N and Perić L 2009. The effect of genotype of broiler chickens on carcass quality in extensive housing system. Acta Veterinaria 59, 91-97.
2. Bogosavljević-Bošković S, Mitrović S, Radović V and Dosković V 2007. The age and housing system effects on the growth of broilers. Biotechnology in Animal Husbandry 23, 519- 525.
3. Branciari R, Mugnai C, Mammoli R, Miraglia D, Ranucci D. Dal Bosco A and Castellini C 2009. Effect of genotype and rearing system on chicken behavior and muscle fiber characteristics. J. Anim. Sci. 87, 4109-4117.
4. Burity CHF, Pissinatti A and Mandarim-de-Lacerda CA 2004. Stereology of the Liver in Three Species of Leontopithecus (Lesson, 1840) Callitrichidae – Primates. Anat. Histol. Embryol. 33, 183–187
5. Disbrey BD and Rack JH 1970. Histological Laboratory Methods. E. & S. Livingstone. Edinburgh.
6. Gerebtzoff MA 1970. Bulletin de l'Academie Royale de Medecine de Belgique. VII Serie, Tom X, 6.
7. Drabekova J, Uhrin V, Pivko J, Macak V, Pavlik V, Jancova A and Faragova N 2005. Comparison Of Quantitative Microscopic And Submicroscopic Analyses Of The Liver In Cows Bred In Various Polluted Regions. Bull. Vet. Inst. Pulawy 49, 79-84.
8. Franco D, Rois D, Vazquez JA and Lorenzo JM 2013. Carcass morphology and meat quality from roosters slaughtered at eight months affected by genotype and finishing feeding. Spanish Journal of Agricultural Research 11, 382-393.
9. Mobini B and Asaid Khoshoii A 2013. A comparative histomorphometrical study of Quadiceps femoris muscle fiber between commercial broiler and domestic fowls. World Applied Sciences Journal 22, 1506-1509
10. Mobini B 2013a. Histological differences in intramuscular connective tissues composition between dark and light colored muscles in broiler chickens. Global Veterinaria 10, 360-364.
11. Mobini B 2013b. Characteristics of the Pectoralis Profundus of Native and Broiler Chickens. International Journal of Basic Science & Applied Research 2, 445-448.
12. Mobini B 2013c. Comparative Histological Studies of Intramusculare Connective Tissues of Muscle Petoralis Profundus from Native and Broiler Chickens. Middle-East J. Sci. Res. 14, 267-272.

13. Pavlovski Z, Škrbić Z, Lukić M, Vitorović D and Petričević V 2009. Naked Neck – Autochthonous Breed of Chicken in Serbia: Carcass Characteristics. Biotechnology in Animal Husbandry 25, 1-10.
14. Pissinatti A, Burity CHF and Mandarim-de-Lacerda CA 2003. Stereology of the myocardium in Leontophitecus (Lesson, 1840) in callitrichidae – primates. J. Med. Primatol. 32, 139-147.
15. Stojanović S, Uščebrka G, Žikić D and Kanački Z. (2013) Skeletal muscle characteristics of broiler chickens under modified incubation factors. Avian Biology Research 6, 281-288.
16. Sundrum A. (2001): Organic livestock farming. A critical review. Livestock Production Science 67, 207–215.
17. Šijački N, Pribiš V and Uščebrka G 1986. Histohemijske osobine mišićnih vlakana grudi i bataka živine zavisno od rase i pola. Zbornik Biotehniške fakultete Univerza v Ljubljani 23, 69-75.
18. Škrbić Z, Pavlovski Z, Lukić M, Petričević V and Milić D 2013. Production performance and carcass quality of coloured broilers differentiated genetic potential for growth. Biotechnology in Animal Husbandry 29, 615-624.
19. Uščebrka G, Žikić D, Matavulj M, Ristić Z and Milošević V 1999. Stereološka analiza mišića grudi i bataka poljskih jarebica (Perdix perdix L.). Proceedings of the 5th Symposium of Stereology, Novi Sad, Serbia, 9-13.
20. Weibel R 1979. Stereological method: Practical methods for biological morphometry. Academic Press. New York.

Invited paper

EFFECTS OF INORGANIC AND ORGANIC SELENIUM SUPPLEMENTATION ON BLOOD AND MILK SELENIUM CONCENTRATION IN DAIRY COWS

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Abstract

Selenium is an important trace element in the nutrition of dairy cows because it prevents oxidative damages of tissue and in that way protects the animals from the incidence of various disorders. Addition of various levels of selenium in food leads to its increase in the milk what is important for postnatal calves development in which in the first weeks of life the milk is the only source of selenium. Into the food for dairy cows the inorganic selenium is added in the forms of – sodium selenite or sodium selenate (SS) or organic selenium – seleniium-enriched yeast (SY). Numerous studies have shown that organic selenium (SY) added into food for dairy cows provides better bioavailability than inorganic selenium (SS), hence the content of selenium in blood and milk of cows fed organic form of selenium is higher than in the inorganic selenium. The opinions about the effect of organic selenium on the activity of seleno-enzyme glutathione peroxidase (GPx) have not been reconciled yet. Adding selenium into food for dairy cows has no effect on the quantity of produced milk nor on the milk composition (proteins, fats and lactose). Selenium reduces the number of somatic cells in milk and in that way prevents the occurrence of the disease of mammary gland.

Key words: *blood, dairy cows, milk, selenium*

Introduction

Selenium is an essential element in the nutrition of diary cows. It reduces oxidative and metabolic stress, has immunostimulative effect, affects the muscular and neuromuscular functions and it is also important for physiological reproductive processes most notably it significantly reduces the incidence of retained placenta in dairy cows after the parturition (Jovanović et al., 2013). Colostrum and milk are good sources of selenium for calves in the first weeks of life.

Numerous regions in Europe, including Serbia, are poor or very poor in selenium (Thorn et al., 1978; Froslie et al., 1980; Maksimović et al., 1991; Mihailović et al. 1996; Jovanović et al., 1998). Concentration of selenium in cereals and forage feeds from these regions is low or very low. In order to ensure adequate levels of selenium for dairy cows the National Research Council (NRC, 2001) has recommended that the food for dairy cows should contain from 0.1-0.3 mg Se/kg DM, depending on the physiological state and the age of animal. However, the form of selenium supplement is not determined – whether it shoul be the organic or inorganic. Many authors (Weiss and Hogan, 2005; Slavik et al., 2008; Ran et al., 2010) established that adding the organic selenium into food for dairy cows leads to

higher increase of selenium in blood and milk in relation to the same levels of inorganic selenium. However, the same levels of different forms of selenium lead to no significant differences in the activity of serum selenoenzyme glutathione peroxidase (GPx3) (Enjalbert et al., 1999; Ran et al., 2010).

Concentration of selenium in serum and whole blood can be used as an indicator of the selenium status so that the increase of its concentration in these body fluids leads to the enhanced function of neutrophiles, decrease in the number of somatic cells and the occurrence of mastitis (Weiss et al., 1990; Cebra et al., 2003; Ibeagha et al., 2009).

The content of selenium in colostrum on the first day postpartum is considerably higher in cows which received selenium supplemented food in relation to the individuals that received no such food. However, the content of selenium in colostrum on the third day is considerably lower both in treated and untreated animals taking into account that in cows that received SY the concentration of selenium was 37% lower, in animals treated with SS by 67%, and in cows that received no selenium by 32%. The fall in the concentration of selenium in all trial animals in colostrum on the third day is associated with the transition of colostrum into milk and greater binding of selenium into compounds with the milk proteins (Debski et al., 1987). Adding the selenium into food for dairy cows (SS or SY) leads to no significant increase of the quantity of milk, content of proteins and lactose, but the percent of milk fat was higher in the individuals that received SY than SS (Carlos et al., 2014).

Biological availability of selenium

Selenium is used for different physiological and metabolical processes in the organism in which the part of selenium is being transformed in various metabolic forms necessary for developing many physiological processes. One part of dietary selenium is being lost by respiratory, urinary and faecal path. A remaining part of selenium can be incorporated specific proteins and numerous other proteins of small molecular mass. The mostly of selenium in Se-yeast products is in the form of selenomethionine. No evidence exists that mammalian cells can differentiate selenomethionine from methionine during protein synthesis. However, absorption of selenomethionine from the intestine can result in incorporation of Se during protein synthesis into any protein that contains methionine. When inorganic selenium is fed, the Se is more likely to be associated with seleno-specific proteins (Weiss and Hogan, 2005). Resorption of selenium from food, organic or inorganic is about 70%, but it can vary considerably depending on the source of selenium. However, total quantity of resorbed selenium is not physiologicaly available because one part is being lost through lungs whilst the remaining part is transformed into selenium compounds – polypeptide and proteins of which only selenoenzyme GPx has certain physiological function.

Biological availability of selenium depends on numerous physiological and metabolic processes.There are many different procedures for determination of biological availability of selenium, however different values have been obtained for the same selenium source, using different methods. It is assumed that the established differences are consequences of different efficacy and utilisation of selenium for certain physiological processes in different kinds of animals and animal species. However, it was determined in many studies that seleno-aminoacids and selenium in plants have good bioavailability while inorganic forms of selenium have lower bioavailability (Mihailović, 1996).

Absorption of different sources of selenium

Mechanisms of digestive absorption of inorganic and organic selenium are completely separate, therefore the factors which reduce the absorption of inorganic selenium little affect the organic form (Weiss, 2005). Intestinal resorption of selenium is considerably higher in monogastric animals than in ruminants (Cousins and Cairney, 1961). Selenite (SeO_3) and selenomethionine cannot be resorbed from the sheep rumen and pig stomach (Wright and Bell, 1966).

Ruminal metabolism and intestinal absorption of inorganic and organic selenium is different. In ruminants the selenates (SeO_4) are being reduced to selenites (SeO_3) in rumen, although certain quantity of selenates passes through rumen and is being resorbed in small intestines. A part of intaken selenites in rumen is transformed into small insoluble molecules of selenium which cannot be used by the animal. A part of selenites in rumen is used for the synthesis of seleno-aminoacids, mostly selenocysteines, which are being incorporated into microbial proteins. Because it is very difficult to determine the quantity of various selenium components, their distribution from ruminal contents is limited (Weiss, 2005).

Of total quantity of selenites added into food, 30 to 40% is being transformed into insoluble forms, 10 to 15% is incorporated into microbial proteins and 40 to 60% remains in the form of selenites (Serra et al., 1994). When the animals are fed diets containing the organic selenium a considerably higher percent of selenium in the form of seleno-aminoacids (mostly seleno-methionine) reaches the intestinal tract in comparison to the animals fed inorganic selenium.

Concentration of selenium in blood and serum

Concentration of selenium in blood depends on the level, form (organic or inorganic selenium) and on the time of consuming the food supplemented by selenium. Ran et al. (2010) determined a considerably higher levels of selenium in blood of cows fed organic or inorganic selenium, than in the individuals that received no selenium in their food (control group). The authors also report that in cows fed organic selenium (SY), on 60th, 75th and 90th day after calving the concentration of selenium in blood was higher 10, 11 and 14% in comparison to the individuals fed inorganic selenium (SS). Similarly to previous reports, Slavik et al. (2008) determined the highest concentration of selenium in the blood of cows fed diets supplemented by organic selenium (SY group 93.0 μg/L; SS group 68.0 μg/L; group that received no selenium in food 35,1 μg/L). Ortman and Pehrson (1999) report that dairy cows fed diets containing 0.1-0.12 mg Se/kg food (organic or inorganic) had 65%, that is, 35% higher levels of selenium in blood than individuals that received no selenium. Research conducted by Weiss and Hogan (2005) also suggest that in dairy cows fed the same levels of organic and inorganic selenium (0.3 mg/kg food) on the 28th day after calving the concentration of selenium in blood serum differed significantly. In the individuals fed organic selenium the concentration of selenium in blood serum was 0.074 mg/L, and in the individuals fed inorganic selenium 0.054 mg/L. In addition, higher levels of selenium in blood serum were determined on the third day after birth in calves whose dams were fed organic selenium in relation to calves whose dams received inorganic selenium. The values of selenium concentration in calves serum were 0.062, that is, 0.042 mg/L. Gunter et al. (2003) also determined 1.85 times higher concentration of selenium in blood of calves whose dams were fed organic selenium in relation to calves whose dams were fed with inorganic selenium. The limit of selenium deficiency in cows is 0.056 mg/L

selenium concentration in whole blood and 0.02 mg/L selenium concentration in blood plasma (Mihailović, 1996).

The activity of glutathione peroxidase (GPx) in blood and plasma

Its biological role selenium performs through enzyme of glutathione peroxidase (GPx), which in its active place contains selenium in the form of selenocysteine. The activity of this enzyme increases in plasma with the increase of its level in food what indicates its biological availability (Mihailović et al., 1991), although only at low levels of selenium. Becuase of that the determination of GPx3 in plasma is used as an appropriate method to determine the nutritive status of selenium. The activity of GPx3 in blood plasma is numerically higher during feeding the dairy cows with organic selenium what has been confirmed in many studies, although only in two studies the statistically significant differences were established (Weiss, 2005). The research by Knowles et al. (1999) suggests that there are no differences in the activity of GPx in cows whose ration was supplemented by 4 mg daily organic or inorganic selenium. However, the author reports that by administering 2 mg selenium daily the activity of selenoenzyme was 50% higher when the organic selenium was added into food. The research regarding the activity of GPx in calves plasma whose dams were fed organic or inorganic selenium are also not reconciled. Gunter et al. (2003) state that the activity of GPx in the plasma of calves whose dams were fed organic selenium was 75% higher than the activity of enzyme of calves delivered by the dams treated by inorganic selenium. However, Awadeh et al. (1998) found out that administration of organic selenium to dairy cows did not have any additional effect on increased activity of GPx in their calves, compared to those fed inorganic selenium. Ran et al. (2010) found out that both inorganic and organic selenium can lead to significant increase of erythrocyte GPx1 in dairy cows in relation to the individuals receiving no selenium (control group). However, the same authors did not determine significant differences in the activity of erythrocyte GPx between treated groups what is in harmony with the research of Weiss (2003). Moreover, Ortman and Pehrson (1999) report the increase of the activity of GPx1 erythrocytes in all treated groups and that the activity of this enzyme reached plateau on 75th day after the beginning of administration. Awadeh et al. (1998) report that only small quantities of administered selenium are associated with the activity of GPx serum. The lack of the effect of administering the selenium on GPx3 serum suggests that maximal activity of enzyme is obtained at adequate status of selenium (Todorović et al., 1999).

Content of selenium in colostrum and milk

Dairy cows fed organic selenium have higher concentrations of selenium in all tissues in relation to the cows fed inorganic selenium, the greatest portion of it being incorporated into the proteins as selenomethionine. The increase of selenium body reserves is very important for calves as well. Calves generating from dams fed organic selenium have higher concentration of selenium in tissues and higher GPx activity than calves produced by cows which received inorganic selenium in their food. Moreover, the colostrum of cows fed SY contains higher levels of selenium than the colostrum of cows fed SS (Weiss, 2005). Weiss and Hogan (2005) determined that in both groups of cows fed 0.3 mg Se/kg feed SY or SS, the concentration of selenium in colostrum was 3.8 times higher than in milk. The authors also state that the concentration of selenium in colostrum is considerably higher in cows fed SY than in cows fed SS. Ran et al. (2010) established that the cows fed diets supplemented by organic selenium (5 mg daily) had on 60th, 70th and 90th day after

calving 43, 39 and 53% higher concentration of selenium in milk than cows fed with SS. Organic selenium is much more efficient than inorganic one at increasing the selenium concentrations in milk what is the result of its better bioavailability (Ortman and Pehrson, 1999; Ceballos et al., 2009). In line with previous reports Phipps et al. (2008) established that when supplementing food for dairy cows with 0.16 mg/kg SS and 0.30 mg/kg SY and 0.45 mg/kg SY, the highest values of selenium in milk were found in the milk of cows which received the highest levels of organic selenium.

Better transfer of selenium of organic origin into milk can be explained by higher level of methionine in milk which is about two times higher than in blood plasma. Hence the selenomethionine from SY is being easily incorporated into the milk proteins (Weiss, 2005). Concentration of selenium in milk increases lineary at adding SY into cows ration. However, at adding SS its concentration does not change significantly. Nutrition of dairy cows fed rations supplemented by SY for the last 60 days of gestation has a positive effect on the calves health (Weiss, 2005). Addition of organic or inorganic selenium into the dairy cows ration leads to no increased production of milk, increased level of lactose and proteins but it leads to the increase in the percent of milk fat (Wang et al., 2009). The individuals into whose food was added organic or inorganic selenium had 4.15, that is, 3.96% milk fat. This increase can be associated with reduced number of somatic cells (SCC) and lower percent of the incidence of mastitis what can affect the milk quality as well (Carlos et al., 2014). The influence of selenium on the composition of fatty acids in milk was studied by Ran et al. (2010). These authors report that in cows treated with SY and SS there occurred the increase only in C16:1 fatty acid which is formed under the influence of enzyme delta-9 desaturase in mammary gland. C18:1 fatty acid is produced also under the influence of the same enzyme but its higher concentration has not been determined. The opinion that the activity of mentioned enzyme can be increased at adding selenium into cows rations is not fully justified. The same authors established a significant increase of polyunsaturated fatty acids (PUFA) and linoleic acid (cis-9, cis-12) in milk fats during the treatment of cows with organic selenium. However, saturated fatty acids (SFA) had a tendency to decrease. Cabré et al. (1992) reported that there existed a positive correlation between the selenium concentration in serum and the percent of essential fatty acids and PUFA what is combined with antioxidative action of selenium. However, the authors Ran et al. (2010) report that their result are not complete and that it is necessary to continue the research.

The concentration of selenium in milk depends also on a physiological state of the animal. In early lactation the concentration of selenium is lower than in later stages of lactation what can be explained by the effect of dilution (Wichtel et al., 2004). The studies on distribution of selenium into different milk fractions showed that the highest concentration of selenium is in whey and the lowest in milk fats (Muňiz-Naveiro et al., 2005a). However, in their subsequent researh these authors (Muňiz-Naveiro et al., 2005b) report that adding of organic or inorganic selenium into food for dairy cows results in its increase in milk but the quantity in separate milk fractions (whey, casein and fat) does not differ.

Conclusion

Decades long research on physiological role of selenium has not given any complete explanations yet. Besides the fact that it is difficult to define bioavailability of inorganic and organic selenium in dairy cows many authors state that it is more profitable to use organic selenium in cows nutrition than the inorganic selenium. There are several reasons for that: 20-30% higher bioavailability and reduced toxicity, higher body reserves of

selenium, considerably increased concentration of selenium in milk what is good for human health, increased concentration of selenium in colostrum what is important for the calf health state, antagonists do not significantly decrease the absorption of organic selenium.

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References

1. Awadeh FT, Abdelrahman MM, Kincaid RL and Finley JW 1998. Effect of selenium supplements on the distribution of selenium among serum proteins in cattle. J. Dairy Sci. 81, 1089-1094.
2. Cabré E, Periago JL, Mingorance MD, Fernández-Bañares F, Abad A, Esteve M, et al. 1992. Factors related to the plasma fatty acid profile in healthy subjects, with special reference to antioxidant micronutrient status: a multivariate analysis. Am. J. Clin. Nutr. 55, 831-837.
3. Carlos EO, Pinheiro MG, Miranda MS, Arcaro JRP, Castelani L, Toledo LM, Ambroslo LA, Leine PR, Manella MQ and Arcaro IJ 2014. Selenium sources in the diet of dairy cows and their effects on milk production and quality, on udder health and on physiological indicators of heat stress. Italian Journal of Animal Science 13, 292, 48-52.
4. Ceballos A, Sanches J, Stryhn H, Montgomery JB, Barkema HW and Wichtel JJ 2009. Meta-analysis of the effect of oral selenium supplementation on milk selenium concentration in cattle. J. Dairy Sci 92, 324-342.
5. Cebra CK, Heidel JR, Crisman RO and Stang BV 2003. The relationship between endogenous cortisol, blood micronutrients, and neutrophil function in postparturient Holstein cows. J. Vet. Intern. Med. 17, 902-907.
6. Cousins FB and Cairney IM 1961. Some aspects of selenium metabolism in sheep. Flust. J. Agr. Res. 12, 927-942.
7. Debski B, Picciano MF and Milner JA 1987. Selenium content and distribution of human, cow and goat milk. J. Nutr. 117, 1091-1097.
8. Enjalbert F, Lebreton P, Salat O and Schelcher F 1999. Effects of pre- or postpartum selenium supplementation on selenium status in beef cows and their calves. J. Anim. Sci. 77, 223-229.
9. Froslie A, Karlsen JT and Rygge J 1980. Selenium in animal nutrition in Norway. Acta Agric. Scand. 30, 17-25.
10. Gunter SA, Beck PA and Phillips JM 2003. Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves. J. Anim. Sci. 81, 856-864.
11. Ibeagha AE, Ibeagha-Awemu EM, Mehrzad J, Baurhoo B, Kgwatalala P and Zhao X 2009. The effect of selenium sources and supplemntation on neutrophil functions in dairy cows. Animal 7, 1037-1043.
12. Jovanović IB, Pešut O, Mihailović M and Kosanović M 1998. Selenium content in feedstuffs from Vojvodina (Serbia), Acta Veterinaria 48, 5-6, 339-344.

13. Jovanović I, Pešut O, Gvozdić D, Stojić V, Šamanc H, Fratrić N, Kirovski D, Vujanac I and Dimitrijević B 2007.Selenium and iodine status relationship in calves and heifers from selenium and iodine deficient areas in Serbia.Lucrari Stiintifice, Medicine Veterinaria Timisóara, XL, 75-82.

14. Jovanović IB, Veličković M, Vuković D, Milanović S, Valčić O and Gvozdić D 2013. Effects of different amounts of supplemental selenium and vitamin E on the incidence of retained placenta, selenium, malondialdehyde, and thyronines status in cows treated with prostaglandin F2α for the induction of parturition. Journal of Veterinary Medicine, Art.ID 867453, 6 pages, http://dx.doi.org/10.1155/2013/867453

15. Knowles SO, Grace ND, Wurms K and Lee J 1999. Significance of amount and form of dietary selenium on blood, milk, and casein selenium concentrations in grazing cows. J. Dairy Sci. 82, 2, 429-437.

16. Maksimović Z, Jović V, Đujić I and Ršumović M 1991. Selenium deficiency in Yugoslavia and possible effects on health. Environmental geochemistry and health 14, 1-5.

17. Mihailović M, Todorović M and Ilić V 1991. Effects of dietary selenium of glutathione peroxidase activity and body weight of growing turkeys. Acta Veterinaria 23, 75-80.

18. Mihailović M 1996. Selen u ishrani ljudi i životinja. Veterinarska komora Srbije, Beograd.

19. Mihailović M, Lindberg P and Jovanović I 1996. Selenium content in feedstuffs in Serbia, Acta Veterinaria 46, 5-6, 343-348.

20. Muñiz-Naveiro O, Dominguez-Gonzáles R, Bermejo-Barrera A, Cocho JA, Fraga JM and Bermejo-Barrera P 2005a. Determination of total selenium and selenium distribution in the milk phases in commercial cows milk by HG-AAS. Anal. Bioanal. Chem. 381, 1141-1151.

21. Muñiz-Naveiro O, Dominguez-Gonzáles R, Bermejo-Barrera A, Cocho JA, Juan DE, Fraga JM, Bermúdez A, Pereiras AG, Santamariña LZ, Lede IM, Puente JV, Gomez LF and Bermejo-Barrera P 2005b. Selenium content and distribution in cows milk supplemented with two dietary selenium sources. J. Agric. Food Chem. 53, 9817-9822.

22. National Research Council 2001. Nutrient requirements of dairy cattle. 7th rev. ed. National Academy press, Washington, DC, USA.

23. Ortman K and Pehrson B 1999. Effect of selenate as a feed supplement in dairy cows in comparison to selenite and selenium yeast. J. Anim. Sci. 77, 3365-3370.

24. Phipps RH, Grandison AS, Jones AK, Juniper DT, Morales RE and Bertin G 2008. Selenium supplementation of lactating dairy cows: effects on milk production and total selenium content end speciation in blood, milk and cheese. Animal Cambrige Journals 2, 11, 1610-1618.

25. Ran L, Wu X, Shen X, Zhang K, Ren F and Huang K 2010. Effects of selenium form on blood and milk selenium concentrations, milk component and milk fatty acid composition in dairy cows. J. Sci. Food Agric. 90, 2214-2219.

26. Serra AB, Nakamura K, Matsui T, Harumoto T and Fujihara T 1994. Inorganic selenium for sheep. 1. Selenium balance and selenium levels in the different ruminal fluid fractions. Asian-Australian J. Anim. Sci. 7, 83-89.

27. Slavik P, Illek J, Brix M, Hlavicova J, Rajmon R and Jilek F 2008. Influence of organic versus inorganic dietary selenium supplementation on the concentration of selenium in colostrum, milk and blood of beef cows. Acta Vet Scan 50, 1-6.

28. Thorn J, Robertson J and Buss DH 1978. Trace nutrients. Selenium in British food. Brit. J. Nutr. 39, 391-396.

29. Todorović M, Mihailović M and Hristov S 1999. Effects of excessive levels of sodium selenite on daily weight gain, mortality and plasma selenium concentration in chickens. Acta Veterinaria 49, 5-6, 313-320.

30. Wang C, Liu Q, Yang WZ, Dong Q and Yang XM, 2009. Effects of selenium yeast on rumen fermentation, lactation yeast on rumen fermentation, lactation performance and feed digestibilities in lactating dairy cows. Livest. Sci. 126, 239-244.

31. Weiss WP 2003. Selenium nutrition of dairy cows: comparing responses to organic and inorganic selenium forms. Nutritional biotechnology in the feed and food industries, in Proceedings of the 19th Alltech Annual Symposium (ed. by Lyons PT and Jacques KA), pp. 333-343. Nottingham.

32. Weiss WP 2005. Selenium sources for dairy cattle. Proc. Tri-State Dairy Nutrition Conference, Ft. Wayne, pp. 61-71.

33. Weiss WP and Hogan JS 2005. Effect of selenium source on selenium status, neutrophil function, and response to intramammary endotoxin challenge of dairy cows. J. Dairy Sci. 88, 4366-4374.

34. Weiss WP, Hogan JS, Smith KL and Hoblet KH 1990. Relationship among selenium, vitamin E, and mammary gland health in commercial dairy herds. J. Dairy Sci. 73, 381-390.

35. Wichtel JJ, Keefe GP, Van Leeuwen JA, Spongler E, Mc Niven MA, Oglivie TH 2004. The selenium status of dairy herds in Prince Edward Island. Can. Vet. J. 45, 124-132.

36. Wright PL and Bell MC 1966. Comparative metabolism of selenium and tellurium in sheep and swine. Am. J. Physiol. 211, 6-10.

Original paper

ELECTROPHORETIC DISTINCTION OF THE ORIGIN IN DIFFERENT DAIRY PRODUCTS AND MILK SAMPLES

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Abstract

Caseins, lacto-albumin, and lacto-globulin are major milk proteins. These globular proteins could be significant indicators of the milk and dairy products origin. Knowing that caseins, lacto-albumins and lacto-globulins vary in molecular weight and concentration in different types of milk, this fluctuation can be used for determination of milk origin. The aim of this study was to develop an appropriate method for distinction of milk proteins from different origin. Twelve samples of milk, white cheese, yellow cheese and whey cheese from cow, sheep and goat were obtained and studied. The protein separation was made using SDS-PAGE. SDS is an anionic detergent that breaks all inter and intramolecular bonds and leaves the polypeptide subunits of proteins in forms that can be separated on the basis of their molecular weight. Polyacrylamide gels, used as support medium, restrain larger molecules from migrating as fast as smaller molecules. In order to optimize the conditions of the experiment, some of the parameters were modified (polyacrilamide concentration from 10-15% according to the molecules size, duration of electrophoresis, quantity of applied material, sonification treatment of the different samples). Bovine milk proteins standards were used for the determination of the proteins. The results have shown differences, as well as other fractions that can be used for identification of the origin. In yellow and white cheese the differences among the samples from different origins appear in lacto-albumin fractions and some digested fractions below the caseins. The main differences in whey cheese samples were identified in casein fractions. The milk samples showed differences in upper fractions, probably serum albumins that remained in the milk samples.

Key words: *dairy products, milk proteins, origin, SDS-PAGE*

Introduction

The detection of milk species is important in cheese producing branch, especially in those made from one pure species and with protected designation of origin (PDO) (Bottero at al., 2002). Different analytical approaches have been applied for identification purposes, such as: immunological, chromatographic, PCR mass spectrometry and electrophoresis (Zachar at al.,

2011). Usually, capillary electrophoresis and isoelectric focusing were used as reference methods according to the Commission Regulation EC No 273/2008). On the other hand, zonal electrophoresis could be a good choice because of the low expenses and availability of the technique for wider range of laboratories, especially for internal control. Furthermore, the results from SDS PAGE can serve as a basis for 2D electrophoresis and eventual development of antibodies for ELISA testing of adulteration.

Electrophoresis is a commonly used technique in many scientific fields, where charged molecules are separated by using an electrical field. Molecules have different migration rates depending on their total charge, size and conformation. The separation of particles during electrophoresis depends on the following factors: the sample, the electric field, medium and buffer (Palashevski and al., 2001).

The mobility or rate of migration, of a molecule increases by increased applied voltage and increased net molecule charge. Conversely, the mobility of a molecule decreases with increased molecular friction, or resistance to flow through the viscous medium, caused by molecular size and shape, total actual movement of the molecules increases with increased time (AES, 2003).

Most electrophoretic systems use an equal and constant voltage on all of the cross-sectional areas of different matrices employed in the electrophoretic separation. These electric fields are best defined in terms of volts per linear centimeter. However, according to the Ohm's law voltage is function of current and resistance. The resistance of the system is important because it will determine the amount of heat generated during electrophoresis. The "smiling" pattern often seen on slab gel electrophoresis is the result of non-uniform heating of the gel ((Bottero at al., 2002).

Because many biological compounds have charges and ionisable groups, electrophoresis is frequently utilized in biochemical research, as a tool for separation of the complex biological molecules such as proteins, nucleic acids, peptides (Gersten, 1996).

For separation of proteins, polyacrylamide electrophoresis (PAGE) is commonly used. Sodium dodecyl sulfate (SDS) - an anionic detergent is applied to protein sample to linearize proteins and to impart a negative charge to linearized proteins, and proteins are separated only by length of their polypeptide chains (Palashevski at al., 2001).

Gels are formed as acrylamide monomer polymerizes into long chains that are linked together by big molecules (N,N'-methylenebisacrylamide). In order that polymerization can start, ammonium persulfate (APS) and N,N,N',N'- tetramethylethylenediamine (TEMED) are added to form the free radicals. TEMED accelerates the decomposition of persulfate molecules into sulfate free radicals and these, in turn, initiate the polymerization. This study reports optimization of the conditions for identification of the origin of milk and dairy products with SDS-PAGE (AES, 2003).

Milk is a complex biological emulsion produced by the mammals whose composition makes it an important source of nutrients and a protector of the immune system. The major milk components are water, lactose, fats, proteins, minerals, vitamins, etc. (Barlowska at al., 2007). The total protein content of milk is composed of different specific proteins. The primary group of milk proteins are the caseins (distinct molecules but similar in structure) which are highly digestible in the intestine and represent high quality source of amino acids (Jensen, 1995). All other proteins found in milk are grouped together under the name of whey molecules. The

major whey proteins are β-lactoglobulin and α-lactoalbumin. Other whey proteins are the immunoglobulins and serum albumin (Hurley at al., 2010).

Authentication of milk and milk derived products is important because fraudulent incorporation of non-declared kind of milk during technological processing can later cause problems for reasons related to intolerance or allergy, ethical objections and legal requirements (Zuchar at al., 2011).

Materials and methods

As an initial material we used cow, sheep and goat milk, and derived whey cheese, white cheese and yellow cheese. Bovine α-casein, β casein, κ-casein and α-lactoalbumin were used as standards.

Sample preparation. The samples were prepared using 200 mg of each dairy product separately, immersed in 0.4 mL of distilled water. Then, the samples were homogenized using vortex for 10 min at 2500 min^{-1}. Afterwards, the samples were treated with ultrasonic homogenizer 3 minutes at 4 watts, and finally centrifuged for 15 minutes at 5000g. The separated supernatant in amount of 100 µL was further mixed with 200 µL reducing buffer 2x. Then these mixtures were heated 3 min, at 99°C, for denaturing of the proteins. The milk was prepared using 10 µL 2x reducing buffer which is added to 10 µL milk. Then the mixture was heated for 3 min, at 99°C and the samples stored in a freezer.

Gel preparation. The gel used for electrophoresis was divided into an upper stacking gel with lower concentration and lower resolving gel with smaller pores. The stacking gel has a role to deposit the proteins at the top of the resolving gel as a narrow band. In the electrophoretic set the two gels can be cast. For preparation of two resolving gels, the needed reagents are listed in Table 1 (Macdonald, 2011).

For preparation of 3% stacking gel we used 4.8 mL dH_2O, 1.8 mL 4X Upper TRIS, 0.9 mL protogel, 15 µL TEMED, and 60 µL APS, and for preparation of SDS- PAGE reagents, the needed components are given in Table 2 (Jensen, 1995).

The electrophoresis was done under the following conditions: voltage of 100 -150 V, duration of 1h – 2 h, staining of 1.5 hour, and overnight distaining. The analysis of the gels was done with Gene Tools software using G-box device.

Table 1. *SDS-PAGE Gel Formulae for resolving gel*

Reagents(mL)	12.5% gel	15% gel
dH_2O	6.6	4.8
4X Lower TRIS	5.1	5.1
Protogel	8.7	10
TEMED	30µL	30µL
10% APS	80µL	80µL

Table 2. *SDS-PAGE reagents*

4x Lower TRIS pH 8.8		**4x Upper TRIS pH 6.8**		**Running buffer(10X)**	
Tris BASE	18.17 g	Tris BASE	6.06 g	Tris BASE	60 g
10% SDS	4.0 mL	10% SDS	4.0 mL	Glycine	288 g
q.s. with H_2O to	100 mL	q.s. with H_2O to	100 mL	SDS (add last)	20 g
pH to 8.8 before adding SDS		pH to 6.8 before adding SDS		q.s. with H_2O to	2.0 L
Reducing buffer (2X)		**Coomassie Brilliant Blue R-250 gel stain**			
10% SDS	3.0 mL	Coomassie Brilliant Blue R-250			400 mg
Upper Tris(4x)	1.25 mL	Methanol			400 mL
b-mercaptoetanol	500µ L	Glacial acetic acid			400 mL
Glycerol	1.0 mL	q.s. with dH2O to 1L			
Bromphenol Blue	Pinch	**Destain**	**Protogel**		
q.s. with H2O to	10.0 mL	10% acetic acid	30% acrylamide	0.8% bis	

Results and discussion

The analysis that can be used for identification of the milk and dairy products origin is based on the electrophoretic determination of protein profile. Milk, white cheese, yellow cheese and whey cheese samples from cow, sheep and goat were studied in order to determine the optimal conditions and factors that affect this method and to prove the distinction between different samples.

During the experiment, some of the parameters were adjusted, in order to acquire proper conditions for good resolution of protein fractions from milk and dairy products samples. The protein standards fractions together with milk samples are shown in Figure 1, where the electrophoresis lasted 50 minutes on 150 V, 1.5 hour staining and overnight distaining.

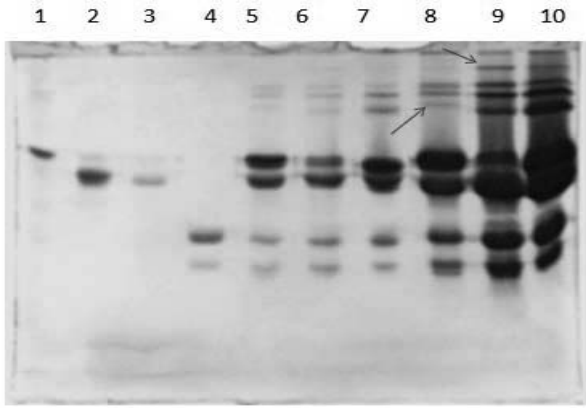

Figure 1. *12.5% SDS-PAGE: 1. α-casein, 2. β-casein, 3. κ-casein, 4. α-lactoalbumin, 5. Sheep milk, 6. Cow milk, 7. Goat milk, 8. Sheep milk (5x), 9.Cow milk (5x), 10. Goat milk (5x)*

The current technique related to control of the milk origin is isoelectric focusing of g caseins after plasminolysis knowing that whey proteins are thermally unstable, especially during the processing and cheese producing (10). In order to avoid complicate preparation procedures, we tried to distinguish different milk and diary product samples using common homogenizing approach.

The casein fractions are well resoluted in the samples from lanes 5, 6 and 7 and applied in the amount of 1.5 μL. In the lanes 8, 9 and 10, because of a higher quantity of the applied material (10 μL), the separation of casein fractions is not very clear. However, good separation is acquired in the upper fractions what was the intention when applying 10 μL. There is a possibility that these upper fractions are the remains of serum albumins in milk. Furthermore, these fractions distinguish from one another in band that is present in the milk from sheep, but not in the other milk samples, and it is with higher molecular weight. There is also another specific band that appears only in the cow milk sample. Every lane on the gel ends nearly at the half of the gel length, so it can be concluded that the duration of the electrophoresis was short in this case and should be extended.

In order to obtain better protein profile, the concentration of the gel was increased up to 15% (Figure 2), and the conditions were also modified: 130V for 1hour and 50 minutes, 1.5 h staining and overnight distaining.

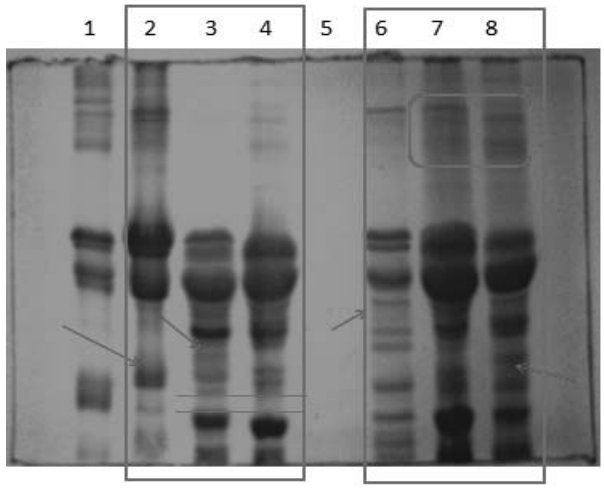

Figure 2. *15% SDS-PAGE: 1. Milk standards-mix, 2. Cow yellow cheese, 3. Goat yellow cheese, 4. Sheep yellow cheese, 5./6. Cow white cheese, 7. Goat white cheese, 8. Sheep white cheese*

Because of the increased concentration of the acrylamide in the gel and lower voltage applied, more protein fractions are visible. Differences among the yellow cheese samples are identified in lacto-albumin fractions. Every lacto-albumin fraction from lanes 2, 3 and 4 differs in the rate of migration, which means that they all have different molecular weight. There is another fraction that appears only in the yellow cheese sample from goat and it is located under the casein fractions. As for the white cheese, the distinction can be made by some digested fractions below the caseins. There is a band located under the casein fraction, present only in

the sheep white cheese sample, and another band from the cow white cheese sample, also specific only for this kind of white cheese.

Figure 3 shows also 15% SDS PAGE, where the differences among protein fractions from yellow cheese are even more visible. The electrophoresis was run 2 hours on 100V.

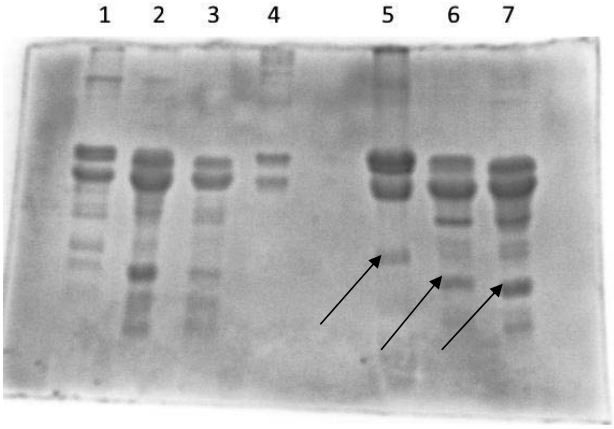

Figure 3. *15% SDS-PAGE: 1. Cow white cheese, 2. Goat white cheese, 3. Sheep white cheese, 4. Milk standards - mix, 5. Cow yellow cheese, 6. Goat yellow cheese, 7. Sheep yellow cheese*

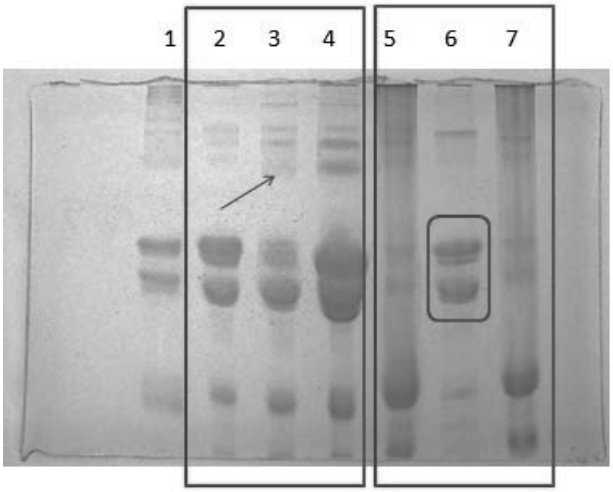

Figure 4. *15% SDS-PAGE: 1. Milk standard - mix, 2. Cow milk, 3. Goat milk, 4. Sheep milk, 5. Cow whey cheese, 6. Goat whey cheese, 7. Sheep whey cheese*

Differences among the yellow cheese samples are more visible in Figure 3. Three different bands, each in one sample can be clearly noticed (marked with arrows in the figure).

Moreover, below the casein fractions of cow yellow cheese sample a band does not appear, unlike the other two samples in lanes 6 and 7.

The whey cheese samples were analyzed (Figure 4) using less quantity on 15% gel, under the following conditions: 130V, 1 hour and 50 min.

Figure 4 again shows the whey cheese samples. The same differences can be identified as in the first figure, only here, because of the lower amount applied, they are more visible. The samples in lanes 5 and 7 appear to be smeared, probably due to a lipoprotein complex that was not disrupted during the homogenization. With another analysis of the milk samples, the differences in the upper fractions were confirmed.

Conclusion

Using SDS PAGE as a technique for distinguishing the origin in different dairy products and milk samples is suitable. Best results are obtained with 15% concentration polyacrylamide gel. Longer duration of electrophoresis (that comes with lower applied voltage) aids in better resolution. The samples should not undergo centrifugation, neither filtration, because some of the proteins stay in the precipitate.

This approach allows its use as an internal checking of milk and dairy products and can eventually result in finding some specific protein fraction in different samples which can be used for development of appropriate antibodies for ELISA testing.

References

1. AES Application Focus 2003. Gel Electrophoresis of proteins (p 6-7).
2. Barłowska J, Litwińczuk Z, Florek M and Kędzierska-Matysek, M 2007. Milk yield and its composition of 4 Polish goat breeds with different genotypes of αs1-casein (in Polish). Vet. Med. 63(12), 1600-1603.
3. Bottero MT, Civera T, Anastasio A, Turi RM and Rosati S 2002. Identification of cow' milk in "buffalo" cheese by duplex polymerase chain reaction. J Food Prot. 65 (2), 362-366.
4. Gersten DM 1996. Gel Electrophoresis (Essential Techniques Series). New York: Wiley.
5. Hurley LW, Arora R, Bhatia S, Mishra BP, Sharma R, Pandey AK, Prakash B and Jain A 2010. Milk Protein.
6. Jensen RG 1995. Handbook of Milk Composition, Academic Press, San Diego.
7. Macdonald Wick 2011. Laboratory Manual, Ohio State University, Department of Animal Science.
8. Mateva SN 2012. Participation of serum proteins in white brined cheese production with ultrafiltration, PhD, Ss Cyril and Methodius Univ.
9. Palashevski B, Popovski ZT, Dubrova-Mateva N, Andonovska V and Naletovski Z 2001. Polymorphisms of □--casein and □-lactoglobulin in cow milk Symposia for Livestock production, Year Book,45 – 50 pp.
10. Zachar P, Šoltés M, Radovan K, Novotný J, Novikmecová M, Marcinčákova D 2011. Analytical methods for the species identification of milk and milk products, Mljekarstvo 61 (3), 199-207.

Original paper

THE USE OF THE YEAST KLUYVEROMYCES FRAGILIS B0399 IN THE PRODUCTION OF PROBIOTIC YOGURT

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Abstract

Rising interest for probiotics in the recent years was caused by the possibility of their use in prevention and cure of different types of human and animal intestinal disorders. During 20th century many research studies were concentrated on finding new types of probiotic cultures. In this work, for the production of probiotic yogurt, we used commercially available, new generation probiotic lactic yeast "Turval B0399", produced by Italian company Turval Laboratories. Turval B0399 is the culture of yeast species Kluyveromyces marxianus fragilis B0399. This yeast is characterized by the unique ability of fermenting with the enzyme β-galactosidase and by production of lactic acid, a fundamental substance in cell metabolic reactions. This probiotic yeast, naturally resistant to antibiotics, mitigate negative effects of antibiotics - by competitive colonisation of intestine it regulates intestinal dismicrobism by preventing the growth of pathogens, such as *Candida albicans*, while increasing the number of residential bifidobacterias. It keeps intestinal homeostasis, improves immunity (in *in vitro* studies it was shown to decrease the production of proinflammatory cytokines, while in studies on patients with atopic dermatitis it decreased the IgE level). It improves the general metabolism and is very successful in prevention and treatment of different intestinal disorders (Crohn's disease and Irritable Bowel syndrome).

In this work we studied the growth of the yeast *Kluyveromyces fragilis* B0399 and its influence on the growth of the probiotic bacteria *Lactobacillus acidofilus* LA5 and *Bifidobacterium lactis* BB12 with the final aim of achieving the maximal number of live cells during the production of probiotic yogurt ($>10^6$ cfu/g). The experimental production of the probiotic yogurt with Turval B0399 was done in the Dairy Laboratory of the Department of Animal Science, Faculty of Agriculture, University of Novi Sad, while all microbiological analyses were done in JPS Dairy Institute, Novi Beograd. During the production of probiotic yogurt we followed the activity of the yeast *Kluyveromyces fragilis* B0399 in different concentrations – 0.5%; 1%; and 3% and under different fermentation temperatures - 39◦C; 23,5◦C and 4◦C (in the cooled probiotic yogurt).

Among all studied conditions we managed to obtain the sufficient number of live yeast cells in the final product when adding 1% of Turval product during the fermentation phase on 23,5◦C, when the number of live cells is $3.5x10^7$cfu/g probiotic bacteria and $3.6x10^5$ cfu/g *Kluyveromyces fragilis* B0399. Clinical studies have shown that in order to exhibit its probiotic functions the daily uptake of the yeast *Kluyveromyces fragilis* has to be ≥10 millions of live cells (certified by the Italian Ministry of Health).

Sensor properties of this probiotic yogurt, odour, taste and colour, are preserved up to expiry date of 30 days. Final product is safe for use and has beneficial properties for good intestinal performance and general health of its consumers.

Key words: *Kluyveromyces marxianus*, *probiotic culture, Turval-B0399, yogurt*

Introduction

Food industry companies have rather high expectations in food products that meet the consumers' demand for a healthy life style. This especially addresses foods that are not intended only to satisfy hunger and provide humans with necessary nutrients, but also to prevent nutrition-related diseases and increase physical and mental wellbeing of consumers, so called "functional food" (Granato et al., 2010; Jankovic et al., 2010; Kaplan et al., 2014). One of the most promising areas for the development of functional foods lies in the modification of gastrointestinal tract activity by the utilization of beneficial microbes (probiotics) in diary products or other dietary supplements, which, when administrated in adequate amounts are aimed at promoting human health (Food and Agriculture Organization/World Health Organization (FAO/WHO), 2001; Jankovic et al., 2010). According to a new market report published by Transparency Market Research, "Probiotics Market: Global Industry Analysis, Market Size, Share, Trends, Analysis, Growth and Forecast", the global probiotic market has been in constant growth, of 7.6% over the previous 5-year period, and is expected to reach 22 billion euros by 2015 (Saxelin, 2008; Pedretti, 2012).

Probiotics have a documented therapeutic role in reducing certain human illnesses, particularly gastrointestinal diseases, caused by deficient or compromised gut microflora (Allen et al., 2004; Canani et al., 2007; Enck et al., 2011); anti-cholesterol activity and anti-high blood pressure effect (Lye et al., 2009; Jahreis, 2002); alleviation of lactose intolerance symptoms by active lactose hydrolysis (Yoshida et al., 2010), promotion of beneficial immune responses (Leyer GJ et al., 2009); beneficial skin effects and reduction of allergies' symptoms (Krutmann, 2009); antimicrobial, anticarcinogenic and anti-mutagenic activities (Orrhage et al., 1994; Rea et al., 2007). There are several proposed molecular mechanisms underlying these functions, including short-chain fatty acid (SCFA) production, the enhancement of the barrier function of the intestinal epithelium, the suppression of the growth and binding of pathogenic bacteria, and alterations of the immune activity of the host (Tuomola et al., 1999; Ventura et al., 2009; Aragon et al., 2010). Furthermore, probiotics can alter colonic fermentation and stabilize symbiotic microbiota (Spiller, 2008), improving the dynamic interplay between the resident bacterial community and the host.

Increasing evidence about the therapeutic potential of probiotics is substantiating constant research and selection of novel microbial species and strains with greater probiotic potential and better properties according to selection criteria (Havenaar et al., 1992), such as: total safety for the host, resistance to gastric acidity and pancreatic secretions, adhesion to epithelial cells, antimicrobial activity, inhibition of adhesion of pathogenic bacteria, evaluation of resistance to antibiotics, tolerance to food additives and stability in the food matrix. The probiotics used today have not been selected on the basis of all these criteria, but the most commonly used probiotics are specific strains of lactic acid bacteria (LAB), belonging mainly to the genus *Bifidobacterium* or *Lactobacillus*. Less frequently used organisms are strains of *Propionibacterium freudenreichii*, bacilli, or yeasts (Wassenaar and Klein, 2008). *Kluyveromyces marxianus fragilis* (found also as *Kluyveromyces fragilis* or *Kluyveromyces marxianus*) is lactic yeast isolated from different dairy products, mainly

kefir (Zhou et al., 2009; Bolla et al., 2011). The importance of this species in food development and fermentation is well documented, while its probiotic activities have been recently well recognized for one strain, named *K. marxianus* B0399, isolated from whey and curds of cow's milk and deposited at the Belgian Coordinated Collection of Microorganisms (BCCM) (accession number MUCL 41579) by Turval Laboratories Italy. Well characterized, health-promoting, probiotic properties of this strain include: strong adhesion to mucosal surfaces and persistence in the intestine; capacity to finely tune the immune response by decreasing the production of the proinflammatory cytokines TNF-α, IFN-γ, IL-6 and the chemokines IP-10 and IL-8 (known to play a crucial role in host defense mechanisms) in PBMCs and Caco-2 cells in the presence of inflammatory stimuli such as LPS, IL-1β, or enteropathogenic bacteria (Maccaferri et al., 2012a and 2012b). Furthermore, two studies have independently proved that this yeast can improve the growth and survival of bifidobacteria in complex food matrices and proximal and transverse colon (Rada, 1997; Maccaferri et al., 2012). It is capable of survival during gastric transit, maintaining its vitality and fermentation capacity (Mustacchi et al., 2010). These scientific evidences are supporting the valuable therapeutic effects of this strain, seen in multiple clinical studies such as: Irritable Bowel Syndrome (Andreoli and Lovrovich 2009; Roda and Cornia 2009), Irritable Colon (Andreoli, 2006), candidiasis (Cettolo and Riul, 2006; Mustacchi et al., 2009), antibiotic-associated diarrhea (Vaughan, 2002) etc. For all these reasons, *K. marxianus* B0399 is the active ingredient in different functional foods currently marketed in several countries worldwide and it is included in the European Food Safety Authority (European Food and Safety Authority (EFSA), 2010) list of qualified presumption of safety (QPS) biological agents added to food and feed (EFSA, 2010). As such, this strain is of particular interest for the global functional food industry.

Since the highest consumption of probiotic products in Europe is associated with probiotic yogurt (Wassenaar and Klein, 2008), our aim in this work was to optimize the production conditions of the new generation, yogurt-like, probiotic dairy beverage, containing mixed probiotic population: yeast *Kluyveromyces fragilis* B0399 and probiotic bacteria *Lactobacillus acidofilus* LA5 and *Bifidobacterium lactis* BB12. We studied the *Kluyveromyces* growth and its influence on the growth of the probiotic bacteria. The final aim was to achieve the maximal number of live cells upon the production of probiotic yogurt ($>10^6$ cfu/g) and after a desired, 30 day, storage period. Production process was further optimized to reach both the 'therapeutic minimum' of live *Kluyveromyces* B0399 cells in the final product ($> 8x10^4$ cfu/g of 125g yogurt package or $> 1x10^7$ cfu/diem, indicated by the Italian Ministry of Health (Bottona et al., 2008)) as well as the lower cost of the production process, acceptable for the Serbian market.

Materials and methods

Milk

Pasteurized and homogenized milk with 1.6% of milk fat was used for the production of fermented dairy beverages. Milk was taken from "Mlekara Dana", Vrbas, and the quality of milk was in accordance with "The regulations on the quality of raw milk" (the Official Gazette of the RS 21/2009) and "The regulations on the quality of dairy products and starter cultures" (the Official Gazette of the RS, 33/2010 and 69/2010).

Starter cultures

Two different types of starter cultures were used for the production of the fermented, probiotic, dairy beverages. A classic probiotic yoghurt (commercial name) was produced

with mixed, ABY-6 starter culture (Chemibiotech s.r.l, Italy), which contains classical yogurt strains: containing *Lactobacillus delbreukii* subsp.*bulgaricus* and *Streptococcus salivarius* subsp.*thermophiles,* as well as two probiotic strains, *Lactobacillus acidofilus* LA5 and *Bifidobacterium lactis* BB12. In addition, the new generation probiotic product was produced using bacterial ABY-6 starter together with a non-conventional, semi-processed, liquid yeast starter, TURVAL B0399® (Turval Laboratories, Udine, Italy). Turval B0399 starter contains probiotic lactic yeast strain *K. marxianus* B0399 in the concentration $\geq 1{,}4x10^7$ yeast cells/ml.

Production of fermented dairy beverages

Milk used for the production of probiotic beverages is low fat milk, standardized to 1.6% milk fat, homogenized and pasteurized initially at 72° for 20s, subjected to bio-chemical analysis of the milk quality, then additionally pasteurized at 92-96°C for 10 and cooled to the inoculation temperature. During the production of the new generation probiotic yogurt, yeast *K. marxianus* B0399 was inoculated in three different concentrations – 0.5%, 1% and 3%, and under different fermentation temperatures – 39°C, 23.5°C and 4°C (in the cooled fermented product). Precisely, in 1000 ml of milk, cooled to a certain inoculation temperature, bacterial and yeast inoculum were added in three different ways as follows: A) alongside, both cultures were added to heated milk, at a temperature of 39°C; or B) alongside, both cultures were added to milk at room temperature (23.5°C); and C) separately, bacterial inoculum was added to heated milk, at a temperature of 39°C while yeast inoculum was added at the end of the fermentation process, in the cooled product (4°C), before mixer homogenization.

In all cases fermentation was interrupted when the fermented product reached 4.75-4.65 pH level and 32-36°SH, which means 4h for 39°C fermentation and about 24h for 23.5°C fermentation.

Fruit containing, yogurt-like fermented milk products with probiotic properties were made in two ways: a) a classical 'fruit containing probiotic yogurt' was made by inoculating ABY-6 probiotic starter culture and 39°C while the new generation 'fruit containing probiotic yogurt' was made as previously described for B). In all cases, according to the manufacturer's suggestion, 10% of fruit paste was added to the cold product (4°C) before mixer homogenization. Apart from 52% of blended strawberries, fruit paste contained small amount of sucrose, water, citric acid, colors and aromas.

All previously mentioned products, containing *K. marxianus* B0399, were further optimized in order to achieve both the 'therapeutic minimum' of live yeast cells in the final product ($\geq 8x10^4$ cfu/g for 125g yogurt package) and the lowest production cost. This optimization was done by varying the size of yeast inoculum form 3% to 0.5%.

ABY-6 probiotic starter culture was added to achieve a concentration of 0.005% in manufacturing yogurt samples.

Products were stored for 30 days in a fridge, at the temperature of 4-8ºC.

Analysis of milk and fermented dairy products

Chemical composition of milk was analyzed right before the yogurt production:

- dry matter was measured with direct method at the temperature of 102±1 ºC;
- milk fat was measured by the Gerber method; - total proteins by Kjeldahl method.

Organoleptic (taste, smell, colour, consistency) and physicochemical analysis of all products were performed on the day 1 after production, and on the day 30 after production.

For this purpose standard methods of analysis were used: active acidity was measured with pH meter and titratable acidity was measured by Soxhlet-Henkel method.

Microbiological analysis (probiotic bacteria and yeast count) was also performed at two previously mentioned time points (day 1 and day 30 after production). Sample suspensions and serial dilutions were prepared in accordance with the standards defined by ISO 6887-5:2010 (Microbiology of food and animal feeding stuffs -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 5: Specific rules for the preparation of milk and milk products). The number of live *K. marxianus* B0399 cells was determined by the colony count method according to the standards defined by ISO 21527-1:2008 (Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of yeasts and moulds -- Part 1: Colony count technique in products with water activity greater than 0.95). Similarly, the number of probiotic bacteria, *L. acidofilus* LA5 and *B. lactis* BB12, was determined by colony-count technique described in ISO 20128:2006 and ISO 29981:2010 standards respectively (Milk products -- Enumeration of presumptive Lactobacillus acidophilus on a selective medium/ Enumeration of presumptive bifidobacteria -- Colony-count technique at 37 degrees C).

Results and discussion

Production of the innovative, plane, dairy probiotic beverage containing *K. marxianus* B0399

Table 1 shows the 'chemical composition of milk used for the production of probiotic beverages'.

Table 1. *Chemical composition of milk used for the production of probiotic beverages*

Milk fat (g/100g)	Total proteins (g/100g)	Total lactose (g/100g)	Dry matter without fats (g/100g)	Dry matter (g/100g)	Somatic Cells (*1000/ml)
1.59	3.32	4.53	8.61	10.185	149

Probiotic species in a specific probiotic food carrier should be alive to an adequate number in order to exert their positive effects on the health of the host. This attribute is known as 'viability' or 'therapeutic minimum' in literature, defined by the adequate number of live probiotic cells in a food product at the time of consumption (Korbekandi, 2011). Various factors have been recognized to affect the viability of probiotic species during storage of fermented dairy products such as interaction between species present, culture, fermentation time and temperature, level of inoculation, carbohydrate source in the fermentation medium, final acidity, dissolved oxygen, and storage time and temperature (Korbekandi et al., 2011; Mohammadi and Mortazavian, 2011). While synergic growth-promoting effects between *L.acidophilus* and *Bifidobacterium* species are well documented (Kneifel et al., 1993) there is no data in the literature about the co-inoculation effect of kefir-derived yeast *K. marxianus* on the survival of these two probiotic bacteria. We tested this effect by comparing the number of probiotic bacteria between two products: i) fermented product made by co-inoculation of 'ABY6' bacterial culture and 'Turval' yeast culture and ii) yogurt fermented with ABY6 only, where yeast culture was added at the end of the production process, in the cooled coagulum. While in the first product the milk is used as

the growth medium for both probiotic bacteria and yeast, in the later product it is the classical 'probiotic yogurt' that is used as a carrier for the innovative probiotic lactic yeast.

Data in Table 2A ('Maximising number of probiotic species by varying fermentation temperature') show that the presence of the yeast strain *K. marxianus* B0399, when added post-fermentation, (in the cooled coagulum) does not significantly influence the final count of probiotic bacteria. The results in Table 2A also suggest that it is rather a long fermentation time (24h) at low temperature (23°C±1°C), which is beneficial for both bacterial, and yeast growth during the production process. This result is supported by the literature data that lower incubation temperature favours the growth of bifidobacteria (Kneifel et al., 1993), and it also shows that the room T was better choice for *K. marxianus* growth, while typical fermentation T of 39°C seemed to even negatively effect the number of *K. marxianus* cells in the final product. This result is in agreement with the multi year fermentation practice of kefir products containing *K. marxianus* strain (Nambou et al., 2014).

Table 2A. *Maximising number of probiotic species by varying fermentation temperature*

Probiotic culture added to the milk at the fermentation temp.	Fermentation Temperature	Fermentation Time	Probiotic culture added to the cooled coagulum	Acidof.+ Bifidus (cfu/ml)	Yeast cells (cfu/ml)
ABY-6 culture	**39°C**	**7h**	/	**8.1×10^6**	/
ABY-6 culture + **3%** Turval	**39°C**	**7h**	/	**7×10^6**	**2×10^5**
ABY-6 culture	**39°C**	**7h**	3% Turval	**7.5×10^6**	**3.75×10^5**
ABY-6 culture + 3% Turval	**23.5°C**	**24h**	/	**9.4×10^6**	**9.5×10^5**

Table 2B. *Maximising number of yeast cells by varying inoculum size of Turval B0399*

Probiotic culture added to the milk at the fermentation temp.	Fermentation Temp.	Fermentation Time	Probiotic culture added to the cooled coagulum	Acidof.+ Bifidus (cfu/ml)	Yeast cells (cfu/ml)
ABY-6 culture + **1%** Turval	**39°C**	**7h**	/	**7.5×10^6**	**4.8×10^4**
ABY-6 culture	**39°C**	**7h**	1% Turval	**7.1×10^6**	**9×10^4**
ABY-6 culture + 1% Turval	**23.5°C**	**24h**	/	**3.5×10^7**	**3.6×10^5**
ABY-6 culture + 0.5% Turval	**23.5°C**	**24h**	/	**1.9×10^7**	**2.8×10^5**

After we identified the ideal fermentation conditions for our mixed microbial population we tested whether it was possible to decrease the yeast inoculum size with respect to the initial 3% concentration and thus decrease the production cost of the new probiotic product. The 3% inoculum size is the Turval concentration that should be applied when the product is made by adding Turval in the cooled coagulum (4°C) in order to satisfy the 'therapeutic minimum' at the time of the consumption ($\geq 1 \times 10^7$ live yeast cells/diem) (Bottona et al., 2008). We hypothesized that by co-inoculating 1% or even 0.5% of yeast culture under favourable fermentation conditions (24h at room temperature) we could still

obtain the *Kluyveromyces* 'therapeutic minimum' in the final product because of the yeast cell proliferation under these conditions. The results presented in Table 2B ('Maximising number of yeast cells by varying inoculum size of Turval B0399') confirm this hypothesis. Even though 0.5% of inoculum gave the satisfactory number of yeast cells in the final product ($2.8x10^5$ /ml) we recommend 1% yeast inoculum as the best compromise between the high cell number and acceptable production cost (to compensate for the possible decline in the concentration of the probiotic organisms during storage of a probiotic). To question whether the number of probiotic bacteria declines below the proposed 'therapeutic minimum' during refrigerated storage, we investigated the viability of probiotic species (yeast and bacteria) in yogurts stored in cold, 30 days after production.

Table 3. *Microbiological analysis of the new generation fruit probiotic dairy beverage*

Probiotic culture added to the milk at the fermentation temp.	Fermentation Temp.	Fermentation Time	Fruit paste added to the cooled coagulum	Acidof.+ Bifidus (cfu/ml)	Yeast cells (cfu/m)
ABY-6 culture	**39°C**	**7h**	**10%**	**$8.1x10^6$**	**-**
ABY-6 culture + 1% Turval	**23.5°C**	**24h**	**10%**	**$2.9x10^7$**	**$3.3x10^5$**

Table 4. *Stability of physico-chemical characteristics of the new generation probiotic dairy beverages during long term (30 days), refrigerated storage*

Sample type: Probiotic cultures	Fermentation Temp, Time	pH value	Titratable acidity (°SH)	pH value	Titratable acidity (°SH)
		1st day after production		**30th day after production**	
ABY-6 culture	39°C, 7h	4.77	32.0	4.58	35.0
ABY-6 culture + **1% Turval**	23.5°C, 24h	4.72	33.0	4.60	35.0
ABY-6 culture + **0.5% Turval**	23.5°C, 24h	4.73	32.0	4.60	35.0
ABY-6 culture + **10% fruit paste**	23.5°C, 24h	4.79	32.0	4.18	39.0
ABY-6 culture + **1% Turval + 10% fruit paste**	23.5°C, 24h	4.68	34.0	4.20	39.0

The results are shown in Table 5 ('Microbiological analysis of the new generation probiotic dairy beverages after 30 days of refrigerated storage'), showing 1 log decrease in the number of live probiotic bacteria after long-term storage and a lesser increase in the yeast count. Likewise, the viability of probiotic bacteria in products over a long shelf life at refrigeration temperature has been reported before to be unsatisfactory (Rybka and Kailasapathy, 1995, Lourens-Hattingh and Viljoen, 2001b). This is mainly due to a certain level of the 'over-acidification' during storage, which if it reaches pH values under 4.6 may be harmful for probiotic species, particularly for bifidobacteria (Kailasapathy and Chin, 2000, Lourens-Hattingh and Viljoen, 2001b). As shown in Table 4 ('Stability of physico-chemical characteristics of the new generation probiotic dairy beverages during long term (30 days), refrigerated storage') the acidity of both classical probiotic yogurt

(ABY-6 species) and innovative, lactic yeast-based probiotic product (ABY-6 culture + Turval) decreased to pH 4.6 over the storage period. This could explain the slight decrease in the viability of probiotic bacteria, shown in Table 5, although the final count remained acceptable (above the therapeutic minimum). On the other hand, the ability to hydrolyse the residual lactose, utilisation of glucose and galactose produced by LAB and resistance to the low pH of *Kluyveromyces* cells are all plausible explanations for a minor increase in the yeast cell number over a long storage period (Table 5). However, this did not interfere with the sensorial characteristics of the product, which remained the same during the whole storage period.

Table 5. *Microbiological analysis of the new generation probiotic dairy beverages after 30 days of refrigerated storage*

Probiotic culture added to the milk at the fermentation temp.	Fermentation Temp.	Fermentation Time	Probiotic culture or Fruit paste added to the cooled coagulum	Acidof.+ Bifidus (cfu/ml)	Yeast cells (cfu/ml)
ABY-6	**39°C**	**7h**	/	$1.3x10^6$	/
ABY-6 + 1% Turval	**23.5°C**	**24h**	/	**$1.3x10^6$**	**$6 x10^5$**
ABY-6	**39°C**	**7h**	**10% fruit paste**	$2.7x10^6$	/
ABY-6 + 1% Turval	**23.5°C**	**24h**	**10% fruit paste**	**$1.5x10^6$**	**$2.5x10^6$**

Production of the innovative fruit dairy probiotic beverage containing *K.marxianus* B0399

The 1% Turval B0399 inoculum was further used to produce an alternative, fruit-containing, diary probiotic product by its co-inoculation with ABY-6 culture under optimal fermentation conditions (RT, 24h). As shown in Table 3 ('Microbiological analysis of the new generation fruit probiotic dairy beverage') the number of yeast cells in the product was satisfactory and similar to its concentration in the plane yogurt (>$3x10^5$/ml). Sensorial characteristics of this product were similar to the classical 'fruit probiotic yogurt' (ABY-6 culture, 39°C fermentation temp.), while the number of probiotic bacteria in the Turval-based product is slightly bigger than in the classical one, similarly to the plane probiotic products described previously (probably the result of the preferable fermentation conditions). In addition, we investigated the effect of commercial strawberry preparations on the viability of probiotic bacteria and probiotic yeast during storage (30 days) at refrigerated temperature. Again, we could observe the correlation between the post-storage over-acidification of fruit beverages (Table 4) and slightly decreased viability of probiotic bacteria (Table 5). While the number of probiotic bacteria in the fruit products slightly decreased over time but still remained satisfactory until the expiry date (30 days), the yeast cell viability showed exactly the opposite, increasing trend (Table 5). This 1 log increase in the yeast count is mainly due to the presence of proportions of sucrose and fructose derived from the fruit (Kailasapathy et al., 2008). Still, this did not interfere with the sensorial characteristics of the product, which remained the same in terms of taste and odour while a fairly recognisable decrease in acidity could be noticed, just like for the classical 'fruit probiotic yogurt' without lactic yeast.

Conclusion

The results obtained in this study demonstrated that fermented dairy products are great choice as food-carriers for the new generation probiotic yeast strain, *K. marxianus* B0399.

The sensory properties such as: appearance, texture, flavour and overall quality of probiotic dairy beverages containing Turval B0399 probiotic lactic yeast were comparable with standard 1.6% fat probiotic yogurt and they remained stable during the 30 day storage period. The pH values in all samples decreased during storage, where a bit higher dynamics could be observed for the fruit-containing yogurt and was not attributable to the presence of yeast. These innovative products are lacking yeasty flavour typical for dairy products containing Saccharomyces species (Lourens-Hattingh and Viljoen, 2001a), which may be explained by a distinct diapason of aroma compounds produced by *K.marxianus* strain (Fonseca, 2008). Even though it was not directly measured in this study, it is important to mention that *K. marxianus* B0399 produces very small amount of CO2 and ethanol at the end of 35-day shelf life of the commercially available, Italian branded, fruit based bio-yogurt EUFYR (Coop Italia, personal communication). This can be explained by strictly anaerobic metabolism of ethanol production of this yeast strain (Fonseca et al., 2008), eliminating what was known to be the major constraints for incorporating another probiotic yeast strain, *Saccharomyces boulardii* into bio-yogurt (Lourens-Hattingh and Viljoen, 2001a).

References

1. Allen SJ, Okoko B, Martinez E, Gregorio G and Dans LF, 2004. Probiotics for treating infectious diarrhoea. Cochrane Database Syst Rev, CD003048.
2. Andreoli S, 2006. Trial#16: Trials with lactic yeast Kluyveromyces B0399 (Kluyver B0399) on patients suffering from irritable colon (Gastroenterologic Service of the Hospital S. Maria della Misericordia of Udine, Italy). Retrieved May, 2014 from http://www.turval.com/research/humans_and_nutrition/trials-with-bioval-plus
3. Andreoli S and Lovrovich P, 2009. Trial#132: Clinical results of treatment with the lactic yeast Kluyveromyces B0399 for Irritable Bowel Sindrome. This study, based on the gathering of symtomatological data before and after the treatment, demonstrates that the prolonged use of Kluyveromyces marxianus fragilis B0399 significantly improves the clinical picture and above all improves the quality of life of these patients (S.Camillo Hospital, Treviso, Italy). Retrieved May, 2014 from http://www.turval.com/research/humans_and_nutrition/irritable-bowel-sindrome
4. Aragon G, Graham DB, Borum M and Doman DB, 2010. Probiotic therapy for irritable bowel syndrome. Gastroenterol Hepatol (N Y) 6, 39-44.
5. Bolla PA, Serradell Mde L, De Urraza PJ and De Antoni GL, 2011. Effect of freeze-drying on viability and in vitro probiotic properties of a mixture of lactic acid bacteria and yeasts isolated from kefir. J. Dairy Res. 78, 15-22.
6. Bottona EP, G and Zilli M, 2008. EXPERTISE: Valutazone del lievito lattico Kluyveromyces marxianus fragilis B03999 ai sensi delle linee guida del Min. della Salute Dic 2005 All 1.(doc.depositato presso il Ministero della Salute). In. Linee guida dei probiotici . Min. della Salute Dic 2005 All 1.
7. Canani RB, Cirillo P, Terrin G et al., 2007. Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. BMJ 335, 340.
8. Cettolo T and Riul L 2006. Trial#96: In vitro test on the effect of the typified lactic yeast (Kluyveromyces marxianus B0399) on the development of Candida albicans ATCC10231 (Specialized Laboratory of Microbiology of the ASA – CCIAA of Udine, Italy). Retrieved on July 2014 from http://www.turval.com/research/humans_and_nutrition/candida-albicans-kluyveromyces-b0399-actively-competes-against-infection-with-candida
9. Enck P, Klosterhalfen S and Martens U 2011. Probiotic therapy of the irritable bowel syndrome. Dtsch Med Wochenschr 136, 371-5.

10. European Food and Safety Authority (EFSA) 2010. Scientific opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed. In EFSA Panel on Biological Hazards. EFSA J. 8.

11. Fonseca GG, Heinzle E, Wittmann C and Gombert AK 2008. The yeast Kluyveromyces marxianus and its biotechnological potential. Appl. Microbiol. Biotechnol. 79, 339-54.

12. Granato D, Branco GF, Cruz AG, Faria JAF and Shah NP 2010. Probiotic Dairy Products as Functional Foods. Comprehensive Reviews in Food Science and Food Safety 9, 455 - 70.

13. Havenaar R, Ten Brink B and Huis JHJ 1992. Selection of strains for probiotic use. In Probiotics (R Fuller), pp. 209-24. Springer

14. Jahreis G, Vogelsang H, Kiessling G, Schubert R, Bunte C and Hammes WP 2002. Influence of probiotic sausage (Lactobacillus paracasei) on blood lipids and immunological parameters of healthy volunteers. Food Research International 35, 133–8.

15. Jankovic I, Sybesma W, Phothirath P, Ananta E and Mercenier A 2010. Application of probiotics in food products--challenges and new approaches. Curr Opin Biotechnol 21, 175-81.

16. Kailasapathy K and Chin J 2000. Survival and therapeutic potential of probiotic organisms with reference to Lactobacillus acidophilus and Bifidobacterium spp. Immunol Cell Biol 78, 80-8.

17. Kailasapathy K, Harmstorf I and Phillips M 2008. Survival of Lactobacillus acidophilus and Bifidobacterium animalis ssp. lactis in stirred fruit yogurts. LWT - Food Science and Technology 41, 1317-22.

18. Kaplan RM, Storey BE, Vidyashankar AN, et al. 2014. Antiparasitic efficacy of a novel plant-based functional food using an Ascaris suum model in pigs. Acta Trop.

19. Kneifel W, Jaros D and Erhard F 1993. Microflora and acidification properties of yogurt and yogurt-related products fermented with commercially available starter cultures. Int J Food Microbiol 18, 179-89.

20. Korbekandi H, Mortazavian AM and Iravani S 2011. Probiotic and Prebiotic Foods: Technology, Stability and Benefits to Human Health. In Advances in food safety and food microbiology (eds Shah NP), pp. 121-46. Nova Sciences Publishers, Hauppauge, N.Y.

21. Krutmann J 2009. Pre- and probiotics for human skin. J Dermatol Sci 54, 1-5.

22. Leyer Gj LS, Mubshaer Me, Reifer C and Ouwehan Ac. 2009. Probiotics effects on cold and influenza-like incidence and duration in children. Pediatrics 124, 172-8.

23. Lisotti A, Cornia GL, Morselli-Labate AM, Sartini A, Turco L, Grasso V, Cavina P, Mazzella G and Roda E 2011. Effects of a fermented milk containing Kluyveromyces marxianus B0399 and Bifidobacterium lactis BB12 in patients with irritable bowel syndrome. Minerva Gastroenterol Dietol 57(Suppl. 1 al N.2), 1-12

24. Lourens-Hattingh A and Viljoen BC 2001a. Growth and survival of a probiotic yeast in dairy products. Food Research International 34, 791-6.

25. Lourens-Hattingh A and Viljoen BC 2001b. Yogurt as probiotic carrier food. International Dairy Journal 11, 1-17.

26. Lye HS, Kuan CY, Ewe JA, Fung WY and Liong MT 2009. The improvement of hypertension by probiotics: effects on cholesterol, diabetes, renin, and phytoestrogens. Int J Mol Sci 10, 3755-75.

27. Maccaferri S, Candela M, Turroni S, Centanni M, Severgnini M, Consolandi C, Cavina P, and Brigidi P 2012a. IBS-associated phylogenetic unbalances of the intestinal microbiota are not reverted by probiotic supplementation. Gut microbes 3(5):406-413.

28. Maccaferri S, Klinder A, Brigidi P, Cavina P and Costabile A 2012b. Potential probiotic Kluyveromyces marxianus B0399 modulates the immune response in Caco-2 cells and peripheral blood mononuclear cells and impacts the human gut microbiota in an in vitro colonic model system. Appl Environ Microbiol 78, 956-64.

29. Mohammadi R and Mortazavian AM 2011. Review Article: Technological Aspects of Prebiotics in Probiotic Fermented Milks. Food Reviews International 27, 192-212.

30. Mustacchi G, Miclavez A, Turello A, Bearzi F, Collavini F and Valles P 2010. Trial#130.1: Verification of the capacity of colonization of the gastrointestinal tract in healthy subjects, after the utililization of the lactic yeast Kluyveromyces marxianus fragilis B0399, through examination of the feces (Oncological Centre, Local Health N1-Trieste; Trieste University), Retrieved on April 2014 from http://www.turval.com/research/humans_and_nutrition/verification-of-the-capacity-of-colonization-of-the-gastrointestinal-tract-in-healthy-subjects-after-the-utililization-of-the-lactic-yeast-kluyveromyces-marxianus-fragilis-b0399-through-examination-of-the-feces-trial-n-130-1.

31. Nambou K, Gao K, Zhou F, Guo B, Ai L and Wu ZJ 2014. A novel approach of direct formulation of defined starter cultures for different kefir-like beverage production. International Dairy Journal 34, 237-46.

32. Food and Agriculture Organization/World Health Organization (FAO/WHO), 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, a joint FAO/WHO expert consultation (Cordoba, Argentina, 1 - 4 October 2001). Retrieved on June 2014 from http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf

33. Orrhage K, Sillerstrom E, Gustafsson JA, Nord CE and Rafter J 1994. Binding of mutagenic heterocyclic amines by intestinal and lactic acid bacteria. Mutat Res 311, 239-48.

34. Pedretti S 2012. Probiotic market: up or down? Nutrafoods 12, N18-N9.

35. Rada V 1997. Effect of Kluyveromyces marxianus on the growth and survival of bifidobacteria in milk. Folia Microbiol (Praha) 42, 145-8.

36. Rea MC, Clayton E, O'connor PM, et al. 2007. Antimicrobial activity of lacticin 3,147 against clinical Clostridium difficile strains. J Med Microbiol 56, 940-6.

37. Roda E and Cornia G 2009. Trial#125: Evaluation of the effects of a yogurt containing Kluyveromyces marxianus fragilis B0399 in patients suffering from Irritable Bowel Syndrome—a randomized clinical study controlled with placebo - abbreviate text (Gastroenterology Unit of the S'Orsola-Malpighi Hospital of Bologna, Italy). Retrieved on July 2014 from http://www.turval.com/research/humans_and_nutrition/trial-125-evaluation-of-the-effects-of-a-yogurt-containing-kluyveromyces-marxianus-fragilis-b0399-in-patients-suffering-from-irritable-bowel-syndrome2014a-randomized-clinical-study-controlled-with-placebo-abbreviate-text

38. Rybka S and Kailasapathy K 1995. The survival of culture bacteria in fresh and freeze-dried AB yoghurts. The Australian Journal of Dairy Technology 50, 51-7.

39. Saxelin M 2008. Probiotic formulations and applications, the current probiotics market, and changes in the marketplace: a European perspective. Clin Infect Dis 46 Suppl 2, S76-9; discussion S144-51.

40. Spiller R 2008. Review article: probiotics and prebiotics in irritable bowel syndrome. Aliment Pharmacol Ther 28, 385-96.

41. Tuomola EM, Ouwehand AC and Salminen SJ 1999. Human ileostomy glycoproteins as a model for small intestinal mucus to investigate adhesion of probiotics. Lett Appl Microbiol 28, 159-63.

42. Vaughan A 2002. Trial#84: Resistance to antibiotics of the feed supplement TURVAL B0399 - probiotic with typified lactic yeast Kluyveomyces marxianus fragilisB0399 (University of Studies of Perugia Italy, Department of Biology and Agrodietary Biotechnologies). Retrieved on July http://www.turval.com/research/humans_and_nutrition/trial-84-resistance-to-antibiotics-of-the-feed-supplement-turval-b0399-probiotic-with-typified-lactic-yeast-kluyveomyces-marxianus-fragilisb03999

43. Ventura M, O'flaherty S, Claesson MJ, et al. 2009. Genome-scale analyses of health-promoting bacteria: probiogenomics. Nat Rev Microbiol 7, 61-71.

44. Wassenaar TM and Klein G 2008. Safety aspects and implications of regulation of probiotic bacteria in food and food supplements. J Food Prot 71, 1734-41.

45. Yoshida E, Hidaka M, Fushinobu S, et al. 2010. Role of a PA14 domain in determining substrate specificity of a glycoside hydrolase family 3 beta-glucosidase from Kluyveromyces marxianus. Biochem J 431, 39-49.

46. Zhou J, Liu X, Jiang H and Dong M 2009. Analysis of the microflora in Tibetan kefir grains using denaturing gradient gel electrophoresis. Food Microbiol 26, 770-5.

Original paper

THE INVESTIGATION OF SOME MOHAIR MINERAL LEVELS (Mg, Fe, Cu, Zn) OF ANGORA GOATS IN ORIGINAL ZONE OF ANKARA PROVINCE

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Abstract

In this study, determination of some mineral levels of mohair samples obtained from Angora goats *(Capra hircus ancyrensis)* bred in villages of Basayas (1st farm, n=28 goats) and Yagmurdede (2nd farm, n=30 goats) in Ankara province of Turkey was aimed. Totally 58 goats (2-4 years old) in two farms kept under similar managemental conditions and based on pasture were chosen as research materials. Mohair samples from mid-side of the goats were collected to determine the levels of magnesium (Mg), iron (Fe), copper (Cu) and zinc (Zn) by atomic absorption spectrophotometer (AAS) (M series V1, 23). The mohair mineral levels in the 1st and the 2nd farm of Angoras were found as 22.234 ± 1.030 µg/g and 20.952 ± 1.462 µg/g for Mg; 37.716 ± 1.573 µg/g and 32.271 ± 1.397 µg/g for Fe ($P<0.05$); 4.248 ± 0.293 µg/g and 7.169 ± 0.285 µg/g for Cu ($P<0.001$); 60.673 ± 2.395 µg/g and 62.802 ± 3.172 µg/g for Zn, respectively. In the 1st and the 2nd farm, non-significant positive and negative correlations between minerals were discovered. The estimated correlation coefficient between Fe and Cu was negative (r = □ 0.291) significant in total population ($P<0.05$).

Key words: *Angora goat, atomic absorption spectroscopy, mineral level, mohair*

Introduction

Turkey has about 8.2 million heads Hair goat (*Turkish:* Kıl keçisi) and 0.16 million heads Angora goat (*Turkish:* Ankara keçisi). 39.495 head of goats and 118.607 head of young-adults offspring were recorded of them in Angora goats (Anonymous, 2013). Goats are kept for their milk, meat, skin, hair, cashmere (down fibers) and mohair for several centuries in Anatolia (Koyuncu et al., 2005). Angora goats were raised in Ankara province of Turkey, at 39° 52′ 30″ N, 2° 49′ 59.88″ E, at an altitude of 938 m (3.077 ft).

There are some minerals that are necessary for feeding of goats. While macro minerals are needed to increase purchase, micro minerals are required in very small quantities. On the other hand mineral intakes in goats as well as sheep are very important for health. For instance, high Mg deficiency causes grass tetany in both. Similar to Fe level in blood it is important for interfering with the uptake of some other minerals that are in very small quantities, such as Zn. Zn and Cu minerals are required by goat for mohair growth and health for keratinisation. Investigations by Reis et al. (1989) proved that both Zn and Cu are indispensable micro nutrients for quite fast growing tissues such as follicles of fiber-producing. Additionally, balanced mineral intake of goats is effective on not only health

but also on productivity. Especially goat mohair quality is directly related to the mineral levels in the body.

Mineral deficiencies are common in small ruminants grazing poor quality pastures and reared under traditional system (Kawas et al., 2010, Xin et al., 2011). Fleece-eating is an important health problem for some animals such as sheep and goats. This event can be observed from lower concentrations of S and Mo. Yet, in this case, Ca, P, Fe, Mn, Zn, Cu, Co and Se are present in lower concentrations (Patkowska-Sokola et al., 2009).

Mohair is a soft, luxurious fiber with a rich luster used in fine clothing, carpets, blankets and upholstery and the main source of income for Angora goat producers. On the other hand mohair is a beautiful, lustrous, strong fiber with unique characteristics, making it as popular today. It is extremely durable with a soft luxurious texture and unique appearance (Anderson, 2014). The natural colours of mohair fiber are usually white and different shades of white. However, the colored (brownish, grey and silver) goats are frequently encountered in Turkey's east.

Typical Angora goats are fairly smaller than average sheep that are reared for wool but they produce twice as much fiber compared with sheep produce (Gallagher and Shelton, 1972). Besides, mohair is pure protein that is homologous to wool protein content (Parris and Swart, 1975) and a kind of admirable natural animal fiber.

The quality of mohair is classified based on following properties. It is also important to consider issues such as fine and long mohair fiber. It deepens on the quality drops. More numbers of crimps of the mohair are expected. Additionally bright fiber and more elegnation are desirable. However stiff bristles called □dog hairs□ are not inconvenient. In this manner, mohair quality is determined by considering these conditions.

Angora goats have received less research attention than meat or dairy goats, particularly in the last 10 to 20 years since animal numbers have declined in response to change in production profitability (Sahlu et al., 2009). Structural characteristics (Salehi, 2009; Syed Momen et al., 2009; McGregor and Butler, 2010) and mineral compositions (Eryavuz et al., 2002; Shamsaddini-Bafti et al., 2012) of goat natural fibers are highly investigated in the literature. Sufficient number of studies on wool mineral analyses of sheep (Aydin, 2008; Patkowska-Sokola et al., 2009) was presented, however, mohair mineral studies (Ehsani et al., 2005; Shamsaddini-Bafti et al., 2012) were relatively few.

The aim of the present study is the assessment of chemical mineral composition in 1st and 2nd quality mohair samples from Angora goats (Fig. 1 and Fig. 2) of similar performance types in capital of Turkey. In this way, the difference of the mineral content can be introduced between different quality (the 1st and the 2nd) and colour mohair.

Materials and methods

Before starting the research, we obtained some information concerning mohair quality on farms. This study was held in the 2 different villages (Basayas and Yagmurdede) of Ankara. The mohair from the 1st farm has 1st and the 2nd farm has 2nd quality mohair based on "Tiftik Birlik" that number of SS 459 in Ayas, Ankara. In February before shearing, in total 58 Angora goats (n1=28; n2=30) that appeared clinically healthy two private farms belonging to Angora goat were used. 2-4 year old goats were randomly selected from each farm.

Figure 1. *Angora goats in the 1st farm*

Figure 2. *Angora goats in the 2nd farm*

Mohair samples from the selected goats were collected according to standard methods. Specimens of mohair (approximately 5 g) were taken from right side of goat rib with scissors. Firstly, wet incineration method was applied to the mohair before the determination of Mg (macro mineral) and Fe, Cu, Zn (micro minerals). Analyses were performed as reported in the literature (Kumaresan and Kapioh, 1984; Salehi, 2009).

Sample Preparation

The 0.5 g mohair samples were used in experimental work. Each sample in a polyethylene bottle containing 150 ml of a 1% solution of non-ionic detergent was washed by agitating on a mixer for 30 minutes at standard room temperature. After that, it was transferred to a polyethylene filter crucible and rinsed with a total of one liter of deionized water. Dry weight has been expected of about 0.5 g. At a temperature of 110 ºC, samples were weighted and transferred into 50 ml flasks. 6 ml of HNO_3 was added and allowed to react at normal room temperature. The digest was warmed and 1 ml of $HClO_4$ was added. When the process was completed, the temperature was increased to 200 ºC. Solution was transferred to a 5 ml volumetric flask and diluted to volume with deionized water. This solution was used for the determination of Mg, Fe and Cu. A further dilution was required for Zn.

Levels of Mg, Fe, Cu and Zn in mohair were determined by atomic absorption spectrophotometer (AAS) (Salvin, 1968; Field, 1988) after wet incineration method. The amounts of mineral concentrations were determined by measuring with absorption/emission amount of AAS device. Concentrations of the mineral to be determined could be made by subtracting the measurement curve after calibrating the device using standard concentration values. Standard solutions are used for calibration procedures (Demir et al., 2011). Results were measured as ppm. The final results were converted to µg/g according to express literature.

Statistical Analyses

The obtained data were statistically analyzed with SAS software package (SAS, 2002). The relationship between farms was analyzed to put forward for revealing with Pearson Correlation Coefficient at the significance level of $P<0.05$.

Results and discussion

Analyzed results of mohair mineral were presented in Table 1. Mg, Fe, Cu and Zn concentrations of mohair were published with reference ranges for 58 Angora goats in total population. Mineral contents were determined at the ranges of 10.16-39.64 µg/g for Mg, 21.28-58.52 µg/g for Fe, 2.01-8.98 µg/g for Cu and 38.32-96.40 µg/g for Zn, respectively.

Shamsaddini-Bafti et al. (2012) reported that Cu and Zn content of cashmere in Raeini goats averaged 0.00065 % and 0.01276 %, respectively. In a different study conducted on Angora goats (Imik et al., 1998), the levels of Fe, Cu and Zn of mohair were found as 41.83 µg/g, 3.93 µg/g and 83.24 µg/g in (-) control group. The levels of Fe and Zn determined in this study were lower than the values reported by Imik et al. (1998) while Cu level was higher.

Table 1. *Mineral levels of Angora goats (µg/g)*

Farm	Mg			Fe			Cu			Zn		
	N	X + Sx Min-Max	P	N	X + S x Min-Max	P	N	X + S x Min - Max	P	N	X + S x Min - Max	P
1	28	22.23±1.03 14.21-35.81		28	37.72±1.57 21.28-58.52		26	4.24± 0.29 2.01-7.99		28	60.67±2.40 43.36-96.40	
2	30	20.95±1.46 10.16-39.64	>0.05	30	32.27±1.40 22.29-56.56	<0.05	24	7.17± 0.29 4.06-8.98	<0.0001	30	62.80± 3.17 38.32-96.36	>0.05
Total	58	21.57±0.90 10.16-39.64		58	34.90±1.10 21.28-58.52		50	5.65± 0.29 2.01-8.98		58	59.71±2.00 38.32-96.40	

An average value of 4 ppm for Cu in goat mohair and hair has been reported by George and Haenlein (2009). Cu levels of the 2nd farm were higher than the result found in this study. In addition, relative high level differences of Cu may be due to different types of fresh water supply. The 2nd farm had darker-colored mohairs than the 1st. This case might result from copper colour, a metallic tone of red. Moreover, Cu is thought to result from the influence of the color enhancer. The high amount of copper, being darker than the lower is natural. Williams (2004) have also reported the genetic variation between the breeds suggesting that different responses arise from a variation in the efficiency of absorption of Cu.

As known, farms had different qualities of mohair at baseline. We have received some information prior to conducting the research from the Association of Agricultural Sale Cooperative of Angora (mohair) and wool, Ayas/Ankara. Accordingly, the 1st farm had the best (1st) quality mohair and used mains water. For all that the 2nd farm had 2nd quality mohair and used pond water (40°7'37"N 32°20'57"E). Mineral levels in drinking water of goats might be the reflection of mohair mineral levels.

Considering Mg and Zn contents, there were no statistical differences (P>0.05) between the farms of Basayas and Yagmurdede. On the other hand, the differences in Fe and Cu contents between the farms were statistically significant at the significance levels of P values (P <0.05, P<0.0001, respectively) (Table 1).

Table 2. *Correlation coefficients among mohair mineral levels in the 1st farm*

N:28	Mg	Fe	Cu	Zn
Mg	**1.000**	- 0.176 ^{NS}	0.001 ^{NS}	0.150 ^{NS}
Fe		**1.000**	0.065 ^{NS}	-0.032 ^{NS}
Cu			**1.000**	0.032 ^{NS}
Zn				**1.000**

NS: Non significant

The estimates of correlations between mohair mineral levels are shown in Table 2 for the 1st farm and Table 3 for the 2nd farm. In the 1st and 2nd farm, positive and negative correlations between minerals were discovered. The correlation between Fe and Cu was negatively significant (P<0.05) in total population (Table 4). Nevertheless, Shamsaddini-

Bafti et al. (2012) reported that there was a positive correlation between Zn and Cu content in fiber (P<0.05).

Table 3. *Correlation coefficients among mohair mineral levels in the 2nd farm*

N:30	Mg	Fe	Cu	Zn
Mg	**1.000**	0.101^{NS}	0.313^{NS}	-0.035^{NS}
Fe		**1.000**	-0.141^{NS}	-0.124^{NS}
Cu			**1.000**	0.093^{NS}
Zn				**1.000**

NS: Non significant

Table 4. *Correlation of mohair mineral levels in total population*

N:58	Mg	Fe	Cu	Zn
Mg	**1.000**	0.020^{NS}	-0.018^{NS}	0.017^{NS}
Fe		**1.000**	-0.291*	-0.102^{NS}
Cu			**1.000**	0.001^{NS}
Zn				**1.000**

NS: Non significant, *P<0.05

There are so many studies about Merino sheep but not about Angora goats yet. In a study performed on sheep, Purser (1979) reported the importance of adequate vitamins and mineral for wool growth. Folic acid and pyridoxine are especially essential for the wool growth. Zn and Cu are required for the process of fiber growth. Reduction of these minerals can reduce the wool and mohair growth. There is a limited number of studies on the mineral composition of Angora goats. Concentration of minerals of goats fleece reflects feed and nutrition quality as well as environmental states, sex, age and breed's physiological condition that can also affect the quality of hair and mohair. The pigment of sheep or goat coats affects the accumulation of some minerals such as Fe, Cu, Mn, K, Na and Mg (Patkowska-Sokola et al., 2009). The richness of minerals depends on the soil and plant concentration. Gallo et al. (1996) studied the relationship of minerals in soil, plant and animal tissue. Environmental status can also affect the mineral content of wool. High concentration of some toxic metals (Cd, Cu, Pb, Zn) could be the result of industrial emission from the air. Angora goats in this study were chosen from the ecologically clean regions. On the other hand, in Turkey Angora goat farming in areas with continental climate is based on pasture. Vegetation in areas with a continental climate with plenty of rainfall is rich only in the months of March and April. For this reason, depending on pasture conditions it is seen that the mineral and vitamin needs of animals are not met. In order to succeed in producing high quality Angoras, the fact that mineral and vitamin needs of animals should be met is crucial and will have a positive impact.

Difference between water resources might be effective on mohair quality and mineral content. It is necessary to say that Angora goat mohair quality and color are possibly associated with the mineral content. Large amounts of copper might probably be darker than mohair. Needless to say that genetic factors could not be neglected in such cases.

Conclusion

Ponds water with unknown content and fresh water of unknown origin might especially cause the accumulated minerals in the body. Investigation of water resources mineral levels with genetic factors can be effective in determining the quality and color of mohair as well as the genetic relationships between different flocks of the Angora goats

concerning the quality of mohair. The further analyses of great number of mohair minerals among blood minerals of animals and fresh water supply would be useful.

Acknowledments

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References

1. Anderson L 2014. Angora goats: A ‘’Shear’’ delight!, Revieved, May 10 from http://sanangelo.tamu.edu/files/2011/11/AngoraGoatsAShearDelight_1.pdf.
2. Anonymous 2014. Revieved, May 8 from http://tr.wikipedia.org/wiki/Ya%C4%9Fmurdede,_Aya%C5%9F
3. Anonymous 2013. Livestock statistics. Turkish Statistical Institute. http://tuikapp.tuik.gov.tr/hayvancilikapp/hayvancilik.zul .
4. Aydin I 2008. Comparison of dry, wet and microwave digestion procedures for the determination of chemical elements in wool samples in Turkey using ICP-OES technique. Microchemical J. 90, 82-87.
5. Demir AO, Mert N, Karakus F, Bingol M and A. Kor A 2011. An investigation of the mohair quality and some blood serum mineral levels of Angora goats breed in different farm conditions. 7th National Zootechnic Scientific Congress, September, Adana, Turkey.
6. Ehsani M, Salehi M and Shakeri P 2005. Study of relationship between mineral content and fiber dimeter of Raeini Cashmere goat in original zone of Keman Province. Sheep and Goat Conference. Animal Science Research Institute of Iran, 159-160.
7. Eryavuz A, Durgun Z and Keskin E 2002. Effects of ration supplemented with zinc on some rumen and blood parameters, mohair production and quality in faunated and defaunated Angora goats. Turk J. Vet. Anim. Sci. 26, 753-760.
8. Field AC 1988. Mineral requirements of ruminant livestock in Turkey report.*IAEA*, Vienna.
9. Gallagher JR and Shelton M 1972. Efficiencies of conversion of feed to fiber of Angora goats and Rambouillet sheep. J. Anim. Sci. 34:319.
10. Gallo M, Gallo J, Sommer A and Flack P 1996**.** Influence of emission fall-out movement of heavy metals in the cycle soil, plant, animal. Arch Tierz 39, 195-202.
11. George F and Haenlein W 2009. Recent advances in mineral nutrition of goats, Cooperative Extension Dairy Specialist. University of Delaware. Agriculture Research Services USA Department Agriculture USA, Retrieved October 20.
12. Imik H, Gucus AI and Cetinkaya N 1998. Effects of supplementation of Angora goat diet with minerals and vitamins on live weight gain, mohair produce, quality and its mineral content with blood mineral concentration. Ankara Univ. Journal of Vet. Med. 45, 83-95.
13. Kawas JR, Andrade-Montemayor H and Lu CD 2010. Strategic nutrient supplementation of free-ranging goats. Small Ruminant Research. 89, 234-243.
14. Koyuncu M, Tuncel E and Uzun SK 2005. Present status of goat breeding in Turkey. Animal Production and Natural Resources Utilisation in the Mediterranean Mountain Areas. EAAP Publication, No.115, Ioannina, Greece, 340-347.
15. Kumerasan A and Kapioh MA 1984. Hair as indicator of mineral status in Yankassa sheep. Rev. Elev. Med. Vet. Pays. Trop. 37(1): 61-64.

16. McGregor BA and Butler KL 2010. Phenotypic associations with fibre curvature standard deviation in cashmere. Small Rumin. Res. 91, 193–199.
17. Patkowska-Sokola B, Dobrzanski Z, Osman K, Bodkowski R and Zygadlik K 2009. The content of chosen chemical elements in wool of sheep of different origins and breeds. Archiv Tierzucht 52, 4, 410-418.
18. Purser DB 1979. Effects of minerals upon wool growth. In: J. L. Black and P J Reis (Ed.) Physiological and Environmental Limitations to Wool Growth. P 295. Univ. of New England, Armidale, New South Wales, Australia.
19. Parris D and Swart LS 1975. Studies on the high sulphur protein of reduced mohair. The isolation and amino acid sequence of protein SCMKB-M 1.2. Biochem. J. 145:459.
20. Reis PJ, Tunks DA and Sharry LF 1989. Corporation of abomasal and intravenous doses of [35 s} cysteine and 35 s methionine in the wool. J. Agri. Sci. 112, 313–319.
21. Sahlu T, Dawson LJ, Gipson TA, Hart SP, Merkel RC, Puchala R, Wang Z, Zeng S and Goetsch AL 2009. ASAS Centennial Paper: Impact of animal science research on United States goat production and predictions for the future. J. Anim.Sci. 87, 400–418.
22. Salehi M 2009. In Specified hair fibers. In Iranian mega guided project of animal skin and fibers. Edited by HR. Ansari – Renani, M Salehi, N Taheropur – Dari. Animal Science Research Institute of Iran, 285–286.
23. Salvin W 1968. Atomic absorpsiyon spectroscopy. Interscience Publication. New York. Chemical Analyses 25, 87-90.
24. SAS 2002. Proprietary Software, Version 9.0. Licensed to Suny at Stony Brook, Site 0013402001. Cary. NC. USA: SAS Ins.
25. Shamsaddini-Bafti M, Salehi M, Maghsoudi A, Tehrani AM, Mirzaei F and Momen SMS 2012. Effect of sex and rearing system on the quality and mineral content of fiber from raeini cashmere goats. J. of Anim. Sci. and Biotechnology. 3:20.
26. Syed Momen M, Fazaeli H, Salehi M, Beheshti M, Chegini SH, Ameri N and Ehsani M 2009. Study of two species atriplex Canescens and Lentiformis on the growth and fiber production in Raeini goats. Animal Science Research Institute of Iran, 77 pp.
27. Williams CL 2004. The effects of molybdenum, iron and sulphur on copper metabolism and physiology of sheep. Ph.D. Thesis. Harper Adams University College.
28. Xin GS, Longa RJ, Guo XS, Irvine J, Ding LM, Ding LL and Shanga ZH 2011. Blood mineral status of grazing Tibetian sheep in the Northeast of the Qighai-Tibetian Plateu. Livestock Science. 136, 102-107.

Original paper

THE INFLUENCE OF FATTENERS DRY AND LIQUID DIET ON SLAUGHTER TRAITS OF CARCASS SIDES

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Abstract

The study was conducted on 700 fattening pigs, three breed half blood with Duroc as a terminal breed ((Large White x Landrace) x Duroc). The pigs were divided into the two groups according to diet: dry and liquid nutrition. Each group consisted of 350 fattening pigs and used the same feed mixtures in prefattening (CP-3) and fattening (ST). During the period from 24.8 to 60kg they were fed with a CP-3, a crude protein content of 16.37%. During the period from 60kg until the end they were fed with ST, a crude protein content of 15.3%. Muscle tissue processed half-carcasses in slaughterhouses were determined by a device that determines the value of S (fat thickness) and M (muscle thickness) using "method one point." Fat thickness skin in mm, measured 7 cm lateral to the central (median) cutting, in the amount between the second and third ribs of the tail. The thickness of the muscle in mm was measured at the same place as the thickness of the bacon. The results show that the fatling fed dry food had significantly higher carcass weight (80.41: 78.51 kg, $p<0.05$), backfat thickness (16.55: 15.31 mm, $p<0.05$), weight (muscle 55.80: 53.82, $p<0.05$), but a lower percentage of meat (56.6: 57.3, $p<0.05$) as compared to pigs fed liquid food. In finishing pigs fed dry food, between carcass weight and backfat thickness and muscle thickness a positive and significant correlation (0.4267 and 0.4290, $p<0.05$) was found and between carcass weight and lean meat a significant negative correlation (-0. 4236 and $p<0.05$). Between backfat thickness and lean meat in the carcass a negative and significant correlation (-0.8534, $p<0.05$) was found and between muscle thickness and lean meat a positive and significant correlation (0.2857, $p<0.05$). In finishing pigs fed liquid food, between carcass weight and backfat thickness and muscle thickness a positive and significant correlation (0.1800 and 0.3705, $p<0.05$) was found and between carcass weight and lean meat a significant negative correlation (-0. 2178; $p <0.05$). Between backfat thickness and percentage of meat in the carcass negative and significant correlation (-0.8692, $p<0.05$) was found and between muscle thickness and lean meat a positive and significant correlation (0.3168, $p<0.05$).

Keywords: *carcass quality, dry diet, fattened, fat thickness, liquid diet, meat percentage*

Introduction

The importance of pig production in the Republic of Serbia is reflected in the possibility of utilization the existing capacities to produce pigs that in the previous period, due to fluctuations in market prices have remained empty. The potential is reflected in a shorter time period for renewal of livestock (Radović, 2010), using high-quality genetic material, breeding for leanness, modern technology and nutrition (Radović et al., 2012).

In the past, customer requirements were much more different than today's demands. Sometimes, great importance was given to lard and fatty parts of pork, unlike today, when more and more people look for the pure meat of superior quality. Of the total amount of meat produced in Serbia, the share of pork amounted to 57% or 269,000 tones (Statistical Yearbook of the Republic of Serbia, 2011), or 36.9 kg of pork per capita. According to Christensen et al. (2012), carcass leanness directly affects the market value of the meat, as well as NaSkO cost of fattening pigs (Flutura et al., 2010). Carcass quality traits vary influenced by genetic factors, the impact of race (Petrović et al., 2004; Pusic and Petrović, 2004; Petrović et al., 2006), the influence of fathers (Radović et al., 2007) and paragenetic environment factors (Kosovac et al., 2008), methods of breeding, age and weight of fattening pigs at slaughter (Radović et al., 2007; Kušec et al., 2008; Kuželov et al., 2011) castration, diet, season, EUA before, during or after slaughter (Karabasil et al., 2013).

Given that in recent years the cost of food has been constantly increasing and that for many years, preparing a meal for pigs was mainly guided towards meeting the needs of animals for energy and protein to improve daily gain of fattening pigs, there is an increasing interest in improving the quality of pork in recent years and growing need for harmonization of food technology, which would have a positive impact on the quality traits of carcass and meat (Kušec et al., 2010). The aim of this study was to assess the phenotypic variability of traits of carcass quality compared to dry and liquid feeding.

Materials and method

The study was conducted on 700 fattening pigs, genotype with Duroc as a terminal breed ((Large White x Landrace) x Duroc). The pigs were divided into two groups according to diet: dry and liquid nutrition. Each group consisted of 350 fattening pigs and used the same feed mixtures in pre-fattening (CP-3) and in fattening (ST). During the period from 24.8 to 60kg they were fed with a CP-3, a crude protein content of 16.37%. During the period from 60kg until the end they were fed with a ST, a crude protein content of 15.3%. The group liquid diet consumed CP-3 and ST mixed with water in 1:3.5. The average initial weight of fattening pigs was 24.8 kg and 100.5 kg of final. The average age at the beginning of fattening was 69 days. Total duration of fattening was 86 days. The average weight of finishing pigs fed dry food in the end was 100.5 kg and those fed with liquid food 99.9 kg.

The study included following traits: carcass weight, backfat thickness, thickness of muscle and meat content in carcass.

Share of muscles of the carcass processed in slaughterhouse was found by means of a device that determines the value of S (fat thickness) and M (muscle thickness) using "one point method". Fat thickness skin in mm was measured 7 cm lateral to the central (median)

plane cutting, in the amount between the second and third ribs of the tail. The thickness of the muscle in mm was measured at the same place as the thickness of the bacon. Descriptive statistics, analysis of variance and the association of these characteristics were performed in the statistical program Statistics 12 (StatSoft, Inc. 2005).

Results and discussion

Average values and variability in quality of carcass traits are shown in Table 1, where we can see that the fatteners fed dry food had higher carcass weight at the end of trial compared to pigs fed liquid food (80.4:78.5 kg). Analysis of variance was statistically significant at $P<0.05$. According to Sencic et al., 2005, final body weight in fattening is a genetically determined trait which in certain genotypes exists at a lower final weight, increasing the share of adipose tissue as a result of less efficient use of food. The same authors state that with very fleshy fatling, the limiting factor shifted to higher final body weight. In the same table we can see that the fatteners fed dry feed had a greater thickness of back fat (16.5: 15.3 mm) and the thickness of the muscle (55.8: 53.8 mm) as compared to the pigs fed liquid diet. Analysis of variance was statistically significant at $P<0.05$. According to Flutura et al. (2010), a difference that occurs in the thickness of the back fat and muscle is in a different efficiency of the use of food, which is prepared and distributed according to the application of certain diet technologies. Vincek et al. (2008) in their results highlight that the impact of the food treatment of fattening pigs greatly affects fat thickness and muscle. Kušec (2008) points out that feeding regimen represents a major biotic factor that directly affects the increase in appropriate circumstances which can fully exhibit genetic potential. The same author Kušec et al. (2010) states that a restrictive diet and installments positively increase carcass leanness.

In the same table it can be seen that the fattening swine fed liquid diet had a higher percentage of meat than fattening pigs fed dry diet, although they had a lower final weight and less thickness of the muscle (56.6: 57.3%). Analysis of variance was statistically significant at $P<0.05$. This research partly corresponds with the study of M. Petrović et al. (2006) in which a very weak negative correlation between the total mass and share of "France" processing of carcasses was considered, as well as research by Doeschl-Wilson et al. (2005) that highlights the impact of the final mass of the content of some parts of the hemisphere.

Table 1. *Average values and variability characteristics of carcass weight, backfat thickness, muscle thickness and carcass leanness*

Performance	Mean		Std.Dev.		Var.Coef.		Standard	
	dry food	liquid food	dry food	liquid food	dry food	liquid food	dry food	liquid food
Mass carcass, kg	80.4	78.5	8.44	7.49	10.50	9.54	0.451	0.400
Backfat thickness, mm	16.5	15.3	2.53	3.22	15.29	21.06	0.135	0.172
Muscle thickness, mm	55.8	53.8	6.66	6.96	11.94	12.93	0.356	0.372
Meatiness, %	56.6	57.3	2.56	2.78	4.52	4.85	0.137	0.149

The phenomenon of growth in pigs was intensively studied for a long time as the material basis of pig production. Given the complexity of the problem of growth the research was carried out with different approaches. According to Vincek et al. (2008) the most common is a temporal increase in the size which implies an increase in the body per time unit and the algometric approach which involves an increase in carcass parts (tissues or organs) in relation to the increase in weight of mercury. Therefore, according to the same authors, in this case, we often speak of the differential or relative growth.

Table 2. *Phenotypic correlation carcass weight, backfat thickness, muscle thickness and carcass leanness in finishing pigs fed dry food*

Performance	Mass carcass, kg	Mass carcass, kg	Muscle thickness, mm	Meatiness, %
Mass carcass, kg	1.0000			
Backfat thickness, mm	0.4627*	1.0000		
Muscle thickness, mm	0.4290*	0.0983	1.0000	
Meatiness, %	-0.4236*	-0.8534**	0.2857*	1.0000

*correlations are significant at p<0.05
**correlations are significant at p<0.01

Phenotypic correlation of carcass traits were different strengths (from very weak to complete) and signs (tables 2 and 3). In both groups of fattening pigs the positive correlation was demonstrated between carcass weight and backfat thickness and muscle (0.4627 and 0.4290, 0.1800 and 0.3705) at a significance level of p<0.05. This is consistent with the research of M. Petrović et al. (2006) which states that between net daily gain and hot carcass fat thickness a positive, weak to very weak correlation was determined. Between the thickness of the muscle and leanness, in both groups of fattening pigs positive correlation at a significance level of p<0.05 (0.2857 and 0.3168) is manifested.

Table 3. *Phenotypic correlation carcass weight, backfat thickness, muscle thickness and carcass leanness in finishing pigs fed liquid food*

Performance	Mass carcass, kg	Mass carcass, kg	Muscle thickness, mm	Meatiness, %
Mass carcass, kg	1.0000			
Backfat thickness, mm	0.1800*	1.0000		
Muscle thickness, mm	0.3705*	0.0672	1.0000	
Meatiness, %	-0.2178*	-0.8692**	0.3168*	1.0000

*correlations are significant at p<0.05
**correlations are significant at p< 0.01

The research of Petrović et al. (2006) states that the phenotypic correlation between fat thickness and meat content in carcass was complete, the negative and statistically

significant (-0.904 and -0.911) which is consistent with our results, where we found a negative phenotypic correlation, a complete and highly statistically significant (-0.8534 and -0.8692). The author also expressed phenotypic negative and significant correlation between carcass weight and leanness, in both groups of fattening pigs (-0.4236 and -0.2178, $p<0.05$). These results suggest that the application of certain diet technologies can influence the expression of genetic predisposition and finishing pigs as stated in the research by Kušec et al. (2010). Wrong diet technologies can cause greater deposition of fat rather than muscle tissue deposition. Vincek et al. (2012) states that according to the results obtained by monitoring the biological maximum and coefficient of asymmetry, during this period in dissected fatteners, they represent the basis for the definition of efficient mathematical model for predicting the growth of live weight and muscle tissue. This is why tests are aimed at testing the temporal and differential growth in order to obtain mathematical models for testing the impact on growth and development of carcass, where special emphasis is placed on the impact of technology of the diet (Vincek et al., 2008; Kušec et al., 2008).

Conclusion

The use of appropriate diet technology for fattening pigs, using the dry or liquid diet greatly affects achievement of greater leanness and thus of more effective expression of genetic predisposition in porkers by which the efficiency of production can be achieved.

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References

1. Doeschl-Wilson A B, Green DM, Fisher AV, Carrol SM, Schoefeld CP and Whittemore CT 2005. The relationship between body dimensions of living pigs and their carcass composition. Meat Sci. 70, 229-240.
2. Flutura S, Etleva D and Anila K 2010. The effect of pellet and granulated feed On production parameters of fattening pigs. Universitatea de Ştiinţe Agricole şi MedicinăVeterinarăIaşi. Lucrări Ştiinţifice - vol. 56, Seria Zootehnie pp. 359-363.
3. Karabasil N, Vasiljević M, Dimitrijević M, Vučinić M, Đorđević V, Ivanović J and Kureljušić J 2013. Examination of the transport of pigs to the slaughterhouse. Meat Technology 54, 1, 1-7.
4. Kosovac O, Živković B, Smiljaković T and Radović Č 2008. Quality of carcass sides, yield and distribution of certain tissues in carcasses from pigs fattened in conventional way and on deep litter. Vet. glasnik 62 (3-4) 179 – 188.
5. Kušec G, Kralik G, Djurkin I, Baulain U, Kallweit E 2008. Optimal slaughter weight of pigs assessed by means of the asymmetric S-curve. Czech J. Anim. Sci. 53, (3): 98–105.

6. Kušec G, Kralik G, Đurkin I, Vincek D, Petričević A and Baulain U 2008. Asymmetric S-Function In The Prediction Of Growth Characteristics Of Hybrid Pigs. Acta agriculturae Slovenica, suplement 2, 173–179.
7. Kušec G, Baulain U, Đurkin I, Lukić B, Kralik G and Petričević A 2010. Influence of feeding on slaughtering traits of hybrid pigs. Krmiva 49, Zagreb, 5; 247-252.
8. Kuzelov A, Taskov N, Angelkova T, Atanasova A and Mladenov M 2011. Impact of live weight on the quality of pigs Halves and meat of the large white breed. Biotechnology in Animal Husbandry 27 (3), 819-824.
9. Petrović M, Pušić M, Radojković D, Mijatović M, Radović Č, Živković B 2006. Fenotipska i genetska varijabilnost osobina kvaliteta polutki i mesa. Biotechnology in Animal Husbandry 22 (5-6), 1-10.
10. Radović Č, Petrović M, Živković Ž, Kosovac O, Radojković D, Mijatović M and Stojanović Lj 2007. The influence of various factors on fixed carcass quality of pigs. Biotechnology in Animal Husbandry 23 (1-2), 71-80.
11. Radović I 2010.Conditions pig production in Serbia and the results of selection measures for improving pig production in Vojvodina. Proceedings of the Eighth Symposium on "Health care, selection and reproduction of pigs", Silver Lake, 03-05.June, p.12-22nd
12. Radović I, Kosovac O, Živković B, Petrović M, Mihal F and Radović Č 2007. Uticaj starosti tovljenika pri klanju na klanične vrednosti svinja rase švedski landras. Savremena poljoprivreda, 56 (1-2), 25-31.
13. Radović I, Trivunović S, Stančić I, Stančić B, Dragin S and Urošević M 2012. The Feed Conversion, Daily Gain, Average Backfat Thickness and Meat Percentage in Perfomance Test of Landrace Boars (Genotypic Parameters). Contemporary Agriculture ISSN 0350-1205, 61(1-2), 44-53.
14. Radović I, Trivunović S and Gvozdenović N2011. Results of Feed Conversion, Daily Gain Average Thickness of the Back and Side Bacon and Percent of Meat in Performance Test of Boars Landrace Race (Phenotypic Parameters). Contemporary Agriculture, ISSN 0350-1205, 60(3-4), 348-358.
15. Statistički godišnjak R. Srbije, 2011
16. StatSoft, Inc. (2005): STATISTICA (data analysis software system), version 12.0 www.statsoft.com.
17. Uzelac Z, Radović I and Kragić S 2008. Povezanost mase polutke i procenta mesa u polutki = Relationship betwen volume of carcass and per cent of meat in carcass.Zbornik kratkih sadržaja, Simpozijum 'Stočarstvo, veterinarska medicina i ekonomika u proizvodnji zdrastveno bezbedne hrane', Herceg Novi 22-29.jun, str. 94.
18. Vincek D, Đurkin I, Kralik G, Petričević A, Baulain U and Kušec G 2008. Utjecaj hranidbenog tretmana na karakteristike rasta svinja. Krmiva 50, Zagreb, 2; 63-72.
19. Vincek D, Sabo K, Kušec G, Kralik G, Đurkin I and Scitovski R 2012. Modeling of pig growth by S-function – least absolute deviation approach for parameter estimation. Archiv Tierzucht 55 (2012) 4, 364-374.

Original paper

TRANSFER OF AFLATOXIN M1 FROM THE CONTAMINATED MILK INTO CHEESE AND WHEY

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Abstract

Aflatoxin M1 is a major metabolite of aflatoxin B1 which is formed when animals ingest contaminated feed. Aflatoxin B1, when ingested by an animal, is rapidly absorbed in the gastrointestinal tract and transformed into a metabolite aflatoxin M1, appearing in the blood after 15 minutes and then secreted in the milk from the mammary gland. Aflatoxin B1 shows hepatotoxic and carcinogenic effects and aflatoxin M1 has a distinct genotoxicity, carcinogenicity and cytotoxicity. The resistance to heat treatment and mild acidic conditions used in the production of cheese or other dairy products (such as, for example, yogurt, cream, butter, and ice cream) has been accounted for the contamination of such products. It is known that aflatoxin M1 is bound to milk proteins, mainly casein, and therefore the toxin is more concentrated in the cheese than in the milk used in cheese production. In practice, aflatoxin M1 can be found in dairy products at levels that are 2-5 times higher than in milk. As a result of the binding of aflatoxin M1 to milk proteins the toxin is distributed more in curd than in whey.

Control of samples of milk from the Vojvodina market was conducted during March and April of 2014, in the Dairy Laboratory and Laboratory for the Analysis of Animal Feed and Animal Products, the Department of Animal Science, Faculty of Agriculture, Novi Sad. The method used was HPLC with fluorescence detection with pre-treatment of milk on immunoaffinity columns. The analysis of milk was performed on milk of 12 dairies whose products can be found on the Vojvodina market. The manufacturer with the highest levels of aflatoxin M1 in milk was chosen and a semi-hard cheese was made of such milk.

The results showed the expected increase of concentration of aflatoxin M1 in cheese and whey. Since Serbia has no regulation that determines the minimum allowable level of aflatoxin M1 in cheese and other dairy products, the results were compared with acceptable values for cheese in some European countries.

Keywords: *aflatoxin M1, cheese, HPLC, milk, whey*

Introduction

Aflatoxins (AFS) are highly toxic secondary metabolites produced by Aspergillus, Penicillium and Rhizopus strains in cultures of herbal products, of which aflatoxin B1 (AFB1) is the most representative (Goldblatt, 1969; Rubio et al., 2011). If AFB1 is present in animal feed during lactation the animals excrete aflatoxin M1 (AFM1) in their milk (Allcroft and Carnaghan, 1963; Cupid et al., 2004; Battacone et al., 2005). It is a toxin classified by the International Agency for Research on Cancer as a possible cause of cancer (IARC, 2002).

Given AFM1 has an impact on the health of consumers through insertion of milk, according to the Codex Alimentarius Commission, the maximum determined recommended concentration is 500 ng kg-1 (Codex Alimentarius Commission, 2001) although the limit (50 ng kg-1) for raw milk, milk products and heat-treated milk is given by regulations in the European Union (EU Commission Regulation, 2003). As for the cheese, only in a few countries (Netherlands, Switzerland, Austria, Turkey and Italy) there are prescribed boundaries within the range of 200-450 ng kg-1 for the presence of AFM1. In Serbia, there are no regulations to control the presence of AFM1 in dairy products.

When the cheese is produced from AFM1-contaminated milk, this toxin will probably be present in the cheese made from that milk. This could be explained by the fact that AFM1 is bound to casein (Applebaum et al., 1982; Battacone et al., 2005) and increase dry matter content (Deveci, 2007).

The affinity of AFM1 was tested not only for this protein, but also for others, such as whey proteins (Mendoca and Venancio, 2005). Therefore, it is necessary to note whether AFM1 is present in the final products such as cheese, and its concentration therein is approximately 2.1 to 4.5 times higher than in the original milk used for cheese production, depending on the cheese type (Van Egmond, 1983; Viseman and Marth, 1983; Blanco et al., 1988; Deveci, 2007; Manetta et al., 2009). Yet in a variety of products, such as yogurt, level of AFM1 is stable and does not affect the production of yogurt (Blanko et al., 1993). Accordingly, AFM1 may also be present in other dairy products such as whey and products obtained from whey.

AFM1 transfer in the amount of 40-60% was recorded in the whey in relation to the AFM1 which is present in milk (Govaris et al., 2001; Oruc et al., 2006; Deveci, 2007; Kamkar et al., 2008; Manetta et al., 2009). However, this scope can be higher, between 70% and 74% (Battacone et al., 2005), or much lower, between 17% and 27% (Lopez et al., 2001). This wide range of AFM1 distribution in cheese production could be due to different factors; for example, techniques used prior to HPLC analysis (Battacone et al., 2005; Kamkar et al., 2008), which is associated with the size of the sample or solvent extraction. Characteristics of the production process, depending on the type of cheese, whether it is hard (Brackett and Marth, 1982; Blanco et al., 1988; Manetta et al., 2009) or soft cheese (Viseman and Marth, 1983; Govaris et al., 2001; Oruc et al., 2006) also may affect the distribution of AFM1. In addition, the type of contamination of milk should also be taken into account, because when milk is naturally contaminated (Virdiset et al., 2008), AFM1 values are usually lower than in the artificially contaminated milk (Deveci, 2007).

This paper aims to investigate the presence of AFM1 in milk and its distribution in milk products that can be found on the market of AP Vojvodina.

Materials and methods

The analysis of chemical composition (fat, protein, lactose and total solids and somatic cells) was done for all samples of milk from the market on the CombiFoss FT + machine (FossElectric, Hillerød, Denmark) calibrated using the certified standards which combine MilkoScanTM FT + (infrared spectrometric analysis), previously calibrated for cow's milk, with FossomaticTM FT + (counting somatic cells based on flow cytometry), in Dairy Laboratory at the Department of Animal Science, Faculty of Agriculture in Novi Sad.

Detection of presence of AFM1 in the milk samples was performed by standard HPLC high performance liquid chromatography in the Laboratory for the Analysis of Animal Feed and Animal Products to the Department of Animal Science, Faculty of Agriculture in Novi Sad.

Milk samples, in which the highest level of AFM1 was detected, were used for production of semi-hard cheese. Cheese production took place using the standard recipe for the production of semi-hard cheese in Dairy Laboratory at the Faculty of Agriculture in Novi Sad. Cheese was made from milk from 6 diaries where AFM1 levels were the highest and where three cheeses per diary were made. Process for the production of cheese was carried out as follows: contaminated milk heated at a temperature of 35 °C, $CaCl_2$, cultures and rennet are added. The renneting of milk is followed by further course of standard technological operations and manipulations such as coagulation, curd draining and pressing to obtain the product. In the production of cheese the whey is separated from the curd.

The obtained cheese and whey were further analyzed for the presence of AFM1, using standard high performance liquid chromatography - HPLC, Agilent Technologies 1260 Infinity LC system, a Hypersil ODS column, with FLD detector and a mobile phase of acetonitrile-water (v/v, 25:75).

Testing: Cuvette for centrifuge with volume of 50 cm^3 was filled with a sample of milk to the top and heated up to a temperature between 37-40 °C, about 30 minutes. Then the milk sample is shaken for fat to disperse, and cuvettes with the samples are centrifuged at 3000 rotations/min for 10 minutes. The layer of fat was removed, and the sample is filtered through a quantitative filter paper (black belt). After that, 50 cm^3 of the filtrate is heated at 37 °C for about 10 minutes. The sample was passed through immunoaffinity column by free fall. The column is washed with 20 cm^3 of deionized water and eluted with approximately 4 cm^3 of acetonitrile. The contents of the tube are evaporated in the thermoblock at 60 °C in a stream of nitrogen. Then, add 200 µl of n-hexane and 200 µl trifluoroacetic acid and stir the vortex for about 1 minute. The contents of the tubes were then incubated in the thermoblock 40 °C exactly 10 minutes, after which a stream of nitrogen at the same temperature evaporate it to dryness. The residue was then dissolved in 300 µl mobile phase by vortex mixing for about 1 minute and transferred to an HPLC vial.

Chromatography was performed on a C-18 column size 4.6 x 100 mm, 5 mm, at a flow rate of the mobile phase (acetonitrile-water (v/v, 25:75)) of 1 ml/min and a column temperature of 40 °C. Injection volume is 20 µl. The detection is performed using FLD detector at the following wavelengths: 365 nm excitation, 440 nm emission.

For a proper interpretation of the results, analysis of variance (ANOVA) and Tukey's post-hoc test were used for significance between the observed characteristics, while the coefficient of correlation was determined using the Pearson test, in the Statistica 12 software.

Results and discussion

Table 1 shows the results of the analysis of the samples of milk and dairy products that were monitored on the market of AP Vojvodina for the presence of AFM1.

Table 1. *Results of analysis of milk and milk products from AP Vojvodina market on AFM1*

Dairy No.	Product	Fat%	Aflatoxin M1 (ppb) µg/kg
1.	Pasteurized milk	2.8	0.048
1.	Yoghurt	2.8	0.000
2.	Yoghurt	2.8	0.209*
2.	Pasteurized milk	2.8	0.150*
2.	Trapist cheese	min 45%	0.478*
2.	Pasteurized milk	2.8	0.124*
2.	Pasteurized milk	2.8	0.124*
3.	Pasteurized milk	2.8	0.075*
3.	Yoghurt	2.8	0.035
3.	Yoghurt	2.8	0.000
4.	Pasteurized milk	2.8	0.021
4.	Yoghurt	2.8	0.039
4.	Cheese made using ultrafiltration	min 45%	0.145*
5.	Pasteurized milk	2.8	0.127*
5.	Yoghurt	2.8	0.094*
5.	Cheese made using ultrafiltration	min 45%	0.281*
6.	Sour milk	2.8	0.028
6.	Pasteurized milk	2.8	0.070*
6.	Trapist cheese	min 45%	0.233*
7.	Sour milk	2.8	0.074*
8.	Pasteurized milk	2.8	0.019
9	Pasteurized milk	2.8	0.113*
10	Pasteurized milk	2.8	0.046
11	Pasteurized milk	2.8	0.070*
12	Pasteurized milk	2.8	0.000

* Levels of AM1 exceeding maximum allowed concentration

Numbers from 1-12 represent dairies that sell milk and milk products on the market of AP Vojvodina and whose products are analyzed.

Based on the results of analysis of samples of pasteurized milk, milk with a high concentration of AFM1 from the dairies: 2, 3, 5, 9, 10, 11, fermented cheese and whey, which are further analyzed for the presence of AMF1 and thereby analyzed for the transfer of toxins from milk into milk products.

Table 2. *Results of analysis of cheese and whey from dairies No. 2, 3, 5, 9, 10, 11*

Dairy No.	Product	Fat%	Aflatoxin M1 (ppb) µg/kg
2.	Pasteurized milk	2.8%	0.124*
2.	Cheese from pasteurized milk, dairy No. 2		0.296
2.	Whey separated from curd of cheese made from pasteurized milk, dairy No. 2		0.047
3.	Pasteurized milk	2.8%	0.075*
3.	Cheese from pasteurized milk, dairy No. 3		0.160
3.	Whey separated from curd of cheese made from pasteurized milk, dairy No. 3		0.019
5.	Pasteurized milk	2.8%	0.127*
5.	Cheese from pasteurized milk, dairy No. 5		0.303
5.	Whey separated from curd of cheese made from pasteurized milk, dairy No. 5		0.030
9.	Pasteurized milk	2.8%	0.113*
9.	Cheese from pasteurized milk, dairy No. 9		0.339
9.	Whey separated from curd of cheese made from pasteurized milk, dairy No. 9		0.035
10.	Pasteurized milk	2.8%	0.046
10.	Cheese from pasteurized milk, dairy No. 10		0.181
10.	Whey separated from curd of cheese made from pasteurized milk, dairy No. 10		0.018
11.	Pasteurized milk	2.8%	0.070*
11.	Cheese from pasteurized milk, dairy No. 11		0.281
11.	Whey separated from curd of cheese made from pasteurized milk, dairy No. 11		0.027

* Levels of AM1 exceeding maximum allowed concentration

Based on the obtained results, we can perceive that out of 25 analyzed samples of milk and milk products from 12 manufacturers placing their products on the market in the territory of AP Vojvodina the AFM1 was detected in 23 samples. Of the total number of 14 samples of pasteurized milk samples 10 samples have been contaminated with AMF1 in concentration above 0.05 mg/kg, which is specified as the maximum permissible concentration according to the current Regulation on amendments to the Regulation on the maximum level of residues for protection found in food products and feed and the animal food and feed which are determined as the maximum allowable amount of residues found in plant nutrition (Official Gazette of RS, 2014). Seven fermented milk products were analyzed, and in six samples the presence of aflatoxin M1 is evident, while three samples have levels of aflatoxin M1 above 0.05 mg/kg. Further, in the four analyzed samples of cheese, all four had high levels of aflatoxin M1, although in Serbia there are no legal regulations that restrict the presence of toxins in the cheese and in other milk products where their presence is evident and sometimes have several times higher concentration than in milk from which it has been produced.

At the global level, the maximum allowed level of aflatoxin M1 in cheese differs as it is presented in Table 3.

Table 3. *The maximum permitted level of aflatoxin AFM1 in cheese in different countries (Anfossi et al., 2011)*

Country	Maximum permitted level (ng/kg)
Argentina	500
Austria	250
Switzerland	250
Egypt	0
Honduras	250
Italy	250 (450*)
Romania	0
Netherlands	200
Turkey	250

* Level of AFM1 in hard cheeses

Most countries have set a limit and decided for the maximum permissible level of 250 ng/kg, which corresponds to the assumption that the cheese is made from milk which is in accordance with the regulations (ie. contaminated at levels below 50 ng/kg) and the concentration of AFM1 can grow up to 5-times due to dehydration. However, some countries have opted for a strategy of zero tolerance (Romania and Egypt) to ensure maximum protection of the health of consumers at the expense of producers of milk and cheese. By contrast, in 2004, Italy raised the limit of AFM1 in hard cheese to 450 ng/kg to protect the production of Parmesan cheese which in that year had big problems due to a high contamination of animal feed with AFB1.

In all six of the analyzed samples of cheese made in the Laboratory of contaminated milk with a high concentration of AFM, there were detected two to three times higher levels than the concentration of AFM1 in milk.

In the samples of cheese made from milk of dairy No. 2, the concentration of AFM1 doubled and was 0.296 mg/kg compared to the concentration of AFM1 in milk of 0.124 mg/kg ppb. The whey, which is obtained during the production of cheese from contaminated milk, was also analyzed and the presence of AFM1 was detected at the concentration of 0.047 mg/kg.

In the samples of cheese made from milk produced in dairies No. 3 and 5, the concentration of AFM1 detected was 2.13 and 2.3 times higher in relation to the milk from which they were made and amounts to 0.160 mg/kg and 0.303 mg/kg. In the sample of cheese made from milk of dairy No. 9, AFM1 concentration was three times higher than in milk from which it was made and amounts to 0.339 mg/kg.

In the cheese sample from milk of dairy No. 10, the increase of AFM1 concentration was 0.181 mg/kg compared to the concentration found in milk which was used for manufacturing the same cheese being 0.046 mg/kg. Whey, which is obtained during the production of the cheese samples from contaminated milk had AFM1 at the concentration of 0.018 mg/kg. It has been noted that the concentration in sample cheese from 11th dairy is three times higher than

in milk from which it was made - AFM1 concentration was 0.07 mg/kg, and in cheese from that milk was 0.281 mg/kg.

Based on the results of analysis of whey samples it can be seen the transfer of AFM1 from milk to whey in the amount of 23.6% to 38.5% and ranges from 0.018 mg/kg to 0.046 mg/kg in the analyzed samples of whey.

Software for statistical analysis of the data Statistica 12 shows the correlation between milk and cheese as well as the correlation between milk and whey and based on the results observed in both cases there is a high correlation between the studied parameters ($r = 0.76$ and $r = 0.80$) with statistically significant difference $p \leq 0.01$.

Conclusion

Based on the results obtained in this study it is evident that 2-3 times increase in the concentration of AFM1 in cheese is in relation to contaminated milk from which it is produced. The above facts should be the guidelines to initiate the establishment of regulations concerning the allowable limit of aflatoxin M1 in cheese and other dairy products in the Republic of Serbia.

The results of research in this paper also point to failures and lack of control of animal feed, which is indirectly reflected in the distortion - in this case the quality of milk and milk products that are used in human nutrition. These results are worrying because we must take into account that the effect of AFM1 is toxic and carcinogenic to human health.

This is the reason why we should systematically take all measures for the production of healthy food for the animals which would provide production of milk and milk products within the permissible values for the member states of the European Union.

References

1. Allcroft, R., Carnaghan, R.B.A. 1963. Groundnut toxicity: an examination for toxin in human food products from animal fed toxic groundnut meal. Veterinary Research 75, 259-263.
2. Anfossi, L., Baggiani, C., Giovannoli, C., Giraudi, G. 2011. Occurrence of Aflatoxin M1 in Dairy Products, Aflatoxins - Detection, Measurement and Control, Dr Irineo Torres-Pacheco (Ed.), ISBN:978-953-307-711-6, InTech
3. Applebaum, R., Brackett, R.E., Wiseman, D.W., Marth, E.H. 1982. Aflatoxin: toxicity to dairy cattle occurrence in milk and milk products. Journal of Food Protection 45, 752-777.
4. Battacone, G., Nudda, A., Palomba, M., Pascale, M.,Nicolussi, P., Pulina, G. 2005. Transfer of aflatoxin B1 from feed to milk and from milk to curd and whey in dairy sheep fed artificially contaminated concentrates. Journal of Dairy Science 88, 3063-3069.
5. Blanco, J.L.,Carrión, B.A., Liria, N., Díaz, S., García, M.E., Domínguez, L., Suárez, G. 1993. Behaviour of aflatoxins during manufacture and storage of yoghurt. Milchwissenschaft 48, 385-387.
6. Blanco, J.L., Domínguez, L., Gómez-Lucía, E., Garayzabal, J.F.F., Goyache, J., Suárez, G. 1988. Behaviour of aflatoxin during manufacture, ripening and storage of Manchego-type cheese. Journal of Food Science 53, 1373-1376.

7. Brackett, R.E., Marth, E.H. 1982. Fate of aflatoxin M1 in Cheddar cheese and in process cheese spread. Journal of Food Protection 45, 549-552.
8. Codex Alimentarius Commission 2001. Comments submitted on the draft maximum level for aflatoxin M1 in milk. Codex committee on food additives and contamination 33rd session, The Hague, The Netherlands.
9. Cupid, B.C., Lightfoot, T.J., Russel, D., Gant, S.J.,Turner, P.C., Dingley, K.H. 2004. The formation of macromolecular adducts in rat and human at dietary levels of exposure. Food and Chemical Toxicology AFB1 42, 559-569.
10. Deveci, O. 2007. Changes in the concentration of aflatoxin M1 during manufacture and storage of White Picked cheese. Food Control 18, 1103-1107.
11. European Commission, Commission Regulation No.2174/2003 of 12 December 2003, amending Regulation (EC) No 466/2001 as regards aflatoxins. Official Journal of the European Community L326, 12-15.
12. Goldblatt, L.A. 1969. Aflatoxin. Scientific Background, Control and Implications. L.A. Goldblatt (Ed.),Academic Press, New York and London, pp 1-54.
13. Govaris, A., Roussi, V., Koidis, P.A., Botsoglou, N.A. 2001. Distribution and stability of aflatoxin M1 during processing, ripening and storage of Telemes cheese. Food Additives and Contaminants 18, 437-443.
14. International Agency for Research on Cancer (IARC) 2002. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. IARC Monographs Vol.82, Lyon, France.
15. Kamkar, A., Karim, G., Shojaee Aliabadi, F., Khaksar, R. 2008. Fate of aflatoxin M1 in Iranian white cheese processing. Food and Chemical Toxicology 46, 2236-2238.
16. López, C., Ramos, L., Ramadán, S., Bulacio, L., Perez, J. 2001. Distribution of aflatoxin M1 in cheese obtained from milk artificially contaminated. International Journal of Food Microbiology 64, 211-215.
17. Manetta, A.C., Giammarco, M., Di Giuseppe, L., Fusaro, I., Gramenzi, A., Formigoni, A., 19. Mendoça, C., Venâncio, A. 2005. Fate of aflatoxin M1 in cheese whey processing. Journal of the Science of Food and Agriculture 85, 2067-2070.
18. Official Gazette of RS 72/14, 2014. Regulation on the maximum level of residues for protection found in food products and feed and the animal food and feed which are determined the maximum allowable amount of residues found in plant nutrition
19. Oruc, H.H., Cibik, R., Yilmaz, E., Kalkanli, O. 2006. Distribution and stability of aflatoxin M1 during processing and ripening of traditional white pickled cheese. Food Additives and Contaminants 23, 190-195.
20. Rubio, R., Moya, V.J., Berruga, M.I., Molina, M.P. 2011. Aflatoxin M1 in the intermediate dairy products from Manchego cheese production: distribution and stability; in the intermediate dairy products, Mljekarstvo 61 (4), 283-290 283.
21. Van Egmond, H.P. 1983. Mycotoxins in dairy products.Food Chemistry 11, 289-307.
22. Vignola G., Lambertini L. 2009. Distribution of aflatoxin M1 during Grana Padano cheese production from naturally contaminated milk. Food Chemistry 113, 595-599.
23. Virdis, S., Corgiolu, G., Scarano, C., Pilo, A.L., De Santis, E.P.L. 2008. Occurrence of Aflatoxin M1 in tankbulk goat milk and ripened goat cheese. Food Control 19, 44-49.
24. Wiseman, D.W., Marth, E.H. 1983. Behaviour of aflatoxin M1 during manufacture and storage of Queso Blanco and Backers' cheese. Journal of Food Protection 46, 910-913.

Original paper

INFLUENCE OF KEEPING AND MILKING OF COWS ON THE HYGIENIC QUALITY OF MILK

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Abstract

The quality and quantity of milk is significantly influenced by housing conditions, care and feeding of dairy animals. Hygienic correct milk can be obtained if the cows or other dairy cattle are kept in hygienic conditions. The aim of this study was to investigate the effect of housing conditions and milking of cows on the microbiological and chemical quality of milk. We examined six samples of bulk tank milk cows from 6 different farms from the Municipality of Podgorica, where cow milking is done by machines. Chemical parameters of milk - fat, protein, lactose, free fat dry matter and somatic cells were investigated on the device Combi-Foss (Foss Electric, Denmark), and the total number of bacteria on the appliance BactoScan. All obtained data were processed using modern variation statistics. Statistical parameters: mean value, standard deviation, maximum and minimum values, are calculated. In order to assess the hygienic conditions of keeping and milking of cows at these farms a certain building, microclimate and hygienic parameters were studied - by modified method of Hristov and Reljić (2009). Based on the established state, each test parameter is rated from 0 to 5 points. Average value of the fat content in bulk tank milk was 3.39 % - ranged from 2.92 % to 3.94 %, protein content 3.13% - ranged from 2.78 % to 3.65 %, lactose 4.27% - ranged from 4.14 % to 4.47%, the fat free dry substance 8.13 % - ranged from 7.75 % to 8.77 %. The value of the number of somatic cells in bulk tank milk at the first farm was 52000/mL, second 85000/mL, third 63000/mL, fourth 1920000/mL, fifth 1373000/mL and sixth 200000/mL. The total bacteria count in the bulk tank milk at the first farm amounted to 5000/mL, second 41000/mL, third 124000/mL, fourth 1002000/mL, fifth 467000/mL and sixth 31000/mL. Physical, microclimate and hygienic parameters of dairy cows housing are rated with good rating on farms 1, 2, 3, 4 and 6 and on the farm 5 with a sufficient rating. The fourth and fifth farms did not use disinfection of teats after milking and disinfection of milking machines. The fourth and fifth farm had significantly higher number of somatic cells in bulk tank milk. The fifth farm had significantly higher total number of bacteria/mL in bulk tank milk - compared to the number that is permitted under applicable regulations. This can be linked with inadequate building, microclimate and hygiene of cows and failing disinfection of the udder and milking machine after milking.

Key words: *hygiene, milk, milking, somatic cells, total bacteria count*

Introduction

Milk is a food of high nutritional value only if it is obtained from healthy animals. Proper breeding, keeping and exploitation of dairy animals provide a healthy product and production of hygienic proper milk. The main sources of milk contamination by microorganisms are diseased udders, udder skin, air, equipment for milking and storage of milk, and man. When leaving the mammary glands of healthy animals the milk contains a small number of non-pathogenic microorganisms - to 300/mL originating from teat canal. Postsecretory level of contamination of milk depends on the way and milking hygiene and procedure with milk after milking. The number of somatic cells in milk of cows directly indicates the state of health of the mammary gland. The number of somatic cells in milk is influenced by numerous factors, such as factors that cause mastitis (pathogen microorganisms, toxins), physiological and pharmacological factors (stage of lactation, breed), stressful factors (changes in food, transportation, housing conditions, method of milking). Healthy mammary glands of milk contain polymorphonuclear leucocytes, macrophages, lymphocytes, erythrocytes and epithelial cells. In the case of mastitis, the number of polymorphonuclear leukocytes is increased and may be up to 100 % of the total cells in milk. The average number of somatic cells in milk from healthy udders is 50 000/mL and in most cases it is less than 150 000/mL (Katić et al., 2003.). Changes in the composition of milk caused by mastitis affect its suitability for technological processing. Milk of cows with mastitis has a reduced percentage of free fat dry matter, casein, lactose, sodium, calcium, magnesium and an increased amount of chloride, serumalbumines, serumglobulines, and increased pH. In the production of cheese from milk with high somatic cell count a coagulation time is prolonged while the yield and sustainability of cheese is reduced.

Given the importance of production proper hygienic milk in human nutrition, the aim of our study was to investigate the effect of keeping and milking hygiene of cows on the microbiological, chemical quality and the number of somatic cells in milk.

Materials and methods

We have examined six bulk tanks milk samples of cows from six farms of the Municipality of Podgorica, where milking cows is done by machines. Milk samples were collected in sterile plastic containers with an added preservative. Immediately after taking, the samples were placed in the hand refrigerator at a temperature of 4-5^{0}C and transported to the laboratory in 12 hours maximally. Milk samples were tested for the following parameters: fat content, protein content, lactose content, free fat dry matter content, number of somatic cells and the total number of bacteria. Determination of chemical parameters and the number of somatic cells in bulk tank milk of cows was done on the machine Combi - Foss (Foss Electric, Denmark) and the total number of bacteria on the appliance BactoScan. All data were analyzed using modern variation statistics. The following statistical parameters were calculated: mean value, standard deviation, maximum value and minimum values.

In order to assess the hygienic conditions of keeping and milking the cows were determined visually by some space, microclimate and hygienic parameters, using modified method of Hristov and Reljić (2009).

Rating of spatial conditions was performed by measuring the dimensions of stables, bearings, windows, doors and outlets. We have taken into account the position of the stables, the type

and condition of ventilation, as well as the characteristics of the material for the construction of walls, ceilings and floors, their current status of functionality and possible damage, and the type and amount of litter. Evaluation of sanitation was carried out on the basis of visual assessment of hygiene litter, bearings, floors, walls, ceilings, windows, outlets, feeders, drinkers, channels and manure corridors and ventilation ducts. Assessment of microclimate conditions was performed using the following parameters: dust, noise, ventilation, air circulation, harmful gases and illumination. Based on the established state of each test parameter was evaluated with a minimum of 0 to a maximum 5 points (5 - excellent, 4 - very good, 3 - good, 2 - satisfactory, 1 – insufficient - there are resources to improve, 0 – insufficient - no resources to improve). Dividing the total number of points with the number of estimated parameters we obtained final rating of a minimum 0 to a maximum 5 (evaluation is done as follows: 0 - 1.99 insufficient, 2.00 - 2.49 sufficient, 2.5 - 3.49 good, 3.5 - 4.49 very good and 4.5-5.00 excellent).

Results and discussion

Results of chemical composition of bovine bulk tank milk are shown in Table 1.

Table 1. *The results of examination of chemical and microbiological parameters and the number of somatic cells in bovine bulk tank milk samples*

Farm	Fat content (%)	Protein content (%)	Lactose content (%)	Free fat dry matter (%)	Freezing point	Somatic cells number	Total bacteria number
1	3.32	3.18	4.47	8.38	0.534	52000	5000
2	2.92	2.91	4.14	7.79	0.517	85000	41000
3	3.32	2.96	4.17	7.86	0.525	63000	124000
4	3.65	3.65	4.39	8.77	0.531	1920000	1002000
5	3.19	3.28	4.24	8.25	0.524	1373000	467000
6	3.94	2.78	4.24	7.75	0.514	200000	31000
Average	3.39	3.13	4.27	8.13	0.524	615500	18000
Max	3.94	3.65	4.47	8.77	0.534	1920000	1002000
Min	2.92	2.78	4.14	7.75	0.514	52000	5000
Sd	0.357	0.314	0.128	0.404	0.007	818826.7	18.385

The results in Table 1 show that in the bulk tank milk samples fat content varied from 2.92 % to 3.94 %, protein content from 2.78 % to 3.65 %, and the content of lactose from 4.14 % to 4.47%. The content of free fat dry matter varied from 7.75 % to 8.77 %. Freezing point varied from 0.514 to 0.534 and the number of somatic cells from 52000 to 1920000/mL. The number of bacteria varied from 5000 to 1002000/mL of milk.

The results of evaluation of the stable spatial parameters of cows keeping are shown in Table 2.

Table 2. *Results of evaluation of the stable spatial parameters of cows keeping*

Farm	**1**	**2**	**3**	**4**	**5**	**6**
Stable	4	4	5	3	3	5
Bearing	3	3	4	2	2	4
Hallway nutrition	2	3	3	1	2	4
Feeding equipment	3	4	4	3	3	4
Equipment for drinking	4	4	4	3	4	4
Hallway manure	3	4	3	2	1	3
Channel manure	3	2	3	3	2	3
Outlet	4	3	3	3	4	2
Ventilation	2	3	2	1	0	4
Windows	3	4	3	1	1	3
Doors	3	4	2	1	1	3
Walls	4	3	4	2	2	4
Roof	1	2	2	0	1	2
The total number of points	39	43	42	25	26	45
Evaluation	3.00	3.30	3.23	1.92	2.00	3.46
Descriptive rating	good	good	good	insufficient	sufficient	good

Results in Table 2 show that the evaluation of the spatial parameters were insufficient on farm 4, sufficient on farm 5 and good on other farms: 1, 2 , 3 and 6.

The assessment results of microclimate conditions in the stables for keeping the cows are shown in Table 3.

Table 3. *Results of evaluation of microclimate conditions in the stables for cows*

Farm	1	2	3	4	5	6
Dust particles	3	2	3	3	3	3
Noise	4	3	2	4	3	4
The air flow	2	4	3	2	1	3
Hazardous gases	2	2	4	2	2	2
Illumination	2	3	2	3	2	4
The total number of points	13	14	14	14	11	16
Evaluation	2.6	2.8	2.8	2.8	2.2	3.2
Descriptive rating	good	good	good	good	sufficient	good

Rating of microclimate conditions was sufficient on the farm number 5 and good on the other farms.

The assessment results of hygienic parameters in the stables for keeping cows are shown in Table 4.

Table 4. *Results of evaluation of hygiene in stables for cows*

Farm	1	2	3	4	5	6
Hygiene bearings	5	4	4	2	2	4
Hygiene litter	4	3	4	2	1	5
Hygiene drinkers	5	4	5	3	3	4
Hygiene hall for food	3	3	4	3	2	3
Hygiene discharge	3	4	4	4	3	4
Hygiene window	4	3	3	3	2	3
Hygiene walls	3	4	3	3	2	3
Hygiene roof	4	5	3	3	2	4
Total number of points	31	30	30	23	17	30
Evaluation	3.87	3.75	3.75	2.87	2.12	3.75
Descriptive rating	very good	very good	very good	good	sufficient	sufficient

The results presented in Table 4 show that the evaluation of the hygienic conditions in the stables was sufficient on the fifth farm, good on the fourth farm and very good on the other farms. The lowest rating (rating 2) on the fourth farm had parameters for hygiene of bearings and litter. On the fifth farm a litter hygiene was evaluated the lowest rating (rating 1).

The results of total evaluation of accommodation conditions on the farms are shown in Table 5:

Table 5. *Total rating of accommodation conditions on tested farms*

Evaluated parameters	Farm					
	1	2	3	4	5	6
Spatial	3.00	3.30	3.23	1.92	2.00	3.46
Microclimatic	2.60	2.80	2.80	2.80	2.20	3.20
Hygienic	3.87	3.75	3.75	2.87	2.12	3.75
Rating	3.15	3.28	3.26	2.53	2.10	3.47
Descriptive rating	good	good	good	good	sufficient	good

The results presented in Table 5 show that the rating of the accommodation conditions for cows on farms 1, 2, 3, 4 and 6 was good and on the farm 5 insufficient.

The results of implementation of washing and disinfection of udder and milking machines are shown in Table 6:

Table 6. *Test results of implementation of washing and disinfection of udder and milking machines*

Farm	Washing the udder by lukewarm water before milking	Teat disinfection after milking	Washing of milking machines by detergent and lukewarm water	Disinfection of milking machines
1	Yes	Yes	Yes	Yes
2	Yes	Yes	Yes	Yes
3	Yes	Yes	Yes	Yes
4	Yes	No	Yes	No
5	Yes	No	Yes	No
6	Yes	Yes	Yes	Yes

The results in Table 2 show that washing the udder with lukewarm water before milking is enforced on all farms. Washing the milking machines on all six farms was conducted using detergent and lukewarm water. Teat disinfection after milking and disinfection of milking machines is not implemented on the fourth and fifth farm.

The modes of keeping and feeding the dairy animals significantly affect the quality and quantity of obtained milk**.** Hygienically correct milk can be obtained only if the cows are kept in hygienic conditions. The proper treatment of animals is closely related to their welfare, and to technological, hygienic and other requirements that provide the necessary comfort and hygiene to dairy farm. Raw milk is usually contaminated as a result of mistakes in the implementation of hygiene during milking and storage of milk. Cows suffering from mastitis secrete a large number of bacteria through milk, which is significant source of contamination. Streptococci and staphylococci, as the most common causes of mastitis, are transmitted from animal to animal through milking. Reduction of new infections can be achieved by milking teat disinfection after milking, proper handling of the milking machine, disinfection of teat cups, washing the udder with lukewarm running water and other hygienic – sanitary measures. Teat cup, if the milking technology is poor, may be an important factor in the transmission of mastitis pathogens. Mastitis increases the number of somatic cells, in particular those caused by *Streptococcus agalactiae* and *Staphylococcus aureus* (Katić et al., 2003).

Stable climate, as zoohygienic significant factor, is determined by the characteristics of the roof, walls and floors of the stable**.** Regular ventilation and outdoor air significantly affect the indoor climate. Construction materials used to build the stable, should be adapted to the geographical area (Stojanović and Katić, 2004). Appropriate microclimate in the stable and cleanliness of floors provides a constant dry and clean udder, which is an important prerequisite for mammary gland health and hygienic quality of milk.

For the preparation of dairy animals for milking, washing teats and udder, which requires lukewarm water and clean cloths for cleaning, must be regularly performed. Improved hygiene

of milking and milking machines can reduce the number of somatic cells in milk. Control of milking machines has great importance in the prevention of new infections in the herd (Hristov et al., 2005). Stable air contains particles of dust, which during hand milking contaminate milk by microorganisms (Katić and Stojanović, 2004). According to Article 87 of the Rule Book on the specific hygiene requirements for food of animal origin, Gazette of Montenegro, no. 14/2009, the allowed number of bacteria in bovine raw milk is 100000/mL and of somatic cells 400000/mL. If the raw milk is used for the manufacture of products from milk, the maximum number of bacteria is 300000/mL milk. With these criteria in mind, bulk tank raw milk originating from farms 3, 4 and 5 does not correspond to the criteria for the total number of bacteria. On the fourth and fifth farm significantly higher number of somatic cells in bovine bulk tank milk was found compared to the number permitted under the Rule Book of the specific hygiene requirements for food of animal origin, Gazette of Montenegro 14/2009 (Table 1).

Katić et al. (1994) determined the mean number of somatic cells in cow milk to be 93361/mL, in the milk of cows with subclinical mastitis challenged with *S. aureus* 182163/mL, in the milk of cows with subclinical mastitis caused by *S. uberis* 2214000/mL and the in milk of cows with nonspecific mastitis 980750/mL. In the bulk tank milk of healthy cows they found the mean fat content of 3.39%, protein 3.37%, lactose 4.81% and free fat dry matter 8.71%. According to the Regulations on the quality and other requirements for milk and milk products and starter cultures (Gazette SRJ 26/2002, 56/2003, 4/2004 and 5/2004) fat content should not be less than 3.3%, protein content 2.9% and free fat dry matter 8.5%. The value of the free fat dry matter content in milk obtained in our tests (8.13±0.404) is below the minimum value of the dry matter prescribed by the regulations. Lactose content obtained in our study on bovine bulk tank milk is 4.27% ± 0.128 which is lower value compared to the lactose content in milk of healthy cows (4.7%) (Table 1).

Rating of spatial parameters was insufficient on farm 4, sufficient on farm 5 and good on other farms: 1, 2, 3 and 6. On the fourth farm, with minimum rating, the following parameters were evaluated: the hall for food, ventilation, windows, doors, roof, air flow, harmful gases, hygiene bearings and hygiene litter (Table 2). On the fifth farm the lowest rating had the parameters: hallway manure, ventilation, windows, doors, roof, air flow and hygiene litter (Table 2). Rating of microclimate conditions was sufficient on farm 5 and good on other farms (Table 3). Rating of hygiene in stables was sufficient on the fifth, good on the fourth and very good on the other farms. The lowest rating (rating 2) on the fourth farm obtained parameters: hygiene of bearings and litter. On the fifth farm the lowest rating was for hygiene of litter (rating 1) (Table 4). Total rating of conditions for accomodation of cows on farms 1, 2, 3, 4 and 6 was good, while the rating on the farm 5 was sufficient (Table 5).

Washing and sanitizing the milking equipment is a critical point in the process of milking, because it affects the level of bacterial contamination of bovine bulk tank milk. Proper cleaning of milking machine provides better milk quality. The residual milk or water after washing the milking machine promote the growth of various microorganisms (Bava et al., 2009). Poor hygiene during milking, with failures in disinfection, particularly of the teats and hand milker, facilitate milk contamination from the environment (Hristov et al., 2005.). If hygiene is not maintained, milking machines are always the source of milk contamination. The main sources of contamination from the milking machine are: teat cups, hoses, main

sewer and dairy milk container. Milk is usually contaminated by psychrotrophic gram negative organisms from equipment for milking,

From the equipment the milk can also become contaminated by the sporogenic aerobic bacteria of genus *Bacillus* and the sporogenic anaerobic bacteria of genus *Clostridium.* A common source of bacterial contamination of equipment is unfit water. Proper sanitation of milking machines and their proper application produces milk with a small number of microorganisms (Stojanović and Katić, 2004). Irregular, rough machining or hand-milking has a significant role in the incidence of mastitis.

Improper preparation of the udder for milking leads to transfering the causes of mastitis from cow to cow. In our tests, washing the udder with warm water before milking was enforced on all farms. Washing the milking machine on all six farms was conducted using detergent and warm water. However, teat desinfection after milking and disinfection of milking machine was not conducted on the fourth and fifth farm (Table 6). In view of these results, the high number of somatic cells and microorganisms in bulk tank milk of cows on farms 4 and 5 can be related to inadequate space, microclimate and sanitation of accomodation conditions for cows, non-implementation of disinfection of the udder after milking and non-implementation of disinfection of milking machine.

Conclusion

In bulk tank cow milk samples fat content ranged from 2.92% to 3.94%, protein content from 2.78% to 3.65 % and content of lactose from 4.14% to 4.47%. The content of free fat dry matter varied from 7.75% to 8.77%. Somatic cells count ranged from 52000 to 1920000/mL and number of bacteria from 5000 to 1002000/mL milk. Total rating of conditions for accomodation of cows on farms 1, 2, 3, 4 and 6 was good, and on the farm 5 was sufficient (Table 5). The high number of somatic cells and microorganisms in bulk tank milk of cows on farms 4 and 5 may be associated with inadequate space, microclimate and hygiene conditions of accomodation, as well as by failed teat disinfection after milking and failed disinfection of milking machine.

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References

1. Bava L, Zucali M, Brasca M, Zanini L and Sandrucci A 2009. Efficiency of cleaning procedure of milking equipment and bacterial quality of milk. Ital. J. Anim. Sci. 8 (Suppl. 2), 387-389.

2. Hristov S, Stanković B and Relić R 2005. Klinički i subklinički mastitis u krava. Biotechnology in Animal Husbandry 21, 1-2, 29-39.
3. Hristov S and Relić R 2009. Ocena uslova smeštaja sa osvrtom na dobrobit krava. Zbornik naučnih radova 15, 3-4, 79-88.
4. Katić V, Babić Lj, Popović J and Tayeb H. 1994. Uticaj mastitisa na kvalitet mleka, Veterinarski glasnik 48, 3-4, 271-276.
5. Katić V, Stojanović L and Bojanić M 2003. Uticaj nespecifičnih uzročnika mastitisa na broj somatskih ćelija u mlijeku. Peto savjetovanje iz kliničke patologije i terapije, Budva, 9-13. jun. Clinica veterinaria, 177-182 pp.
6. Regulations on the quality and other requirements for milk and milk products and starter cultures (Gazette SRJ 26/2002, 56/2003, 4/2004 and 5/2004).
7. Rule book of the specific hygiene requirements for food of animal origin, Gazette of Montenegro, 14/2009.
8. Stojanović L and Katić V 2004. Higijena mleka, Aranđelovac.

Original paper

THE EFFECT OF SUPPLEMENTATION ON SELENIUM AND ZINC CONTENT IN BLOOD AND MILK OF DAIRY COWS

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Abstract

Milk is an important source of microelements for calves during the suckling period as well as in human nutrition. Concentration of trace elements in blood and their secretion via milk can significantly change depending on food intake and composition. Proper control of selenium and zinc content in blood and in milk can improve the status of these microelements, so that the occurrence of deficiency or excessive quantity due to their increased intake can be prevented. This paper presents the results of the study on the concentration of selenium and zinc in the blood and milk of diary cows whose rations have been supplemented by organic forms of selenium (0.2 mg/kg DM) and zinc (40 mg/kg DM) during the last ten days of dry period and early lactation. Supplemented cows in trial groups A and B achieved significatly higher concentrations of these microelements in blood (Se 186.70±8.50 μg/L vs. 118.80±7.05 μg/L), blood serum (Zn 1204.70±109.5 μg/L vs. 1095.40±130.2 μg/L) and milk (Se 57.30±8.05 vs. 21.30±4.60 μg/L; Zn 2893.90±120.15 μg/L vs. 1952.10±130.50 μg/L) on 60th day postpartum compared to non-supplemented control.

Key words: *blood, dairy cows, milk, selenium, zinc, supplementation*

Introduction

Microelements (selenium, zinc, copper, manganese and iodine) have an important role in preserving dairy cows health, immune system functions and optimal reproductive and production characteristics (Joksimović and Davidović, 2006 and 2007; Siciliano-Jones et al., 2008; Spears and Weiss, 2008). The concentration of microelements in milk varies depending on the content of these elements in soil and in dairy cow feeds (Malbe et al., 2010). Supplementation of animal feeds with critical microelements has become a common practice in order to prevent defficiency disorders and secure maximum productivity. However, the range of increase of their concentration in milk is limited by biochemical homeostatic mechanisms (Pechová et al., 2008).

Selenium (Se) is an essential microelement for human and animal health. Selenomethionine (Se-Met) is the most important nutritional form of organic selenium in different cereals (Weiss and Hogan, 2005). The main advantage of organic selenium is its improved retention in tissues which thereby increases the selenium body reserves. These

reserves are particularly important in stress conditions, during disease and gestation when the need for selenium increases and food supply is usually decreased due to lower food consumption (Gunter et al., 2003). In stress conditions, Se-Met is being released through protein catabolism and used as selenium source for newly synthesized selenoproteins such as GSH-Px, thioredoxin reductase and methionine sulphoxide reductase. These enzymes extinguish a variety of free radicals contributing to maintaining animals health and high productive and reproductive in stress conditions (Joksimović Todorović et al., 2012).

Recommendations of the National Research Council (2001) are that inorganic Se be added into the dairy cow rations in the quantity from 0.1 to 0.3 mg/kg dry matter (DM); however FDA (2003) ammended the regulation by approving the use of up to 0.3 ppm daily selenized yeast in the nutrition of dairy and fattening animals. Maximum tolerant level can amount to 0.5 ppm (Steven and Elliott, 2007). Concentration of selenium in milk increases linearly with the intake of Se in the form of selenized yeast (SY) or other high-Se food, but only to a certain level. Bioactivity of selenium from selenized yeast is estimated to be 20% higher than in inorganic selenium (Weiss, 2005).

It is confirmed that addition of organic forms of Se increases the Se concentration in milk (Juniper et al., 2006; Knowles et al., 2006; Muňiz-Naveiro et al., 2006). Small Se anions are rapid transported through digestive tract membrane (Pechová et al., 2008). Ran et al. (2010) proved that on day 60, 75 and 90 of lactation the selenium concentration in blood of cows supplemented by selenized yeast was 10, 11 and 14% higher in comparison to the cows supplemented inorganic selenium. Many authors indicate that the concentration of microelements is generally higher in colostrum than in ripe milk (Pavlata et al., 2004). After the colostral period, selenium concentration decreases (Hosnedlová et al., 2005). Negative correlation of Se content in relation to lactation days, can be caused by emptying the Se body reserves because of its gradual loss via milk. Weiss and Hogan (2005) determined that Se concentration in colostrum was about 3.8 times higher than in milk ($P<0.01$) in dairy cows supplemented by 0.3 mg/kg DM selenized yeast or sodium selenate during the dry period and first 28 days of lactation. Selenium concentration in colostrum and milk were 1.5 to 2 times higher ($P<0.01$) in cows fed SY compared to those supplemented with selenate.

Zinc (Zn) is an essential microlement and represents an integral component of over 300 enzymes. It influences reproductive functions, secretion of gonadotropin, androgen, prostaglandin and prolactin (Suchý et al., 1998) and plays a role of antioxidant (Arthur, 2001). Natural sources of zinc for farm animals can be found in brans, cereals and animal yeast (Suchý et al., 1998). Average concentration of zinc in food is 36 mg/kg DM. The dairy cow requirement for zinc is 50 mg/kg DM (min 45, max 250 mg/kg DM), for lactating dairy cows 850-1200 mg zinc daily (per consumption of 17-24 kg dry matter daily), and for cows in dry period 600 mg daily (per consumption of 12 kg dry matter) (Kruczyńska, 2004). The addition of chelate forms of zinc increases its utilisation by microorganisms in rumen and increases its transport to the blood or tissues (Strusińska et al., 2003). The National Research Council (2001) recommends that dairy cow feeds should be zinc supplemented in the quantity from 40 to 60 mg/kg DM.

Zinc cations are accumulated in blood, tissues and in a great degree in milk independently on the intake what is associated with regulation of intestinal absorption and change of metabolic demands (Windisch, 2002). Zinc absorption is an active process, in which zinc is partly retained in intestine mucosa from where it is slowly released (Pechová et al., 2008). Absorbed zinc can link with albumin and be transported through liver towards tissues. The greatest part of zinc in the organism is situated in muscular tissue, liver, bones and

mammary gland (Suchý et al., 1998). In blood, zinc in plasma is 75% linked primarily to proteins, 33% to erythrocytes and 3% to leukocytes (Bencko et al., 1995). Zinc concentration in plasma is about 15 μmol/L, out of which 84% is linked to albumin, 15% to α_2-macroglobulin and 1% to amino acids (Tapiero and Tew, 2003). Zinc content in blood can be reduced by 50% in the states of high stress, traumas or inflammatory processes (Zadák, 2002). After the colostral period, zinc concentration does not change significantly (Hosnedlová et al., 2005).

Kellogg et al. (2004) suggest that zinc methionine significantly increases lactation performances, milk quantity and milk fat, and improves health state of mammary gland since it reduces the somatic cell count (SSC) by 33.3% improving the structure of keratin in teat ducts. Cope et al. (2009) observed that addition of recommended levels of zinc chelate form in the feeds for dairy cows significantly increases the milk quantity (37.6 kg/day) in relation to inorganic zinc (35.2 kg/day) or lower levels of organic chelate zinc (35.2 kg/day). Yang et al. (2011) report that exchanging 30% inorganic copper, zinc and manganese by chelate forms of these nutrients 6 weeks prepartum up to 305 days of lactation in dairy cows leads to increase of milk production by 6.5% and reduces SCC by 34%.

Dairy cow milk contains 2-6 mg/L zinc and this value is higher than Se concentration which is 2-60 μg/L (Illek et al., 2000; Knowles et al., 2006). Zinc in milk is primarily bound to colloid calcium phosphate in casein micelles (Silva et al., 2001). Almost 90% zinc is bound to casein in ripe milk, while 60% zinc is bound to casein in colostrum (Kincaid and Cronrath, 1992). Rodriguez et al. (2001) determined that concentrations of selenium and zinc in milk vary significantly throughout the season decreasing from March to September and being significantly higher during winter and spring than during summer and autumn. These seasonal differences are probably caused by various contents of microelements in food.

The aim of this paper was to determine the effect of supplementation of organic selenium (selenized yeast, SY) and organic zinc (zinc methionine, ZnMet) into bovine rations on the concentration of these microelements in blood and milk of Holstein-Friesian cows.

Material and methods

The trial was conducted on 30 cows of Holstein-Friesian breed (13 secundipara and 17 third calving), in the intensive keeping system and on 10th day before expected calving randomly divided into three equal groups consisting of ten cows each. Control group was fed basic ration with no supplements of selenium and zinc in food. Trial groups were fed rations supplemented by microelements starting from 10th day before expected calving (-10 d) up to 60th day of lactation (60 d). Trial group A was supplemented by 0.2 mg/kg DM organic selenium in the form of selenized yeast (Sel-Plex 50, Alltech, Inc), and trial group B received 40 mg/kg DM organically bound zinc in the form of zinc methionine (Bioplex Zn, Alltech, Inc). Average consumption of DM was about 20 kg. Cows were fed individually and water was available *ad libitum*. Blood for analysis (20 mL) was taken from each animal in heparinized test tubes by punctioning the *v. jugularis* on 10th day prepartum and 60th day postpartum, in the morning before feeding. Milking was performed twice daily and milk samples were taken on 60th day of lactation during the morning milking. Determination of selenium concentrations in blood, zinc in milk serum, selenium and zinc in milk was performed by hydrid procedure of atomic absorption spectrophotometry (AAS). Results were statistically processed by means of software package SPSS 8.0. Intergroup comparisons were analyzed using Student t-test.

Results and discussion

Table 1 presents the results of the study on average concentration of selenium in whole blood and milk on 10th day prepartum and 60th day of lactation.

Table 1. *Selenium concentration (Mean±SD) in whole blood and in milk on 10th day prepartum and 60th day of lactation*

Period	Concentration of selenium in blood (µg/L)			Concentration of selenium in milk (µg/L)		
	Control group (n=10)	Trial group A (n=10)	Statistical significance between the groups in the same period	Control group (n=10)	Trial group A (n=10)	Statistical significance between the groups in the same period
-10 d	120.10±10.02	119.10±8.40	NS	20.10±4.12	25.50±6.62	NS
60 d	118.80±7.05	186.70±8.50	**	21.30±4.60	57.30±8.05	**
Statistical significance within each group in different periods	NS	**	-	NS	**	-

NS non-significant, P>0.05; **P<0.01

Blood and milk selenium concentration in cows from Trial A and Control did not differ on the tenth day prepartum. On the sixtieth day of lactation selenium concentration in whole blood (186.70±8,50 µg/L) and milk (57.30±8.05 µg/L) of supplemented cows was significantly higher (P<0.01) compared to control. In Trial group A the addition of selenised yeast into dairy cow ration increased selenium concentration in blood and milk by 50% and 100%, respectively.

The results obtained correspond to the results of Ortman and Pehrson (1999), Knowles et al. (2006), Slavik et al. (2008) and Ran et al. (2010), who determined that addition of different forms of selenium in dairy cow rations can increase the concentration of selenium in milk. Ran et al. (2010) proved that addition of selenized yeast in the nutrition of dairy cows in prepartum period and period of early lactation significantly increases the concentration of selenium in milk within first three months of lactation. The results of our research corroborate the results of Ceballos et al. (2009) who determined that addition of 6 mg selenium into bovine rations on a dialy basis within the period of 75 days satisfies the needs of individual and enables the increase of intake of selenium in consuming dairy products. Ortman and Pehrson (1999) added 3 mg selenium daily, and Gunter et al. (2003) added 2.8 mg selenium in feed daily and found that selenized yeast more efficiently increases the concentrations of selenium in the blood of dairy cows in relation to inorganic selenium.

In the trial carried out by Pavlata et al. (2000) the content of selenium in bovine blood serum was not lower than 100 µg/L. Weiss and Hogan (2005) reported that in cows whose ration was supplemented by 0.3 mg/kg DM selenized yeast from the 60th day prepartum up to the 28th lactation day the blood serum selenium concentration at calving (0.059 mg/L) and on the 28th lactation day (0.074 mg/L) was about 1.4 times higher compared to cows supplemented by the same quantity of sodium selenat (0.043 mg/L and 0.054 mg/L). The

results obtained by Maus et al. (1980) and Weiss et al. (1990) show that the concentration of selenium in dairy cows plasma ranges from 1016-1397 nmol/L (or 0.08-0.11 μg/mL) after addition of 0.25 - 0.3 mg/kg DM dietary selenium. Jukola et al. (1996) state that the optimal preventative action against mastitis can be obtained when the whole blood selenium concentration is higher than 0.18 μg/mL and in blood plasma 0.08 μg/mL. Similar values were obtained in our trial by adding selenized yeast to cows ration.

Table 2 displays the results of the research on average concentration of selenium in whole blood and milk on 10th day prepartum and on 60th day of lactation.

Table 2. *Zinc concentration (Mean±SD) in blood serum and in milk on 10th day prepartum and on 60th day of lactation*

Period	Concentration of zinc in blood serum (μg/L)			Concentration of zinc in milk (μg/L)		
	Control group (n=10)	Trial group B (n=10)	Statistical significance between the groups in the same period	Control group (n=10)	Trial group B (n=10)	Statistical significance between the groups in the same period
-10 d	1070.50± 150.2	1055.50± 112.1	NS	1970.20± 140.8	1962.30± 135.1	NS
60 d	1095.40± 130.2	1204.70± 109.5	*	1952.10± 130.5	2893.90± 120.1	*
Statistical significance within each group in different periods	NS	*	-	NS	*	-

NS non-significant, P>0.05; *P<0.05

There was no significant difference in the concentration of zinc in blood serum and milk between Control and Trial group B on the tenth day prepartum. Supplementation of organically bound zinc resulted in significant increase (P<0.05) of zinc concentration in blood serum (1204.70±109.5 μg/L) and milk (2893.90±120.1 μg/L) compared to control on the sixieth day of lactation. Statistically significant increase of zinc concentration in blood serum and milk was confirmed also in Trial B group on the 60th day postpartum.

Our results correspond with the research of Pechová et al. (2008), who during the first half of lactation supplemented their rations with 0.4 mg/kg DM selenium and 105 mg/kg DM zinc, the average consumption of DM being 20·5 kg. Authors determined that the concentration of selenium in whole blood (183.75±29.63 μg/L) was higher than in milk (28.59±7.12 μg/L), while the content of zinc in blood serum (1063.92±181.13 μg/L) was lower than in milk (3855.2±814.7 μg/L). In this respect, Pechová et al. (2008) did not determine significant correlation between the concentrations of these microelements in milk and blood nor between milk production and concentration of these microelements in milk. Zinc content in milk in our trial was within usual values for consumable milk 2-6 mg/L (Knowles et al., 2006).

Transport of zinc into milk is an active process (Kelleher and Lonnerdal, 2003), and in the milk of dairy cows zinc is linked primarily to casein (Kincaid and Cronrath, 1992). Dobrzański et al. (2005) determined an average zinc content of 2.575±644 μg/L in full blood of clinically healthy cows in full lactation and in the age of 4-12 years. Kruczyńská

(1992) confirmed that the level of zinc in blood serum of cows has an average value of 13.0-48.7 μmol/L, and in calves 10.7-39.0 μmol/L. Average value of zinc in dairy cows plasma is 10.5-17 μmol/L (Masoero et al., 1998), while Strusińska and Iwańska (1994) established the value of 15.7 μmol/L zinc in blood plasma in cows 60 days postpartum.

Campillo et al. (1998) determined that the concentration of zinc in fresh cows milk is 3±0.2 μg/mL and in dried milk 23.5±0.3 μg/g.

Hosnedlová et al. (2005) analysed 50 samples of milk of dairy cows collected in the period between May and June and determined an average value of zinc to be 4.67±0.64 mg/L milk. These authors, on all examined farms, obtained higher values than those obtained in our research. The concentration of zinc in milk was highest on the farms with milk production of over 7000l daily (4.96±0.21 mg/L) and lowest on the farms with the production of up to 1000l daily (4.18±1.30 mg/L).

Conclusion

Supplementation of dairy cow rations with organic selenium (selenized yeast, SY) and organic zinc (zinc methionine, ZnMet) from the tenth day before expected calving up to the sithieth day of lactation has a positive effect on the concentrations of these nutrients in cow blood and milk.

Compared to the control group receiving no such supplementation the cows in trial groups on the sixtieth day postpartum had:

- Significantly higher blood selenium concentration (186.70±8.50 μg/L vs 118.80±7.05 μg/L);
- Significantly higher blood serum zinc concentration (1204.70±109.5 μg/L vs 1095.40 μg/L ± 130.2);
- Significantly higher selenium (57.30±8.05 vs 21.30±4.60 μg/L) and zinc concentrations in milk (2893.90±120.15 μg/L vs 1952.10±130.50 μg/L);
- Established differences were at the level of significant ($P<0.05$) for zinc and very significant ($P<0.01$) for selenium.

Within the trial groups the addition of microelements into dairy cow rations resulted in:

- The increase of selenium blood and milk concentrations in Trial group A cows by approximately 50% and over 100%, respectively, the established differences at the beginning and at the end of trial being at the level of very significant ($P<0.01$);
- Statistically significant increase of zinc concentrations in Trial group B cows blood serum and milk on the sixtieth day postpartum compared to the tenth day prepartum ($P<0.05$).

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References

1. Arthur GH 2001. Arthur's Veterinary Reproduction and Obstetries. W.B. Saundres, London, Eight edition: 868.
2. Bencko V, Cikrt M and Lener J 1995. Toxic metals in the human living and working environment (in Czech). Grada Publishing, Prague, 2. ed., 288.
3. Campillo N, Viñas P, López-Garcia I and Hernández-Córdoba M 1998. Direct determination of copper and zinc in cow milk, human milk and infant formula samples using electrothermal atomization atomic absorption spektrometry. Talanta 46, 615-622.
4. Ceballos A, Sánches J, Stryhn H, Montgomery JB, Barkema HW and Wichtel JJ 2009. Meta-analysis of the effect of oral selenium supplementation on milk selenium concentration in cattle. J. Dairy Sci. 92, 324-342.
5. Cope CM, Mackenzie AM, Wilde D and Sinclair LA 2009. Effects of level and form of dietary zic on dairy cow performance and health. J. Dairy Sci. 92, 5, 2128-2135.
6. Dobrzánski Z, Górecka H, Opaliński S, Chojnacka K and Kolacz R 2005. Trace and ultra-trace elements in cows milk and blood (in Polish). Med. Wet. 61, 3, 301-304.
7. FDA 2003. Food additives permitted jn feed and drinking water of animals: Selenium yeast. Federal Register 68 (170), 52339-52340 (September 3).
8. Gunter SA, Beck PA and Phillips JM 2003. Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves. J. Anim. Sci. 81, 856-864.
9. Hosnedlová B, Trávniček J and Chrastný V 2005. Zinc and cooper concentration in milk of dairy cows in the south Bohemia region. ISAH, Warsaw, Poland, 1, 256-259.
10. Illek J, Bečvář O, Lokajová E and Matějíček M 2000. Trace elements in nutrition of cattle-zinc (in Czech). Krmivářstvi 6, 30.
11. Joksimović Todorović M and Davidović V 2006. Selenium-health, productive and reproductive traits of farms animals. Acta Physiologica et Pharmacologica Serbica 42, 3, 239-245.
12. Joksimović Todorović M, Hristov S, Davidović V, Stanković B and Relić R 2006. Uloga selena i vitamina E u sprečavanju zaostajanja posteljice kod mlečnih krava. Clinica veterinaria 2006: zbornik radova [Elektronski izvor] / VIII savjetovanje iz kliničke patologije i terapije životinja sa međunarodnim učešćem, 26-30.06.2006. Neum. ISBN 9958-599-20-1. COBISS.BH-ID 15029766.
13. Joksimović Todorović M and Davidović V 2007. Organski mikroelementi u ishrani goveda. Međunarodni naučni skup "Multifunkcionalna poljoprivreda i ruralni razvoj u Republici Srpskoj", Jahorina 13-14.12. 2007.god. Tematski zbornik, 578-583.
14. Joksimović Todorović M, Hristov S, Božić A, Relić R, Davidović V and Stanković B 2007. Uticaj selena i vitamina E na zdravlje mlečne žlezde krava. Savremena poljoprivreda 56, 1-2, 122-127.

15. Joksimović Todorović M, Davidović V and Relić R 2012. Oxidative stress – mastitis. Proceedings of The First International Symposium on Animal Science, 8-10th November 2012, Belgrade, Serbia, Book 2, 673-680.

16. Jukola E, Hakkarainen J, Saloniemi H and Sankari S 1996. Blood selenium, vitamin E, vitamin A and β-carotene concentrations and udder health, fertility treatments and fertility. J. Dairy Sci. 79, 838-845.

17. Juniper DT, Phipps RH, Jones AK and Bertin G 2006. Selenium supplementation of lactating dairy cows: Effect on selenium concentration in blood, milk, urine, and feces. J. Dairy Sci. 89, 3544-3551.

18. Kelleher SL and Lonnerdal B 2003. Zn transporter levels and localization change throughout lactation in rat mammary gland and are regulated by Zn in mammary cells. J. Nutr. 133, 3378-3385.

19. Kellogg DW, Tomlinson DJ, Socha MT and Johnson AB 2004. Review: Effects of zinc methionine complex on milk production and somatic cell count of dairy cattle: twelve-trial summary. Proc. Anim. Sci. 20, 4, 295-301.

20. Kincaid RL and Cronrath JD 1992. Zinc concentration and distribution in mammary secretions of prepartum cows. J. Dairy Sci. 75, 481-488.

21. Knowles SO, Grace ND, Knight TW, Mcnabb WC and Lee J 2006. Reasons and means for manipulating the micronutrient composition of milk from grazing dairy cattle. Animal Feed Sci. Technol. 131, 154-167.

22. Kruczyńská H 1992. Effectiveness of mineral nutrition of cows and calves in the conditions of Wielkopolska (in Polish). Poznań. Roczniki Akademii Rolniczej w Poznaniu. Rozprawy naukowe. Zeszyt 231, 59.

23. Kruczyńská H 2004. Excess is also unhealthy (in Polish). Hoduj z glowa 3, 9, 12-15.

24. Malbe M, Otstavel T, Kodis I and Viitak A 2010. Content of selected micro and macro elements in dairy cows milk in Estonia. Agronomy Research 8, 11, 323-326.

25. Masoero F, Moschini M, Rossi F and Piva G 1998. Effect of bovine somatotropin on milk production, milk quality and the cheese-making properties of Grana Padano cheese. Livestock Production Science 54, 107-114.

26. Maus RW, Martz FA, Belyea RL and Weiss WF 1980. Relationship of dietary selenium to selenium in plasma. J Dairy Sci. 63, 532-537.

27. Muňiz-Naveiro O, Dominguez-Gonzáles R, Bermejo-Barrera A, Bermejo-Barrera P, Cocho JA and Fraga JM 2006. Study of the bioavailability of selenium in cows milk after a supplementation of cow feed with different forms of selenium. Anal. Bioanal. Chem. 385, 189-196.

28. National Research Council 2001. Nutrient requirements of dairy cattle. 7th rev. ed. National Academy Press, Washington, DC, USA.

29. Ortman K and Pehrson B 1999. Effect of selenate as a feed supplement in dairy cows in comparison to selenite and selenium yeast. J. Anim. Sci. 77, 3365-3370.

30. Pavlata L, Pechová A and Illek J 2000. Direct and indirect assessment of selenium status in cattle - a comparison. Acta Vet. Brno 60, 281-287.

31. Pavlata L, Pechová A and Dvořák R 2004. Microelements in colostrum and blood of cows and their calves during colostral nutrition. Acta Vet. Brno 73, 421-429.

32. Pechová A, Pavlata L, Dvořák R and Lokajová E 2008. Contents of Zn, Mn and Se in milk in relation to their concentrations in blood, milk yield and stage of lactation in dairy cattle. Acta Vet. Brno 77, 523-531.

33. Ran L, Wu X, Shen X, Zhang K, Ren F and Huang K 2010. Effects of selenium form on blood and milk selenium concentrations, milk component and milk fatty acid composition in dairy cows. J. Sci. Food Agric 90, 2214-2219.

34. Rodriguez EMR, Alaejos MS and Romero CD 2001. Mineral concentrations in cows milk from the Canary Island. J. Food Comp. Anal. 14, 419-430.

35. Siciliano-Jones JL, Socha MT, Tomlinson DJ and DeFrain JM 2008. Effect of trace mineral source on lactation performance, claw integrity, and fertility of dairy cattle. J. Dairy Sci. 91, 5, 1985-1995.

36. Silva FV, Lopes GS, Nóbrega JA, Souza GB and Nogueira ARA 2001. Study of the protein-bound fraction of calcium, iron, magnesium and zinc in bovine milk. Spectrochimica Acta Part B 56, 1909-1916.

37. Slavik P, Illek J, Brix M, Hlavicova J, Rajmon R and Jilek F 2008. Influence of organic versus inorganic dietary selenium supplementation on the concentration of selenium in colostrum, milk and blood of beef cows. Acta Vet. Scan. 50, 1-6.

38. Spears JW and Weiss WP 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J. 176, 1, 70-76.

39. Steven A and Elliott PAS 2007. The Selenium Dillema: What is the role for EU approved Sel-Plex®? In Biotechnology in the Feed Industry: Proceedings from Alltech's European, Middle Eastern and North African Lecture Tour 2007, 63-75 pp .

40. Strusińska D and Iwańska AS 1994. Content of minerals in blood plasma, colostrum and milk of cows fed daily rations supplemented with mineral, β carotene and vitamins (in Polish). Zwiazki mineralne w zywieniu zwierzat. Konferencja naukowa 8-9 IX 1994. Poznań, Wydawnietwo Akademii Rolniczej w Poznaniu, 91-97 pp.

41. Strusińska D, Iwańska AS, Mierzejewska J and Skok A 2003. Effect of mineral-vitamin and yeast supplements on concentrations of some biochemical parameters in the blood serum of cows (in Polish). Med. Wet 59, 40, 323-326.

42. Suchý P, Suchý PJR and Straková E 1998. Micro-elements in nutrition of farm animals (in Czech). Krmiva and Výživa 3-4, 18-19.

43. Tapiero H and Tew KD 2003. Trace elements in human physiology and pathology: zinc and metallothioneins. Biomedicine and Pharmacotherapy 57, 399-411.

44. Weiss WP 2005. Selenium sources for dairy cattle. Proc. Tri-State Dairy Nutrition Conference, Ft. Wayne, 61-71 pp.

45. Weiss WP, Hogan JS, Smith KS and Hoblet KH 1990. Relationship among selenium, vitamin E, and mammary gland health in commercial dairy herds. J. Dairy Sci. 73, 381-390.

46. Weiss WP and Hogan JS 2005. Effect of selenium source on selenium status, neutrophil function, and response to intramammary endotoxin challenge of dairy cows. J. Dairy Sci. 88, 4366-4374.

47. Windisch W 2002. Interaction of chemical species with biological regulation of metabolism of essential trace elements. Anal. Bioanal. Chem. 372, 421-425.

48. Yang FL, Li XS and He BX 2011. Effects of vitamins and trace-elements supplementation on milk production in dairy cows: A review. Afrikan J. Biotech. 10, 14, 2574-2578.

49. Zadák Z 2002. Zinek. In: Výživa v intenzivni péči. (Nutrition in intensive care), pp. 165-167. Grada Publishing, Praha.

Original paper

SOME SLAUGHTER AND CARCASS TRAITS OF THE LAMBS OF DALMATIAN PRAMENKA REARED IN THREE DIFFERENT FATTENING SYSTEMS

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Abstract

In order to determine the impact of different fattening system (I - milk, pasture; II - indoors, milk, grains, III - milk, pasture, concentrate) on slaughter value and carcass quality of the lambs of Dalmatian Pramenka, 18 lambs (100 ± 5 days old), 6 in each group, were slaughtered. Lambs of one group were from the same flock and selected by birth weight (2.00 ± 0.20 kg). After slaughtering and cutting the carcasses into halves, in order to determine the share of legs and shoulders as well as certain tissues in the halves, the legs and shoulders were separated from the halves and total dissection was made. The significant differences ($P<0.05$) of slaughter weight (I - 21.17 kg, II - 23.25 kg, III - 26.25 kg) and hot carcass weight (I - 9.98 kg, II - 11.92 kg, III - 12.92 kg) among three groups were found. The legs II (1.65 kg) and III (1.71 kg) were significantly heavier ($P<0.001$) than legs I (1.32 kg), as well as shoulders (I - 0.52 kg, II - 0.65 kg, III - 0.69 kg; $P<0.01$). Total dissection of the halves established these tissues ratio: muscle 51.25 %, fat 10.18 %, connective 13.93%, bone 23.04% and other tissues 2.32%. The biggest quantity of muscle tissue was found in halves III (3.27 kg) what was more ($P<0.05$) than in halves II (2.83 kg) and I (2.50 kg). However, the biggest quantity of fat was found in halves II (0.85 kg) what was more ($P<0.01$) than in halves I (0.33 kg) and III (0.52 kg). Therefore, the addition of concentrate in pasture fattening system (III) increased the muscularity, without significantly increasing the amount of fat in the lamb carcass.

Key words: *carcass dissection, carcass quality, carcass tissue ratio, Dalmatian lamb*

Introduction

Across the Mediterranean region of Croatia, as well as the other Mediterranean countries, sheep are mainly farmed under extensive or semi-extensive systems based on natural pastures.The main features of those systems are minimum investment in facilities, equipment and nutrition. Throughout the year the animals are kept outside, staying on natural pastures and mountain slopes but during bad weather and cold days in winter they are mainly kept in stables and fed with hay (Mioč et al., 2013). With approximately

230,000 heads, native Dalmatian Pramenka is the most numerous sheep breed in Croatia (HPA, 2013). Although this sheep is characterized by combined production characteristics, they are mainly used for meat production, especially famous young Dalmatian lamb (Mioč et al., 2008 and 2012). The production of Dalmatian lamb is traditionally based on slaughtering of light lambs at 18 to 25kg of body weight at slaughter, and at the age of 90 and 120 days, and consequently the average carcass weight (with head) is between 8 and 14 kg. Considering the carcass weight, as well as colour of the meat, and fat covering score, according to the Croatian Regulation (NN 30/2010) the carcass of Dalmatian lamb belongs to the category of light lamb (L), class/grade B/1 or C/1 or C/2 (Krvavica et al., 2013). Lamb carcasses of these characteristics are also preferred by the Croatian market, and they are especially suitable for preparation of the traditional Dalmatian lamb roast on a spit. Consequently, carcass of the Dalmatian lamb always comes on the market with head, kidneys and kidney fat.

Although the sheep farming systems in Dalmatia are mostly extensive, especially if sheep are used just for lamb production, at the sheep farms which besides lambs also produce cheese, the farming system becomes more or less intensive. Besides the extensive system of the lamb fattening, where the nutrition of the lambs and ewes is based on milk and pasture, there are two more semi-extensive fattening systems which are also commonly used in the traditional sheep farming in Dalmatia. One of them implies raising the lambs exclusively indoors until the end of fattening. During the time that ewes and lambs stay together indoors, besides the milk available ad libitum, at the age of four weeks the lamb diet is supplemented with grains (oats, barley, corn). This way of lamb farming is applied until the slaughtering of the lambs (the lambs never go out on pasture and besides the salt and water ad libitum they do not get any other feed supplements). The other version of semi-extensive farming system is more intensive and includes separating the lambs from their mothers and controlled suckling several times a day, and lamb grazing on separate pasture, and adding the feed in daily meal after weaning at age of 60 days, as well.

Numerous studies referred that intensification of the farming system increased carcass weight and dressing percentage in lambs, as well as fatness degree (Diaz et al., 2002; Priolo et al., 2002; Borton et al., 2005; Joy et al., 2008) and affects carcass tissue composition (Carrasco et al., 2009 and 2009a; Majdoub-Mathlouthi et al., 2013).

With respect to the three different fattening systems commonly used in the traditional sheep farming in Dalmatia, the aim of this study was to determine how these differences affect the quality of carcass and meat of the Dalmatian Pramenka lambs.

Materials and methods

A total of 18 lambs from Dalmatian Pramenka breed (birth weight 2 ± 0.2 kg; age 100 ± 5 days) originated from three different extensive and semi-extensive fattening systems (each group was composed of 6 lambs : 3 males and 3 females) which are all commonly used in traditional sheep farming in Dalmatia, were used in the research. All the lambs originate from three different farms (6 lambs per farm) selected according to the three types of fattening system. At the first farm (group I) lambs were raised outdoors in extensive system based on milk and pasture. First 15 days after birth, lambs were kept indoors and fed only by suckling. After that time, the lambs were grazed, but since lambs were raised together with ewes they were sucked ad libitum during the whole fattening period. At the second farm (group II) lambs were raised indoors under the semi-extensive system. During the day when the ewes were on pasture, the lambs were in stable and at the age of four weeks their daily diet was supplemented with grains (oats, barley and corn), ad libitum.

While the ewes were in stable (from late afternoon to early morning) the lambs were with them and they were sucked ad libitum. The lambs have never been out to pasture with ewes. At the third farm (group III) lambs were raised outdoors under the semi-extensive fattening system. First 15 days after birth the lambs were with their mothers all the time and they were sucked ad libitum. After that, the lambs were separated from the ewes and able to suck only few times a day, whereby the number of suckling was gradually reduced up to 2 times a day after 45 days. Also, at this time grazing on separate pasture, as well as small amounts of high quality hay, were introduced in the lamb meal. At the age of 60 days the lambs were weaned, after which they consumed pasture and hay ad libitum, as well as approximately 150 g of concentrate per day, distributed in two to three daily meals. Common characteristics of all three fattening systems were: first 4-5 days after birth ewes stayed indoors together with lambs; good quality hay was available to the lambs while they were indoors with or without ewes (commonly they start to nibble the hay at the age of 15-20 days); salt and water were available to lambs and ewes ad libitum all the times.

At the age of 100 ± 5 days lambs were slaughtered and cold carcasses were dissected according to the procedure described by Krvavica et al. (2013), including the methods of slaughter measurements (live body weight at slaughtering, hot and cold carcass weight, warm and cold dressing percentage). The measurements of the primal cuts and total dissection were conducted only on the right halves of lamb carcasses. Precise total dissection of the previously semi frozen halves was made manually, using a professional butcher knives, in such a way that different tissues, such as muscle, visible fat (external and internal surface fat, kidney fat, intermuscular fat) and connective tissue, and bones with cartilages were separated from each other. The tissues obtained by total dissection of leg shank portion, and shoulders and the rest part of the half carcass were measured separately.

Data were analysed using the SAS/STAT software (SAS, 2008). Effect of lamb fattening system on researched traits was analysed using the GLM procedure according to the ANOVA model that includes also the effect of fattening system and the weight of the hot carcass as a covariate.

Results and discussion

As it is presented in Table 1, the significant differences of some slaughter traits and weight of primal cuts and tissue composition of the lamb halves among the three different fattening groups were found. The heaviest lambs at slaughter were of group III (26.25 kg), which were significantly heavier than group I (21.17 kg; $P<0,001$) and group II (23.25 kg; $P<0.001$). Differences between the groups I and II were also significant ($P<0.05$). Consequently, the hot carcass weight among the groups was also significantly different ($P<0.01$), but not between the groups II and III, while the differences of cold carcass weight were similar as of live body weight. However, the significant differences of dressing percentages among the groups were not found. These specified differences confirmed the assumption that differences in lamb fattening system such as supplementing the lamb diet by grains or concentrate significantly affect the slaughter traits which is similar to the results observed by several authors (Borton et al., 2005; Carrasco et al., 2009; Ekiz et al., 2012). However, in contrast to this research these authors found the differences of dressing percentage affected by fattening system.

Weight of leg shank portion and shoulder of lambs from group I fattened on milk and pasture (1.32 kg and 0.52 kg, respectively) were significantly lower ($P<0.001$ and $P<0.01$, respectively) than in the other two groups, but the same differences were not found

between the groups II and III. According to Ekiz et al., 2013, lambs grazed on pasture and stubble had significantly lighter pelvic limb in comparison to those grazed just on pasture or fed concentrate. However, concentrate supplement in lamb diet did not affect weight of the pelvic limb of Comisana (Maiorano et al., 2009) and Tensina light lambs (Carrasco et al., 2009 and 2009a) as well as weight of leg and shoulder of Barbarine lambs fed with two level of concentrate (Majdoub-Mathlouthi et al., 2013).

Table 1. *Effect of lamb fattening system on some slaughter traits and weight of individual primal cuts and tissue composition of the lamb halves of Dalmatian Pramenka*

	Fattening system				
Slaughter traits	I	II	III	SEM	Effect
Live body weight, kg	21.17^{a}	23.25^{b}	26.25^{c}	0.46	*
Hot carcass, kg	9.98^{a}	11.63^{b}	12.92^{b}	0.35	**
Warm dressing percentage, %	47.22	49.99	49.16	0.98	NS
Cold carcass, kg	9.75^{a}	11.37^{b}	12.65^{c}	0.34	*
Cold dressing percentage, %	46.12	48.85	48.14	0.97	NS
Half, kg	4.85^{a}	5.65^{b}	6.31^{c}	0.17	*
Carcass primal cuts, kg					
Leg shank portion	1.32^{a}	1.65^{b}	1.71^{b}	0.52	***
Shoulder	0.52^{a}	0.65^{b}	0.69^{b}	0.23	**
Rest of the half	2.99^{a}	3.32^{a}	3.88^{b}	0.15	*
Tissue composition of the half, kg					
Muscle tissue	2.50^{a}	2.83^{b}	3.27^{c}	0.09	*
Surface fat	0.21^{a}	0.48^{b}	0.24^{a}	0.04	**
Kidney fat	0.05^{a}	0.19^{b}	0.18^{b}	0.02	**
Total fat	0.33^{a}	0.85^{b}	0.52^{a}	0.06	**
Connective tissue	0.76^{ab}	0.65^{a}	0.92^{b}	0.06	**
Bones	1.20^{a}	1.25^{a}	1.42^{b}	0.05	*
Other tissues	0.08^{a}	0.11^{a}	0.19^{b}	0.02	*

SEM: standard error of mean; NS: not significant; a, b, c: means at the same row with different letters are significantly different; *P≤0.05; **P≤0.01; ***P≤0.001

Half carcass tissue composition was significantly different among the groups. The most of the quantity of muscle tissue was found in group III (3.27 kg) fed with concentrate what was significantly more than in group I (2.50 kg; P<0.001) and group II (2.83; P<0.001), while the difference between groups I and II were on P<0.05 level of significance.

The fattening system had clear influence on the total fat quantity despite the variation according to the fat depots. The treatment of group III (concentrate supplement in grazing pasture) had less effect on internal fat depots (kidney fat) which is similar to Carrasco et al., 2009. Quantity of total fat was the highest in group II (0.85 kg) fattened indoors based on milk and grains, while it was similar in group III (0.52 kg) grazed on pasture with concentrate and group I (0.33 kg) grazed on pasture without feed supplement but significantly lower in comparison to group II (P<0.01). Similar to this study, numerous authors also found highest level of muscle tissue and less fatness level in pasture grazed lambs compared to the lams raised indoors (Diaz et al., 2002; Carrasco et al., 2009 and 2009a; Ekiz et al., 2012) and some of them reported that the reason thereof might be

related with the changes in the metabolism of grazing lambs due to physical activities which cause mobilization of lipids from depots in order to build muscles what subsequently reduce the carcass fat (Diaz et al., 2002; Ekiz et al., 2012). Quantity of connective tissue and bones was highest in group III.

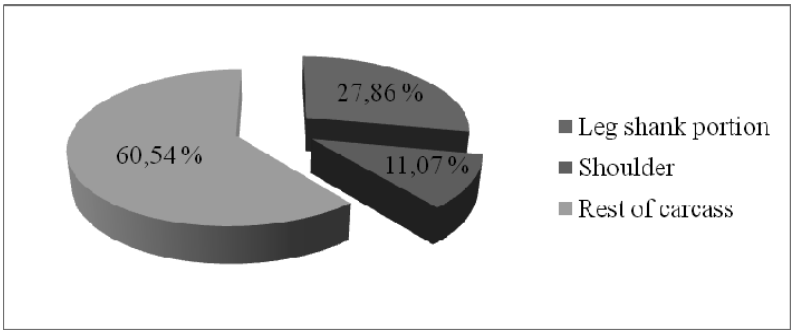

Figure 1. *Percentage of leg and shoulder in the lamb carcass*

The half lamb carcass of Dalmatian pramenka was made approximately from 27.86 % of leg shank portion and 11.07 % of shoulder (Figure 1). Tissue composition of the half carcass was as follows: 51.25 % of muscle tissue, 10.18 % of fat, 13.93 % of connective tissue, 23.04 % bones and 2.32 % other tissues (Figure 2).

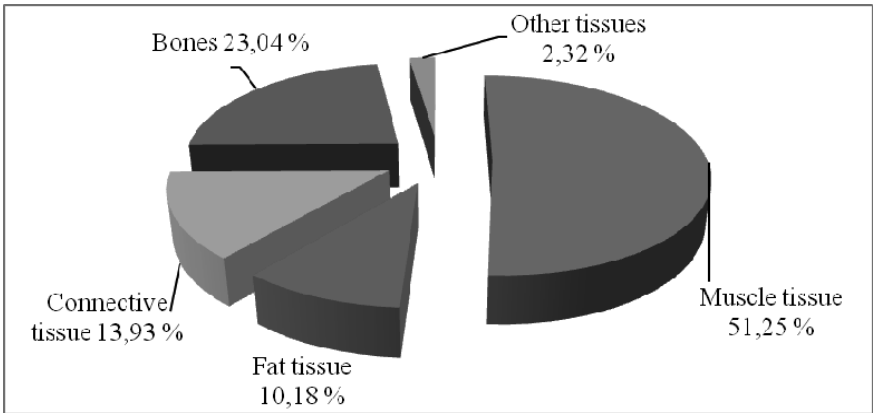

Figure 2. *Proportion of certain tissues in the lamb carcass, %*

Conclusion

Different lamb fattening systems, such as supplementing the lamb diet by grains or concentrate, significantly affect the slaughter traits and weight of primal cuts and tissue composition of the lamb carcass of Dalmatian Pramenka. Moreover, the addition of concentrates in pasture fattening system (group III) has positive effect, increasing the muscularity, without excessively significantly increasing the amount of fat in the lamb carcass. Although the addition of grains also has positive effect on muscle tissue the

indoors rearing system of group II probably has negative impact on increasing the fatness, especially on internal and external surfaces fat depots.

References

1. Borton RJ, Loerch SC, McClure KE and Wulf DM 2005. Comparison of characteristics of lambs fed concentrate or grazed on ryegrass to traditional or heavy slaughter weights. I. Production, carcass, and organoleptic characteristics. Journal of Animal Science 83, 679-685.
2. Diaz MT, Velasko S, Cañeque V, Lauzurica S, Ruiz de Huidobro F, Perez C, Gonzáles J and Manzanares C 2002.Use of concentrate or pasture for fattening lambs and its effect on carcass and meat quality. Small Rumminant Research 43, 257-268.
3. Ekiz B, Yilmaz A, Ozcan M and Kocalk O 2012.Effect of production system on carcass measurements and meat quality of Kivircik lambs. Meat Science 90, 465-471.
4. Ekiz B, Demirel G, Yilmaz A, Ozcan M, Yalcintan H, Kocak O and Altinel A 2013. Slaughter characteristics, carcass quality and fatty acid composition of lambs under four different production systems. Small Ruminant Research 114, 26-34.
5. Carrasco S, Ripoll G, SanzA, Álvarez-Rodríguez J, PaneaB, Revilla R and Joy M 2009. Effect of feeding system on growth and carcass characteristics of ChurraTensina light lambs. Livestock Science 121, 56-63.
6. Carrasco S, Ripoll G, PaneaB, Álvarez-Rodríguez J and Joy M. 2009a. Carcass tissue composition in light lambs: Influence of feeding system and prediction equations. Livestock Science 126, 112-121.
7. HPA 2013. Hrvatska poljoprivredna agencija – Godišnje izvješće za 2012. Ovčarstvo, kozarstvo i male životinje.
8. Joy M, Ripoll G and Delfa R 2008. Effects of feeding system on carcass and non-carcass composition of ChurraTensina light lambs. Small Ruminant Research 78, 123-133.
9. Krvavica M, Rogošić J, Šarić T, Župan I, Ganić A and Madir A 2013. Pokazatelji klaoničke vrijednosti i kvalitete trupa janjadi dalmatinske pramenke. Meso 6, 455-463.
10. Majdoub-Mathlouthi L, Saïd B, Say A and Kraiem K 2013. Effect of concentrate level and slaughter body weight on growth performances, carcass traits and meat quality of Barbarine lambs fed oat hay based diet. Meat Science 93, 557-563.
11. Maiorano G, Ciarlariello A, Cianciullo D, Roychoudhury and Manchisi A 2002. Effect of sucling management on productive performance, carcass traits and meat quality of Comisana lambs. Meat Science 83, 677-583.
12. Mioč B, Držaić V, Vnučec I, Prpić Z, Antunović Z and Barać Z 2013. Some slaughter and meat traits of lambs and kids from an extensive production system.Veterinarski arhiv 83, 263-274.
13. Mioč B, Prpić Z, Vnučec I, Sušić V, Antunović Z, Barać Z and PavićV 2008. Vanjština različitih kategorija hrvatske šarene koze. Stočarstvo 62, 439-447.
14. Mioč B, Vnučec I, Prpić Z, Barać Z and Pavić V 2012. Dalmatinska janjetina.Četrnaesto savjetovanje uzgajivača ovaca i kozara u Republici Hrvatskoj. Trogir, 18. i 19. listopada. Zbornik predavanja 26-42.
15. Priolo A, Micol D, Agabiel J, Prache S and Dransfield E 2002. Effect of grass or concentrate feeding on lamb carcass and meat quality. Meat Science 62, 179-128.
16. SAS 2008.SAS Version 9.2. SAS Institute Inc. Cary, NC.

Review paper

INFLUENCE OF PHYTOESTROGENS ON SKELETAL MUSCLE STRUCTURE

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Abstract

Constant increase of meat quantity along with ensuring its high quality are nowadays being the priority requirements of the market towards modern meat production. With selection and animal nutrition as the basic mechanisms regulating the quantity and quality of meat, in recent years more attention has been devoted to investigations of the effects of different chemical compounds on muscle tissue, while monitoring their potential negative effects on both animals and humans as the end consumers. A group of compounds that is being increasingly studied in the last years are phytoestrogens – substances of plant origin with chemical structure very similar to estrogen, capable of causing either estrogenic or anti-estrogenic reactions in the organism. The most studied phytoestrogens are daidzein and genistein, and due to their ability to mimic estrogen in the body, they are thought to be able of influencing growth and carcass composition in farm animals. This paper gives an overview of the newer results on the effects of phytoestrogens genistein and daidzein on skeletal muscle tissue in farm animals.

Key words: *daidzein, muscle growth, muscle structure, phytoestrogens*

Introduction

Modern meat production, as one of the most important branches within animal production is facing very difficult tasks – at the same time it has to settle often opposite demands of the market: to increase the production so it could meet the needs of end customers, not to undermine basic biological and physiological possibilities of animals by selection, to keep the quality of meat at high level both for end consumers and meat industry, to speed up the process od meat production, while the costs of production should not exceed the limit of profitability. Having in mind that regulation of muscle growth and differentiation of muscle cells includes a number of factors: environmental, genetical, and intrinsic factors such as hormones and growth factors, the results of scientific research within these fields need to be an integral part of the meat production process.

The influence of estrogen on muscles

Ruminants are the only animal species in which an unambiguous positive effects of estrogen on muscle growth, daily gain increasment, feed utilization with minimal increase in food consumption and decrease of fat deposition are observed. After diethylstilbestrol, DES, was synthesized in late 30ies of the twentieth Century, the effects of this chemical on

production characteristics in domestic animals have been investigated, and hence increased daily gain in heifers, sheep and poultry were determined, DES was patented as stilbestrol. It was used until 1979., when it was forbidden as potentially carcinogenic. However, in some countries, similar compounds (17β estradiol, estradiol benzoate, zeranol, dienestrol) are still being used as growth promoters in cattle production.

Estrogens have less effects in non-ruminants. The mechanism of action of estrogen on the growth of pigs is not fully understood. In intact males - boars, administration of estrogen increases food intake and its utilization, thereby increasing growth, reducing the undesirable characteristic smell of the meat, but to a smaller extent, increases the amount of adipose tissue (Hancock et al., 1991). Rempel and Clapper (2002), and Hilleson-Gayne and Clapper (2005) found the dependence of IGF concentrations and 17β-estradiol, where the application of estradiol led to an increase in the concentration of IGF in serum (Rempel and Clapper, 2002), and reduction of estradiol concentration in serum led to a reduction of the IGF concentration (Hilleson-Gayne and Clapper, 2005). According to these authors, therefore, estradiol might indirectly, through IGF axis, have an impact on the growth of pigs. These authors observed the most pronounced effects of estradiol on changes in the IGF concentration in boars.

On the other hand, by examining the effect of estrogens *in vitro* - in pig muscle cell cultures, Mau et al. (2008) have observed almost no effect on the proliferation of muscle cells at physiological concentrations, but the inhibitory effect of estrogen on proliferation of muscle cells, if the estrogen was administered in supraphysiological concentrations, was determined. Tsai et al. (2007) came to similar conclusions, and confirmed the *in vivo* reduction of body weight and the level of IGF in muscles of ovariectomized female rats with implants containing 17β estradiol. Enns and Tiidus (2010), observed the stimulatory effect of estrogen to rebuild muscle tissue in humans after injury through the activation and proliferation of satellite cells. It is possible that different results with respect to the effects of estrogen are a consequence of different gender used in the different experiments, or use of estrogen in combination with anabolic agents.

Plant hormones – phytoestrogens

Phytoestrogens are a group of chemical compounds of plant origin, which have either estrogenic or antiestrogenic effects in the organism. These compounds have been identified in more than 300 different plants (Tham et al., 1998), such as garlic, parsley, soybean, clover, alfalfa, wheat, barley, rice, carrot, potato, apple, pear, grape, etc. In the food standardly used both for human consumption and for animal nutrition, the most represented two categories of phytoestrogens are isoflavones and lignans. In addition to these two groups, there is a third group of phytoestrogens - coumestanes. In contrast to a widely present lignans, isoflavones are found almost exclusively in legumes. Especially rich source of these compounds is soy - plant species inevitable in animal nutrition (Tham et al., 1998; Reinli and Block, 1996).

Two compounds from the group of isoflavones which are now studied the most are daidzein and genistein. A huge attention was directed towards investigation of these isoflavones effects after the discovery of Akyame et al. (1987), who have observed that genistein is a potent inhibitor of protein tyrosine kinases - a group of enzymes which play a key role in the formation and uncontrolled growth of cancer cells. At the same time, the increase of awareness of more and more frequent occurrence of various diseases especially hormone-dependent tumors in Western countries compared to the eastern countries, especially Japan and China. It led the scientific population towards extensive research of

soy, a plant which dominates in the diet of humans in the Far East, and consequently phytoestrogens which are found in soy, and potentially could be used in the prevention and treatment of tumors. Total isoflavone content, and the amount of genistein and daidzein in particular soy products are shown in Table 1.

Table 1. *The amount of isoflavones in different soy products, mg isoflavones/kg feed*

Product	Total isoflavones	Daidzein	Genistein	Reference
Soybean 1	1176	365	640	Tham et al, 1998
Soybean 2	4215	1355	2676	
Roasted soybean	2661	941	1426	
Soy flour	2014	412	1453	
Tofu	532	238	245	
Tempeh	865	405	422	
Miso	647	272	281	
Soybean meal	/	395-488	506-695	Fritsche i Steinhart, 1999
Soybean hulls	/	< 0,1	18,4	
Soybean flakes	/	363-475	1275-1547	

Common characteristics of all phytoestrogens, are that their chemical structure is very similar to that of estrogen (Figure 1), and that in organism they have the ability to cause either estrogen, or anti-estrogenic response, depending on the concentration, the concentration of endogenous estrogen and individual characteristics of an individual, particularly gender and hormonal status (Tham et al., 1998).

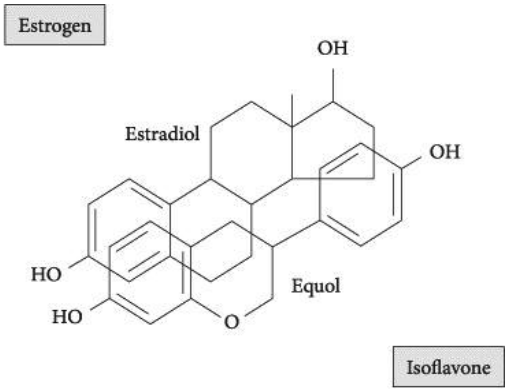

Figure 1. The similarity of the chemical structure of estrogens and isoflavones

These functions phytoestrogens exhibit binding to estrogen receptors ER in tissues: originally known, "classical" estrogen receptors ERα and newly found ERß, possibly have different roles in gene regulation, and also the presence of these receptors in various tissues differs. Thus, in rats the presence of ERα in the uterus, testis, ovary, kidney, epididymis, thyroid gland was observed, and Erß were the most present in the prostate, lung, bladder, brain (Kuiper et al., 1996. a, b, Setchell and Cassidy, 1999). In humans, ERα were determined in the testes, kidneys, and adrenal glands, both ERα and ERß were determined in blood vessels, mammary gland, uterus, ovaries, wnile the presence of only ERß was

found in the brain, lungs, thyroid gland, prostate, bladder, bones (Setchell and Cassidy, 1999). It is very important to stress the fact that the affinity of different estrogenic compounds towards two types of estrogen receptors varies, and specifically phytoestrogens have significantly higher affinity for ERß than to ERα (Kuiper et al. 1998).

Investigations of the presence and role of estrogen receptors in muscle tissue are of recent date. Wiik et al. (2009) reported the existence of both types of receptors in the muscles of humans, of both sexes and various ages. Their research showed the presence of ER in the nuclei of muscle fibers, and in the capillaries surrounding the fibers. The same results are shown by Kalbe et al. (2007), confirming for the first time the presence of ERα and ERß in several muscles of pigs. This means that the muscle tissue is a target tissue for the estrogen action, both endogenous, as well as those compounds that mimic estrogen, including isoflavones. Two compounds from the group of isoflavones whose effects on muscles are now studied intensively are daidzein and genistein.

The influence of isoflavones on muscle tissue

Due to the ability to mimic estrogen, it is possible that isoflavones could affect growth and carcass composition in farm animals. However, the results of authors who have examined the impact of isoflavones on muscle tissue in different animal species, the growth and carcass quality in general, are very different.

According to Lee et al. (2005) and Bidner et al. (1972), the use of different estrogenic compounds in the pig production leads to reduction in carcass fat and increase of muscularity. Payne et al. (2001) examined the effect of three levels of isoflavones in food in castrates and gilts fattening. Castrates received: 1. group: soybean meal (1.14 mg/g total isoflavones); 2.group: soy protein (0.06 mg/g total isoflavones); 3. group: soy protein with addition of isoflavones to the level as in the first group. Gilts received: 1. group: soybean meal (1.14 mg/g total isoflavones); 2. group: soybean meal plus supplement of isoflavones in concentrations twice higher than soybean meal; 3. group: soybean meal plus supplement of isoflavones in concentrations five times higher than in soybean meal. Authors monitored daily gain and food consumption, the cross section of MLD, fat thickness, percentage of muscle in the carcass and technological properties of meat (pH_{24}, meat temperature, meat color, hardness, etc.). Looking at the whole fattening period, isoflavones did not affect daily gain and daily food consumption in castrates, but in the final phase of fattening, the average daily weight gain and food consumption were significantly higher ($p<0.10$) in animals fed soy protein concentrate. Hull length, weight and percentage of lean meat, and lean meat daily gain were increased, while fat thickness decreased in castrates who received additional isoflavones in the diet. However, between barrows fed soybean meal, and barrows fed soy protein with added isoflavones there were no significant differences. Technological properties of meat did not differ ($p>0.10$) in barrows from different experimental groups. In gilts from three experimental groups there were no differences in any of the observed characteristics. Based on the results, Payne et al. (2001) concluded that isoflavones reduce fat and increase the amount of lean meat in fattening barrows but not in gilts, and that the addition of isoflavones above the amount normally found in soybean meal has no effect on the improvement of the carcass characteristics and meat quality.

Jiang et al. (2007) investigated the effects of different doses of isoflavones (0, 10, 20, 40, 80 mg/kg) in male broilers fed the same basal diet without soybean meal. The results showed that the addition of the isoflavones of 10 and 20 mg/kg increased the average daily weight gain and food consumption. Addition of 40 mg/kg isoflavones increased the water holding capacity of meat, and the pH value of the meat. Based on these results, Jiang et al.

(2007) concluded that the addition of isoflavones in the diet of male broilers had a positive impact on growth and meat quality.

Rehfeldt et al. (2007) investigated the effects of adding isoflavones daidzein to sows diet during late gestation on the properties of the muscle tissue in the offspring. Results of this study did not showed statistically significant differences in litter size, piglet birth weight and percentage of muscle, depending on the daidzein addition in sows diet. The interaction treatment x litter size showed a tendency of reducing muscle tissue in favor of the skin in piglets whose mothers received daidzein. The percentage of body fat in piglets originating from mothers treated with daidzein was higher ($p = 0.04$). Addition of daidzein had no effect neither on the weight of *m. semitendinosus* in piglets, nor on the number of fibers in the muscle cross section. Body weight at the end of fattening did not differ in slaughter pigs whose mothers were treated with daidzein compared to progeny of untreated sows, and also there was no difference in the amount of lean meat, backfat thickness and percentage of meat in the carcass. As to the histological features of *m. semitendinosus*, there was no difference in the size of the muscle cross section, the total number of muscle fibers, and the number of nuclei per fiber, but a difference was observed in the presence of different fiber types within the muscle. The offspring of treated sows had a higher number of fast twitch glycolytic (FTG) fibers and a lower number of fast twitch oxidative (FTO) fibers compared to the offspring of untreated sows. Also, a significant difference was observed depending on the gender: barrows had a significantly higher number of muscle fibers ($p=0,04$), smaller diameter ($p=0.03$) and a higher proportions of STO ($p=0.06$) and FTG ($p=0.03$) fibers compared to females. Based on these results authors pointed at a marginal effect of daidzein addition to sows diet during late pregnancy on characteristics of muscle tissue in the offspring.

According to Adamovic (2013) addition of different doses of daidzein in diet of pregnant sows from day 85 of gestation until the end of pregnancy, had no effect on the morphological characteristics of muscle: weight, length, volume and cross-section of the muscle in newborn piglets and slaughter pigs at the end of fattening. In this study, treatment of pregnant sows with daidzein during the late stages of pregnancy did not have statistically significant effects on the histological characteristics of *m. semitendinosus* neither in newborn piglets, nor in slaughter pigs at the end of fattening.

However, in experiments with cultures of muscle cells (*in vitro*), it was shown that isoflavones inhibit the growth and development of muscle cells. Jones et al. (2005) found that phytoestrogens, genistein in particular, at a concentration of $\geq$ 1 mmol / l, inhibits proliferation of rat muscle cells *in vitro*, but does not affect protein degradation. Similar results were obtained by Ji et al. (1999), who found that genistein strongly inhibits proliferation and fusion of myoblasts in rats, that the inhibition strength is dependent on the dose of genistein, and that the effective doses are already from 1 μmol/l. Authors have not observed adverse effects of genistein on protein degradation. Also, it was noticed that the negative effect of genistein on myoblast fusion is present only when genistein was added during the first 24 hours of initiation of miotubules forming. If genistein was added later, after the first 24 hours of initiation, it had no effect on the fusion of myoblasts. Regarding the synthesis of myosin, authors observed a detrimental effect of genistein at high doses and long exposure. At low doses (1 μmol/l), which correspond to a concentration in the serum of humans and rats in which soy is present in the diet, genistein increased the volume of myosin synthesis.

Mau et al. (2008) studied the direct effects of genistein and daidzein on proliferation of muscle cell cultures derived from newborn piglets. In this research doses of isoflavones

0.1, 1, 10 and 100 μM were applied, which could normally be measured in the serum after consuming infant feeding soy based formula, or plant feeds usually used in the pig diet. The results of this study showed that the effects of isoflavones depend on the dose of isoflavones used, and much stronger inhibitory effect of genistein, compared to daidzein, on the proliferation of muscle cells. Such an effect of genistein has been shown already at a dosage of 1 μM. On the other hand, daidzein, even at considerably higher doses - up to 10 μM, did not have a detrimental effect on the growth of pig muscle cells.

Similar research on the cultures of muscle cells of pigs was conducted by Rehfeldt et al. (2009). The same doses of genistein and daidzein (0.1, 1, 10, 20, and 100 μmol/l) were applied and 17β-estradiol (0.1, 1 nmol/l) during the last 26 hours of cell cultivation. Myogenic differentiation was not changed under the action of any of the concentrations of isoflavones or estradiol. At first sight this is inconsistent with the findings of Ji et al. (1999), but these authors found an inhibitory effect of genistein on the proliferation and myoblast fusion when it was administered during the initiation. Therefore Rehfeldt et al. (2009) conclude that the inhibitory effect of genistein is dependent on the stage of development of cell cultures, and therefore, if it is added later, genistein as well as daidzein and estradiol, have no effects on differentiation. However, these authors have confirmed the inhibitory effects of genistein, depending on the administered dose, and absence of effects of daidzein and 17β-estradiol in the synthesis of proteins. It has been found that low doses of genistein, daidzein, and 17β-estradiol decrease the degradation of proteins in cultured muscle cells, which could potentially contribute to increased accumulation of proteins in the pig skeletal muscles.

Conclusion

Most authors agree that application of high doses of isoflavones, especially genistein, would have a detrimental effects on protein synthesis and, if applied during initiation of muscle cells differentiation, it would inhibit normal development of muscle cells. However, some authors report positive effects of isoflavones on daily gain, lean meat content, fat reduction, muscle fiber types proportion etc., which seem to be dependant on the species, gender, dose of isoflavone used, and the moment of application. Since soy, as a very rich source of isoflavones, makes the two-thirds of protein requirements in animal feed, further investigations of possible positive as well as negative effects of those compounds on the muscle tissue should be made, in respect to meat quantity and quality. Also, since soy-based formulas are widely used in newborn children nutrition, the results from already conducted and some future experiments on animals would be very important for evaluation of possible effects of isoflavones in humans.

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References

1. Adamović I 2013. Uticaj dodavanja daidzeina u hranu suprasnih krmača na rast i razvoj mišićnog tkiva potomstva. Doktorska disertacija. Beograd, Univerzitet u Beogradu - Poljoprivredni fakultet.

2. Akyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M and Fukami Y 1987. Genistein, a Specific Inhibitor of Tyrosine-specific Protein Kinases. The Journal of Biological Chemistry 262, 5592-5595.
3. Bidner TD, Merkel RA and Miller ER 1972. Effects of a combination of diethylstilbestrol and methyltestosterone on performance, carcass traits, serum and muscle characteristics of pigs. J. Anim. Sci. 35, 525-533.
4. Enns DL and Tiidus PM 2010. The Influence of Estrogen on Skeletal Muscles: Sex matters. Sports Medicine 40, 1, 41-58.
5. Fritsche S and Steinhart H 1999. Occurrence of hormonally active compounds in food: a review. Eur. Food. Res. Technol. 209, 153-179.
6. Hancock DL, Wagner JF and Anderson DB 1991. Effects of estrogens and androgens on animal growth. In: Pearson, A. M and Dutson, T. R. (eds) Growth regulation in Farm Animals, Advances in Meat Research, Elsevier, London 7, 255-284.
7. Hilleson-Gayne CK and Clapper JA 2005. Effects of decreased estradiol 17β on the serum and anterior pituitary IGF-I system in pigs. Journal of Endocrinology 187, 369-378.
8. Ji S, Willis GM, Frank R, Cornelius SG and Spurlock ME 1999. Soybean isoflavones, Genistein and Genistin, Inhibit Rat Myoblast Proliferation, Fusion and Myotube Protein Synthesis. J. Nutr. 129, 1291-1297.
9. Jiang ZY, Jiang SQ, Lin YC, Xi PB, Yu DQ and Wu TX 2007. Effects of Soybean Isoflavone on Growth Performance, Meat Quality, and Antioxidation in Male Broilers. Poultry Science 86, 1356-1362.
10. Jones KL, Harty J, Roeder MJ, Winters TA and Banz WJ 2005. In vitro effects of soy phytoestrogens on rat skeletal muscle cells. J. Med Food 8 (3), 327-331.
11. Kalbe C, Mau M, Wollenhaupt K and Rehfeldt C 2007. Evidence for estrogen receptor alpha and beta expression in skeletal muscle of pigs. Hostochem. Cell Biol. 127, 95-107.
12. Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S and Gustaffson JA 1996a. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc. Natl. Acad. Sci. U.S.A. 93, 5925-5930.
13. Kuiper GGJM, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S and Gustaffson JA 1996b. Comparison of the ligand binding specificity and transcript tissue distribution of estrogenic receptors α and ß. Endocrinology 138, 863-870.
14. Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe HS, Van Der Saag PT, Van Der Burg B and Gustaffson JA 1998. Interaction of Estrogenic Chemicals and Phytoestrogens with Estrogen Receptor ß. Endocrinology 139, 4252-4263.
15. Lee CY, Ha SH, Baik KH, Sohn SH, Park BC and Park MJ 2005. Effects of estradiol implantation on growth, carcass traits and circulating concentrations of insulin like growth factors (IGFs) and IGF-binding protein-3 in finishing barrows. Livestock Prod. Sci. 96, 149-155.
16. Mau M, Kalbe C, Viergutz T, Nuernberg G and Rehfeldt C 2008. Effects of Dietary Isoflavones on Proliferation and DNA Integrity of Myoblasts Derived from Newborn Piglets. Pediatric Research 63, 39-45.
17. Payne RL, Bidner TD, Southern LL and Geaghan JP 2001. Effects of dietary soy isoflavones on growth, carcass traits and meat quality in growing-finishing pigs. J. Anim. Sci. 79, 1230-1239.
18. Rehfeldt C, Adamovic I and Kuhn G 2007. Effects of dietary daidzein supplementation of pregnant sows on carcass and meat quality and skeletal muscle cellularity of the progeny. Meat Science 75, 103-111.

19. Rehfeldt C, Kalbe C, Nuernberg G and Mau M 2009. Dose-Dependant Effects of Genistein and Daidzein on Protein Metabolism in Porcine Myotube Cultures. J. Agric. Food. Chem. 57, 852-857.

20. Reinli K and Block G 1996. Phytoestrogen content of foods – compendium of literature values. Nutr. Cancer 26, 123-148.

21. Rempel LA and Clapper JA 2002. Administration of estradiol-17beta increases anterior pituitary IGF-I and relative amounts of serum and anterior pituitary IGF-binding proteins in barrows. Journal of Animal Science 80, 214-224.

22. Setchell KDR and Cassidy A 1999. Dietary Isoflavones: Biological Effects and Relevance to Human Health. Journal of Nutrition 129, 758-767.

23. Tham DM, Gardner CD and Haskell WL 1998. Potential Health Benefits of Dietary Phytoestrogens: A Review of the Clinical, Epidemiological and Mechanistic Evidence. The Journal of Clinical Endocrinology and Metabolism 83, 2223-2235.

24. Tsai WJA, McCormick KM, Brazeau DA and Brazeau GA 2007. Estrogen Effects on Skeletal Muscle Insulin-Like Growth Factor 1 and Myostatin in Ovariectomized Rats. Exp. Biol. Med. 232, 1314-1325.

25. Wiik A, Ekman M, Johansson O, Jansson E and Esbjornsson M 2009. Expression of both oestrogen receptor alpha and beta in human skeletal muscle tissue. Histochem. Cell Biol. 131, 181-189.

Original paper

EFFECT OF VITAMIN E, LUTEIN, SELENIUM AND OIL MIXTURE ADDED TO FEED AND COOKING LENGTH ON YOLK COLOR AND EGG QUALITY

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Abstract

The aim of this research was to determine the quality of eggs laid by Tetra SL hens fed standard diet (B) or diet supplemented with 100 mg vitamin E/kg+200 mg/kg lutein+0.5 mg/kg selenium+5% oil mixture (BK). The effect of treatment was determined for yolk color values ($P<0.001$), which were more intense in the BK group (14.35) than in the B group (12.20). Other egg quality indicators (egg mass, portions of albumen, yolk and shell, Haugh units, albumen height, egg shell thickness and strength, pH values of albumen and yolk) exhibited no statistically significant differences ($P>0.05$). However, the treatment had statistically significant effect ($P<0.001$) on yolk color values for fresh eggs (B=12.00 and BK=14.42), as well as for eggs cooked for 7 minutes (B=9.14 and BK=13.00) and for 10 minutes (B=7.28 and BK=8.42). There was statistically significant effect of treatment, cooking length and their interaction on yolk lightness (L*). This value increased in dependence on cooking length (treatment B) and consequently, for the same yolks, intensity of redness (a*) was decreased, while intensity of yolk lightness (L*) in BK treatment was varying. The L* value decreased for eggs cooked for 7 minutes, but increased significantly for eggs cooked for 10 minutes, if compared to fresh eggs ($P<0.001$). The values of egg yolk lipid oxidation (TBARS) measured for fresh eggs (B=0.735 µg MDA/g and BK 0.745 µg MDA/g) and stored eggs (B=0.778 µg MDA/g and BK=0.805 µg MDA/g) were equal for both examined groups ($P>0.05$).

Key words: *lutein, oil mixture, selenium, vitamin E, yolk color*

Introduction

Lipids are the main ingredients of egg yolks (Ahn et al., 1997). Stability and content of lipid fraction affect the quality and sensory traits of eggs (Franchini et al., 2002.). Poultry feeds should contain more plant oils and should be supplemented with antioxidants that protect poultry and poultry meat and eggs from oxidation (Barroeta, 2007). Many research studies proved that higher concentration of PUFA in eggs supported lipid peroxidation and negatively influenced sensory traits and quality of eggs (Grashorn, 2005; Mohiti-Asli et al., 2008). Therefore, poultry feed is supplemented with antioxidants, such as vitamin E, selenium and lutein. Enriching eggs with omega-3 fatty acids combined with abovementioned antioxidants have several advantages. Vitamin E, lutein and selenium protect DHA from oxidation during absorption and metabolism, thus preventing occurrence of unfavorable "fishy" taste. Yolk lipids are necessary for efficient absorption of vitamin E and lutein in human digestive system (Van het Hoff, 2000). Interaction between lutein and vitamin E and phospholipids results in increased yolk antioxidative

potential, which has positive effects on preservation of egg freshness during storage. Selenium, as a part of antioxidative enzyme glutathione peroxidase, protects intestine membrane from lipid oxidation during digestion of DHA (Surai and Sparks, 2001). Besides antioxidative effects, lutein is also a natural pigment and different studies indicated that yolk color in table eggs was an important trait from a viewpoint of consumers (Hernandez et al., 2000; Roberts, 2004). Since it is known that carotenoids cannot be produced by animals, their intake into the organism is necessary through feed. They are stored in fatty tissue, and in egg yolk, therefore adding of some kind of food colorant to the feed is necessary. The aim of this research was to determine the effect of treatment (supplementation of various oils in hens' feed combined with three antioxidants) and the cooking length on yolk color and quality of eggs.

Materials and methods

The research was carried out on 120 hens of the Tetra SL hybrid that lay eggs of brown shell color. Experimental period lasted for 5 weeks, and hens were 40 weeks old at the beginning of experiment. The groups B and BK were formed, differing only in the treatment. The B group was fed standard diet for laying hens, while the BK group was fed diet supplemented with 100 mg/kg vitamin E+200 mg/kg lutein+0.5mg/kg Se+5% oil mixture. Each group consisted of 12 cages with 5 hens in each. Analysis of external and internal quality of fresh table eggs was determined on 40 eggs of the L class. The following external egg quality indicators were analyzed: egg mass, egg shell strength and thickness, egg shell mass. Assessed internal egg quality indicators were: mass of albumen and yolk, color of yolk, albumen height, Haugh units (HU), pH values of albumen and yolk. Portions of main parts in egg were also calculated. Mass of egg and its main parts (albumen, yolk and shell) was determined on the scales PB 1502-S. Egg shell strength was measured in the Eggshell Force Gauge Model-II device. Thickness of egg shell was measured by electronic micrometer providing accuracy of 0.001 mm in the middle of egg shell. HU and albumen height were determined by the Egg Multi-Tester EMT-5200. Values of pH of albumen and yolk were measured by the pH meter MP 120. Yolk color was determined by using DSM Color Fan (values were presented in the table as La Roche; DSM Nutritional Products, Basel, Switzerland). Other indicators of yolk color (L*, a* and b*) were measured by Minolta CR-300 colorimeter (Minolta, Osaka, Japan). A total of 28 yolks (14 fresh and 14 stored) was used to determine oxidation of lipids, according to the modified methods of McDonald and Hultin (1987) and Botsoglou et al. (1994). Eggs were stored for 28 days in a refrigerator at +4°C. Research results were processed by using Statistica for Windows, version 12.0 (StatSoft Inc., 2013). Data processing was completed by applying GLM procedure for calculation of analysis of variance. Examined factors referred to the treatments (B and BK) and the length of cooking (0, 7 and 10 minutes). Differences between groups were determined by post-hoc analysis using the Fisher's LSD test.

Results and discussion

The Table 1 presents the results of fresh egg quality. According to the egg mass of both examined groups, and following the Regulations on egg quality (OJ 115/06 and 76/08), all eggs belong to the market class L (B=68.28 g and BK=67.13 g; P>0.05). Both examined groups of eggs had similar portions of main parts (albumen= 61.92% and 62.30%, yolk 25.69% and 25.27% and egg shell 12.34% and 12.42%, respectively), meaning that treatments did not have any effect on stated indicators. The HU values were higher in the

treatment B than in the treatment BK (78.48 and 76.75, respectively), however, obtained difference was not statistically significant. Specification for the device Egg multi tester used for measuring of HU values above 72 refer to the freshest eggs (extra fresh). Referring to freshness, when comparing values of HU obtained in our experiment with values from specification, it was noticed that eggs in both groups were above lower level for extra fresh eggs, which is also in line with the provisions of the Regulations on egg quality (OJ 115/06 and 76/08).

Table 1. *Indicators of fresh egg quality*

Characteristics	B	BK	SEM	Probability
Egg weight (g)	68.28	67.13	0.710	NS
Albumen percentage (%)	61.92	62.30	0.312	NS
Yolk percentage (%)	25.69	25.27	0.244	NS
Shell percentage (%)	12.34	12.42	0.188	NS
Yolk color (La Roche)	12.20^{b}	14.35^{a}	0.134	<0.001
Haugh units	78.48	76.75	1.927	NS
Height albumen (mm)	6.53	6.52	0.261	NS
Shell thickness (mm)	0.407	0.404	0.008	NS
Shell breaking strength (kg/cm^2)	3.179	2.917	0.135	NS
pH albumen	8.65	8.37	0.103	NS
pH yolk	5.96	5.97	0.015	NS

*B=standard laying hens' mixture; BK=100 mg/kg vit. E+200 mg/kg lutein+0.5mg/kg Se+5% oil mixture.
NS- non-significant. Values in columns marked by different exponents ^{a,b} differ statistically.

More stronger and thicker egg shell was observed in the group B then in BK (3.179 kg/cm^2 and 0.407 mm, compared to 2.917 kg/cm^2 and 0.404 mm, respectively), however, there was no statistically significant influence of the treatment (P> 0.05). The study on effects of egg mass on some indicators of egg quality, Şekerog˘lu and Altuntaş (2009) stated that eggs of medium weight had the thickest shell (0.400 mm), while the thinnest shell was determined for eggs classified as extra-large (0.382 mm). The longer hens remain in production, the thinner egg shell will become. The optimum thickness of shell of brown eggs ranges from 0.330 to 0.340 mm (Kralik et al., 2008). Comparison of our results with the above mentioned values led to conclusion that the thickness of egg shell was above the optimal values in both examined groups. Statistically significantly (P<0.001) more intensive color of fresh yolk was determined in the BK treatment (14.35) than in the B treatment (12.20). It was assumed that such differences in egg yolk color were caused primarily by lutein, which was supplemented in feed of the BK group. Lutein belongs to the group of fat-soluble carotenoids, which are also known as xanthophylls (Yeum and Russell, 2002). Lutein is primarily used in chicken feed as a natural pigment to intensify yellow color of yolk, but later on, its antioxidant activity was also proven (Lim et al., 1992). Jang et al. (2014) also stated that supplementation of lutein in hens' feed significantly affected egg yolk color (P<0.05). Since the vitamin E was also added to feed of the BK group its influence shall be also considered because it can also intensify the egg yolk color (Carrillo-Dominguer et al., 2012). Values of pH in albumen and yolk were similar for both groups of eggs (P>0.05). It was determined that the treatment and the length of cooking had statistically significant effect on intensity of egg yolk color as measured by DSM La Roche Color Fan (Table 2). If compared to the treatment B, more intensive yolk color was determined in the experimental group BK (12.00 and 14.42, respectively). Egg yolk color intensity was significantly reduced as depending on cooking length. Group B with egg yolk color of 12.00 reduced color to 9.14 after 7 minutes of

cooking and to 7.28 after 10 minutes of cooking. The decreasing value for yolk color was also determined in the group BK (14.42 > 13.00 > 8.42, respectively). Fresh eggs exhibited no difference in yolk color, i.e. the L* values were similar (B=61.85 and BK=59.24).

Table 2. *Effect of treatment and cooking length and their interaction on yolk color*

Cooking length (CL; min)	Treatment (*T)	La Roche	Lightness (L*)	Redness (a*)	Yellowness (b*)
0	B	12.00^{c}	61.85^{cd}	9.78^{cb}	52.17^{b}
	BK	14.42^{a}	59.24^{ed}	8.98^{cb}	27.33^{d}
7	B	9.14^{de}	73.77^{b}	5.75^{de}	54.21^{b}
	BK	13.00^{b}	48.12^{f}	14.77^{a}	42.62^{c}
10	B	7.28^{f}	83.28^{a}	4.93^{e}	55.25^{b}
	BK	8.42^{de}	63.25^{c}	10.63^{b}	66.84^{a}
SEM		0.265	1.332	0.662	2.128
Probability	CL	<0.001	<0.001	<0.001	<0.001
	T	<0.001	<0.001	<0.001	<0.001
	CL*T	<0.001	<0.001	<0.001	<0.001

*B=standard laying hens' feed; BK=100 mg/kg vit. E+200 mg/kg lutein+0.5mg/kg Se+5% of oil mixture.
NS- non-significant. Values in columns marked by different exponents $^{a-f}$ differ statistically.

However, by cooking the B group eggs, the L* values statistically significantly increased (61.85<73.77<83.28), while L* values of yolk in the BK group varied (59.24>48.12<63.25). Similar research results were published by Englmaierova and Skrivan (2013). These authors reported that hen groups fed diets supplemented with lutein laid eggs, whose yolk cooked for 5 minutes, decreased the L* values in comparison with fresh yolk, while all the values in yolks cooked for 7 minutes were increased. Since yolks from treatment B increased the L* value along with length of cooking, a significant lowering of value a* (redness) occurred for 10 minutes of cooking eggs, while value b* (yellowness) was balanced. On the contrary, the length of cooking in BK treatment had statistically significant effect on the increase of a* and b* values. Similar results were obtained by Englmaierova and Skrivan (2013). Figure 1 shows values of lipid oxidation in fresh eggs and in eggs stored for 28 days at 4°C. It is concluded that values of TBARS in fresh eggs of both treatment were similar (B=0.735µg MDA/g and BK=0.745µg MDA/g). Slightly intensive lipid oxidation was observed in eggs of BK treatment that were stored for 28 days at 4°C if compared to eggs from treatment B (0.805µg MDA/g and B=0.778µg MDA/g, respectively). Such occurrence can be explained with the fact that treatment BK, unlike treatment B, was given feed supplemented with oil mixture (5%), which could cause slightly higher oxidation of egg yolk lipids. However, TBARS values that indicate lipid oxidation were not influenced by treatment, length of egg storage and their interaction (P>0.05).

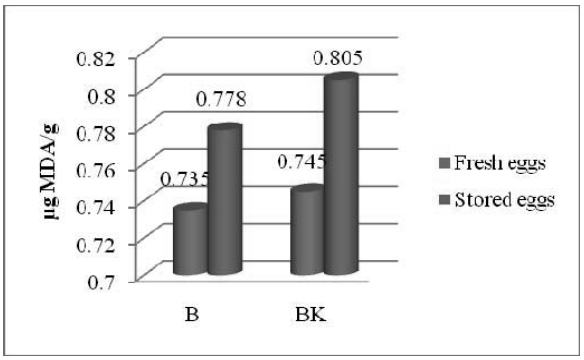

Figure 1. *Values of egg yolk lipid oxidation*

Conclusion

The paper elaborated effects of treatments (B=control group given diets of standard composition and BK=diets supplemented with 100 mg/kg vitamin E+200 mg/kg lutein+0.5mg/kg Se+5% oil mixture), cooking length (0, 7 and 10 minutes) and their interactive influence on yolk color and egg quality. Research results pointed out that supplementation of lutein, vitamin E, selenium and oil mixture had statistically significant influence on occurrence of more intensive color of fresh yolk, while cooked eggs had lighter color of yolks. The B group exhibited less intensive lowering of yolk color when depended on cooking length than the treatment BK. Intensity of yolk color increased when depending on cooking length (treatment B), and consequently the same yolks exhibited decrease of redness intensity (a*), while values for yellowness (b*) were similar. That was not the case in the treatment BK, where the intensity of yolk color (L*) varied. Value L* decreased in eggs cooked for 7 minutes, but in eggs cooked for 10 minutes it significantly increased if compared to fresh eggs. Depending on the cooking length, values a* and b* of BK yolks follow the trend of increase. The treatment, length of storage and their interaction had no effects on obtained results for egg yolk lipid oxidation.

References

1. Ahn DU, Kim SM and Shu H 1997. Effect of egg size and strain and age of hens on the solids content of chicken eggs. Poultry Science 76, 914-919.
2. Barroeta AC 2007. Nutritive value of poultry meat: relationship between vitamin E and PUFA. World's Poultry Science Journal 63(2), 277-284.
3. Botsoglou N.A., Fletouris D.J., Papageorgiou G.E., Vassilopoulos V.N., Mantis A.J. and Trakatelliss A.G. 1994: Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. Journal of Agricultural and Food Chemistry 42(9), 1931–1937.
4. Carrillo-Dominguez, S, Avila GE, Vasquez PC, Fuente B, Cavalo CC, Carranco JME and Perez-Gil Romo F 2012. Effects of adding vitamin E to diets supplemented with sardine oil on the production of laying hens and fatty-acid composition. African Journal of Food Science 6(1), 12-19.
5. Englmaierova M and Skrivan M 2013. The effect of source of carotenoids in diet and cooking on yolk color and egg quality of hens. XV European Symposium on the Quality of Eggs and Egg Products and XXI European Symposium on the Quality of Poultry Meat,

Proceedings World's Poultry Science Journal, Volume (69), Supplement, ID 203, Bergamo, Italy, 15-19.09.2013. EISSN: 1743-4777.

6. Franchini A, Sirri F, Tallarico N, Minelli G, Iaffaldano N and Meluzzi A 2002. Oxidative stability and sensory and functional properties of eggs from laying hens fed supranutritional doses of vitamins E and C. Poultry Science 81, 1744-1750.
7. Grashorn MA 2005. Enrichment of egg and poultry meat with biologically active substances by feed modifications and effects on the final quality of the product. Polish Journal of Food and Nutrition Science 14(55), 15-20.
8. Hernandez JM, Blanch AJ and Roche FHL 2000. Perceptions of egg quality in Europe. International Poultry Production 8(5), 7-11.
9. Jang I, Ko Y, Kang S, Kim S, Song M, Cho K, Ham J and Sohn S 2014. Effects of Dietary Lutein Sources on Lutein-Enriched Egg Production and Hepatic Antioxidant System in Laying Hens. Journal of Poultry Science 51, 58-65.
10. Kralik G, Has-Schon E, Kralik D, Šperanda M 2008. Peradarstvo biološki i zootehnički principi. Sveučilišni udžbenik, Grafika, Osijek. 64-72.
11. Lim BP, Nagao A, Terao J, Tanaka K, Suzuki T and Takama K 1992. Antioxidant activity of xanthophylls on peroxyl radical-mediated phospholipid peroxidation. Biochimica et Biophysica Acta 1126, 178-184.
12. Mc Donald RE and Hultin HO 1987. Some characteristics of the enzymic lipid peroxidation system in the microsomal fraction of Flounder skeletal muscle. Journal of Food Science 52(1), 15-21.
13. Mohiti-Asli M, Shariatmadari F, Lotfollahian H and Mazuji MT 2008. Effect of supplementing layer hen diets with selenium and vitamin E on egg quality, lipid oxidation and fatty acid composition during storage. Canadian Journal of Animal Science 88, 475-483.
14. Regulations on Egg Quality (in Croatian: Pravilnik o kakvoći jaja), Official Journal of the Republic of Croatia 115/06 and 76/08.
15. Roberts JR 2004. Factors Affecting Egg Internal Quality and Egg Shell Quality in Laying Hens. Journal of Poultry Science 41, 161-177.
16. Sekerog˘lu A and Altuntaş E 2009. Effects of egg weight on egg quality characteristics. Journal of the Science of Food and Agriculture 89(3), 379-383.
17. StatSoft, Inc. (2013). STATISTICA (data analysis software system), version 12.0. www.statsoft.com.
18. Surai PF and Sparks NHC 2001. Designer eggs: from improvement of egg composition to functional food. Trends in Food Science and Technology 12, 7-16.
19. Van het Hoff KH 2000. Dietary Factors That Affect the Bioavailability of Carotenoids. Journal of Nutrition 130, 503-506.
20. Yeum KJ and Russell RM 2002. Carotenoid bioavailability and bioconversion. Annual Review of Nutrition 22, 483-504.

Original paper

THE EFFECT OF RAW SOYBEANS IN MIXTURES FOR LAYING HENS ON EGG QUALITY AND EGG SHELL QUALITY

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Abstract

The study was conducted on hens at the age of 49 - 57 weeks to identify opportunities for replacing, with raw grain, thermally processed soybean Lana variety with a reduced level and Lydia variety with a standard level of trypsin inhibitor. The effect of using different levels of participation of both varieties of raw soybean in mixtures for layers on egg quality and eggshell quality was examined. The research was conducted according to the principle of two-factorial experiment (2 varieties x 4 levels of participation of raw soybeans). The average values of properties of the external and internal egg quality were under statistically significant influence of the level of participation of raw soybean in the mixture. Share of 8 % of raw soybeans of both varieties in diets for layers influenced significantly lower eggshape index, albumen height and Haugh's unit in relation to the mixture with a lower level of participation of raw soybeans. Yolk color and eggshell firmness were not significantly influenced by the studied factors.

Key words: *egg quality, egg shell quality, laying hens, soybean, trypsin inhibitor*

Introduction

Various methods of thermal treatment increase the nutritional value of soybean but result in the consumption of large amounts of energy and thus increase the cost of food. Through plant breeding, varieties with reduced content of specific anti-nutritive substances have been created. As a result of the domestic soybean breeding program focused on a reduced trypsin inhibitor (TI) activity, in our conditions, a Lana variety was created with lower TI than standard varieties.

Trypsin inhibitors reduce the utilization of soy protein and are the main antinutritive factor in raw soybean that prevents the utilization of nutrients which this feed is rich in (Zhang et al., 1993). In addition to the TI, lectins are present in the soybean as important antinutritive substances (Douglas et al., 1999).

Comparing the nutritional value of soy with lower TI in the experiments conducted on chickens (Han et al., 1991), layer hens (Zhang et al., 1991) and pigs and chickens (Palacios et al., 2004), better product results have been determined when compared with standard soybean. Cook et al. (1988), in a study on pigs, have found that the negative effects of the use of raw soybeans decreased with the age of the animal. Latshaw and Clayton (1976), found that the increase of the share of thermally unprocessed soybeans in diets for laying

hens diet gradually reduces egg laying capacity and egg weight. Senkoylu et al. (2005) and Koci et al. (1997) established no significant differences in production performance between laying hens fed diets with different levels of participation of full-fat soybean.

The aim of this study was to investigate the effects of replacing a portion of heat-treated soybean of Lydia standard variety and Lana variety with reduced TI, with raw grain of both varieties in the diet for laying hens, on egg quality and egg shell quality.

Material and methods

The study was conducted at the experimental farm of the Institute of Animal Husbandry in Zemun using light line hybrid hens Isa Brown. The mixtures for laying hens contained two local varieties of soybean, heat-treated and untreated, or raw, Lana variety with reduced trypsin inhibitor and Lydia standard variety (Table 1). Effect of different levels of raw soybeans in diets on egg quality and egg shell quality was determined by two factorial experiment 2 x 4 (2 varieties x 4 levels of participation of raw grain in the mixture) with a total of 8 treatments (a total of 512 monitored birds).

Table 1. *Level of trypsin inhibitor in soybean*

Treatment	Raw soybean		Heat-treated soybean	
Variety	Lana	Lydia	Lana	Lydia
TI (mg/g)	17.71	36.74	4.38	14.03

The experiment was conducted on hens aged 49 - 57 weeks. In the preparation of the diet the recommendations for the studied hybrid were used. Raw material composition of the mixture was the same with adjustment for soybean variety and the relationship between heat-treated and raw grain to achieve the objective of the research. Participation of heat-treated soybean of both varieties was 8 % in the mixture and it was included in the two control treatments (K). In trial groups, (I) 6 % of 8 % of whole soybeans contained in the mixture was heat-treated and 2 % was raw soybean. In the group (II) 4 % of heat treated and 4 % of raw soybean was added to the mixture. In the group (III) diet included only 8 % of the raw soybeans. Ingredients of mixtures and chemical composition are given in Table 2.

Random samples of 15 eggs were taken from each treatment over a period of 7 days during the experiment and the quality of eggs and egg shell was investigated. The quality of the eggs was studied on fresh eggs, immediately after collection, and thus the resulting score on the initial quality of table eggs was obtained.

Egg quality traits were divided on the properties of the external and internal egg quality. Determination of the external quality of eggs included egg mass and egg shape index. The following internal properties of egg quality were determined: albumen height, yolk color and Haugh's unit obtained as a logarithmic function of egg mass and height of thick egg white.

Eggshell quality included the determination of the following characteristics: egg shell mass, shell thickness and shell deformation and breaking force were determined according to the method stated by (Pavlovski and Vitorović, 1996).

Table 2. *Ingredients and chemical composition of mixtures–diet for layer hens during the experiment (%)*

Feeds	Groups (Treatments)							
	Lana				Lydia			
	K	I	II	III	K	I	II	III
Heat-treated (extruded) soybean	8	6	4	0	8	6	4	0
Raw soybean	0	2	4	8	0	2	4	8
Corn	59	59	59	59	59	59	59	59
Soybean meal	15	15	15	15	15	15	15	15
Sunflower meal	6	6	6	6	6	6	6	6
Livestock lime granules	8	8	8	8	8	8	8	8
Livestock lime, powder	2	2	2	2	2	2	2	2
Monocalcium phosphate	1	1	1	1	1	1	1	1
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Mikozel	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Σ (%)	100	100	100	100	100	100	100	100
Chemical composition								
ME, MJ/kg	11.84	11.78	11.76	11.70	11.84	11.78	11.76	11.70
Crude protein	15.3	15.6	15.8	15.4	15.9	15.4	15.3	15.1
Crude fat	5.12	5.11	5.16	5.14	4.99	4.99	4.93	4.91
Crude fibre	4.87	4.64	4.51	4.40	4.18	4.33	4.25	4.35
Ash	12.40	12.75	12.43	12.9	13.10	12.50	13.04	13.14
Calcium	3.53	3.97	3.72	3.66	3.69	4.01	3.65	3.87
Total phosphorus	0.65	0.58	0.60	0.63	0.62	0.57	0.59	0.60
Sodium	0.19	0.19	0.18	0.16	0.21	0.17	0.16	0.19

The software package STATISTICA, version 12 (Stat Soft Inc.) was used for statistical analyses. The level of statistical significance of differences between the groups was determined by Tukey-test.

Results and discussion

Average values of egg quality traits obtained during the study are shown in Table 3. In the study, no statistical significance for the mass of eggs depending on diet containing different soybean varieties was observed. Statistical analysis of the level of participation of raw soybeans in mixtures indicated that the differences between the groups were statistically significant ($p<0.01$). With the increase in the share of raw soybeans in mixtures the egg mass gradually decreased. The highest average value of egg mass was found in layers in Lydia-K group (64.80 g) and the lowest in layers in Lydia-III group (62.61 g). The differences in egg mass between the groups were not influenced by the interactive effects of the studied factors. Perez-Maldonado et al. (2000), in the diet for laying hens from 29-48th weeks of age, which included raw soybeans with a lower TI levels, obtained the results showing higher egg mass (63.1 g) compared to the raw standard variety of soybean (62.1 g).

Feeding hens diets containing different soybean varieties during the test did not cause significant changes in egg shape index. A significant ($p<0.05$) influence of the level of raw soybean in diet on the egg shape index was observed. The highest average shape index was measured in eggs of laying hens fed with 2 % raw soybeans (77.74 %), while the use of 8

% of raw soybean influenced the expression of the smallest values of this parameter (77.22 %). Analysis of the interaction of two tested factors indicated very significant differences ($p<0.01$) between the groups. The lowest average values of the shape index during the study form (indicating more oval egg shape) were found in eggs of hens from Lydia-III group while the highest mean egg shape index (the most elongated eggs) were determined in eggs of hens from Lana-I group. Established values of egg shape index (from 76.61 % to 77.82 %) showed the value of this indicator in range of desired values for this property and in accordance with the data obtained by (Mašić and Pavlovski, 1994).

Table 3. *Egg quality properties*

Factor	Variety	Level of raw soybean, %	Parameters	Egg quality properties				
				Egg mass(g)	Shape index	Albumen height(0,1mm)	Haugh units	Yolk colour
Variety	Lana		x	63.30	77.39	81.47	88.20	12.01
			Sd	5.05	2.15	15.49	9.28	0.72
	Lydia		x	63.60	77.42	80.71	87.79	12.00
			Sd	5.30	2.29	14.58	8.78	0.74
Level of raw soybean, %		0%	x	64.48^{x}	77.27^{b}	82.50^{x}	88.55^{x}	11.92
			Sd	4.94	2.32	14.96	8.57	0.73
		2%	x	63.28^{y}	77.74^{a}	81.88^{x}	88.83^{x}	12.09
			Sd	4.79	2.31	14.68	8.69	0.73
		4%	x	63.13^{y}	77.39^{ab}	81.72^{x}	88.30^{x}	12.03
			Sd	5.44	2.15	15.27	8.84	0.77
		8%	x	62.89^{y}	77.22^{b}	78.25^{y}	86.29^{y}	11.99
			Sd	5.39	2.07	14.96	9.81	0.68
Variety x Level	Lana	0% (K)	x	64.20	77.27^{xy}	83.92	89.36	12.01
			Sd	4.56	2.26	14.71	8.27	0.72
		2% (I)	x	63.10	77.88^{x}	81.52	88.44	12.09
			Sd	4.55	2.26	16.10	9.71	0.70
		4% (II)	x	62.70	76.98^{y}	80.96	87.97	11.95
			Sd	5.57	2.02	15.01	8.90	0.80
		8%(III)	x	63.20	77.47^{xy}	79.48	87.02	12.01
			Sd	5.39	1.98	15.94	10.08	0.64
	Lydia	0% (K)	x	64.80	77.28^{xy}	81.09	87.74	11.84
			Sd	5.29	2.38	15.13	8.81	0.74
		2% (I)	x	63.50	77.61^{xy}	82.24	89.23	12.08
			Sd	5.01	2.37	13.17	7.55	0.76
		4% (II)	x	63.52	77.82^{x}	82.48	88.64	12.10
			Sd	5.30	2.21	15.54	8.80	0.73
		8%(III)	x	62.61	76.97^{y}	77.01	85.55	11.98
			Sd	5.41	2.14	13.87	9.51	0.72
Two-factorial variance analysis (p value)								
Variety				0.303	0.836	0.385	0.441	0.755
Level of raw soybean				0.001	0.026	0.003	0.003	0.056
Variety x Level				0.422	0.002	0.194	0.201	0.053

*x-y, The values in each column without common superscript are significantly different at the level of 1%
*a-b, The values in each column without common superscript are significantly different at the level of 5%

The initial quality of the eggs is important for maintaining the qualities for the direct consumer. Under the influence of different varieties there were no statistically significant differences in the height of egg white. It has been found that the level of 8 % of participation of raw soybean in diet affected significantly lower ($p<0.01$) egg white heights as compared to the other groups. Interaction effect of the studied factors showed no statistically significant differences between groups. Realized average height ranged from (77.01) in the Lydia-III group to (83.92) in the Lydia-K group, which indicated a good initial egg quality but also a great variability of these properties both in groups and within groups. Mašić and Pavlovski (1994), reported that the height of egg white has the greatest variability among internal egg quality traits. Senkoylu et al. (2005), reported that the height of egg white among groups of hens fed diets containing different amounts of full-fat soy is not significantly different.

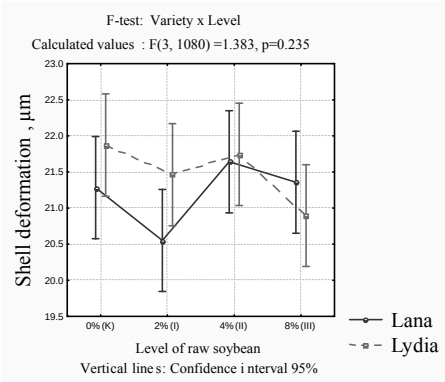
Haugh units featured less variability in relation to the height of egg whites since for an objective assessment of the internal quality of eggs the HU are more appropriate indicator. There was no significant effect of variety on the value of Haugh unit, but it was determined that the level of participation of raw soybean of 8 % in mixtures affect significantly lower ($p<0.01$) values compared to the other groups. In the analysis of the interactive influence of both factors no statistical significance was observed. The average value of Haugh units during the experiment ranged from (85.55) in the Lydia-III group to (89.36) in the group of Lana-K. Senkoylu et al. (2005), reported lower values of the Haugh units (81.8 to 84.2) in comparison to the results obtained in our study.

Soybean varieties and the level of participation of raw soybeans in mixtures, as well as the interaction effect of the studied factors had no statistically significant effect on the intensity of the color of egg yolks in the present research. The average value of the egg yolk color ranged from (11.84) in the Lydia-K group to (12.10) Roshe points in the Lydia-II group. Considering that the mixtures used did not differ in the share of nutrients that can affect the yolk color the average value of this indicator of egg quality was consistent across groups. Jokić et al. (2004), reported that the color of egg yolk may be affected by the increasing participation of xanthophyll- and pigment-rich nutrients that control this feature.

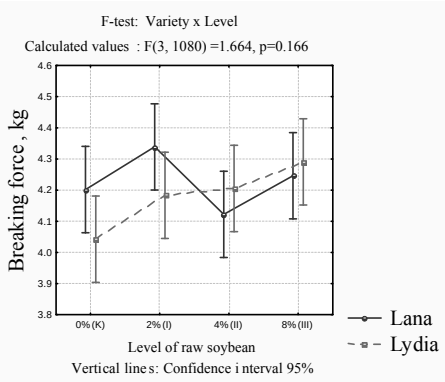
In graphs 1, 2, 3 and 4 the characteristics of eggshell quality are shown. Poor quality eggshell may have a significant economic impact on lowering the value of eggs. The eggshell is the protection of the internal contents of eggs from mechanical damage and contamination. The results indicate that there was no significant effect of soybean varieties and levels of participation of raw soybeans and their interaction effects on indicators of shell strength, deformation, breaking force, mass and shell thickness.

Eggshell deformation is a parameter which indirectly indicates the strength of eggshell. It represents a value that expresses the deflection of eggshell under a pressure of 500 g in the equatorial part of the egg. Lower values of this parameter of the eggshell quality indicate its greater resistance to pressure, or to a potentially tougher/firmer eggshell. The average values of the parameter (Graph 1) in the course of experiment ranged from 20.55 μm in the Lana-I group to 21.87μm in the Lydia-K group. The breaking force indicates a minimum force necessary (expressed in kg) which leads to the breaking of the eggshell. This is a direct indicator of the eggshell strength/firmness. The mean value of this indicator (Graph 2) of eggshell quality ranged from (4.04 kg) in Lydia-K group to (4.34 kg) in Lana-I group. The average value of the eggshell mass (Graph 3) in the study varied from (8.62 g) in Lydia-III group to (8.90 g) in the Lydia-K group, and the average value of the thickness of the shell (Graph 4) varied from (32.49) in the Lydia-K and Lydia-II groups to (33.02) in

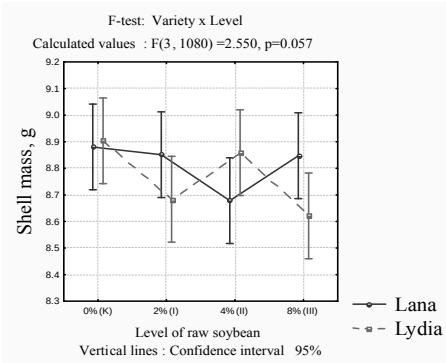
Lydia-III group. Senkoylu et al. (2005), observed no significant differences in egg shell thickness (0.294 mm to 0.298 mm) with a gradual increase in the share of full-fat soybeans in mixtures for laying hens.



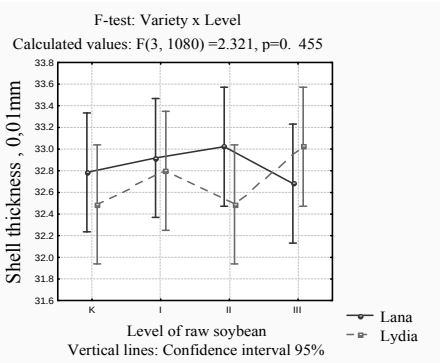
Graph 1. *Average shell deformation, µm*



Graph 2. *Average breaking force, kg*



Graph 3. *Average shell mass, g*



Graph 4. *Average shell thickness, 0.01mm*

Conclusion

Average property values of external and internal egg quality were under the statistically significant influence of the level of participation of raw grain in the mixture. Share of 8% of raw soybean of both varieties in diets for laying hens influenced significantly lower egg mass value, egg shape index, albumen height and Haugh's unit.

Indicators of the strength of egg shell: shell deformation, breaking force, mass, and egg shell thickness were not significantly influenced by the studied factors or under the influence of their interaction effects.

Acknowledgments

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References

1. Cook DA, Jensen AH, Fraley JR and Hymowitz T 1988. Utilization by growing and finishing pigs of raw soybeans of low Kunitz trypsin inhibitor content. J. Anim. Sci. 66, 1686-1691.
2. Douglas M, Parsons C and Hymowitz T 1999. Nutritional evaluation of lectin-free soybeans for poultry. Poultry Science 78, 91-95.
3. Han Y, Parsons CM and Hymowitz T 1991. Nutritional evaluation of soybeans varying in trupsin inhibitor content. Poultry Science 70(4), 896-906.
4. Jokić Ž, Kovčin S and Joksimović-Todorović M 2004. Ishrana živine. Poljoprivredni fakultet, Beograd.
5. Koci S, Kociova Z, Ceresnakova Z, Palanska O, Matrai T 1997. The effect of full fat extruded soya on the performance and produce quality in layers and broilers. Zivocisna Vyroba 42, 67–71.
6. Latshaw JD and Clayton PC 1976. Raw and heated soybeans in laying diets. Poultry Science. 55, 1268-1272.
7. Mašić B and Pavlovski Z 1994. Mala jata kokoši nosilja u različitim sistemima držanja. Monografija, Naučni institut za stočarstvo, Beograd.
8. Palacios MF, Easter RA, Soltwedel KT, Parsons CM, Douglas MW, Hymowitz T and Pettigrew JE 2004. Effect of soybean variety and processing on growth performance of young chicks and pigs. J. Anim. Sci. 82, 1108–1114.
9. Pavlovski Z and Vitorović D 1996. Direktan metod za određivanje čvrstoće ljuske jaja. Nauka u živinarstvu 3-4, 171-175.
10. Perez-Maldonado RA, Mannion PF and Farrell DJ 2000. Raw soybenans selected for low trypsin inhibitor for activity poultry diets. Animal Feed Science and Technology. Proc. Aust. Poult. Sci. Sym.
11. Senkoylu N, Samli HE, Akyurek H, Agma A and Yasar S 2005. Use of high levels of full-fat soybeans in laying hen diets. J. Appl. Poult. Res. 14, 32-37.
12. Zhang Y, Parsons M and Hymowitz T 1991. Effect of soybeans varying in trypsin inhibitor content on performance of laying hens. Poultry Science 70, 2210-2213.
13. Zhang Y, Parsons CM, Weingartner KE and Wijeratne WB 1993. Effect of extrusion andexpelling on the nutritional quality of conventional and kunitz trypsin inhibitor-free soybeans. Poult. Sci. 72, 2299–2303.

Review paper

INCREASE OF THE LUTEIN CONTENT IN HENS' EGGS

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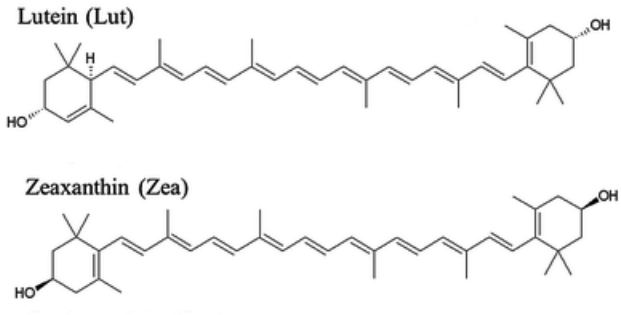
Abstract

Lutein is a plant pigment that belongs to the xantophyll group of carotenoids. In nature it is widespread (prevalent) in green leafy vegetables (spinach, kale, collard greens, lettuce) but also in peppers, tangerines, corn and egg yolk. Since it cannot be synthesized in the body it has to be taken with food. In human body lutein is concentrated in the retina and macula lutea and its content in those tissues rises with increased intake through food or supplements. It has antioxidant activity, protects eyes from high-energy blue light and helps in reducing the risk of developing age-related macular degeneration and cataracts. Until now, lutein was used in poultry industry mostly for pigmenting broiler's meat and skin and egg yolk and lately there have been more studies whose goal is to increase lutein content in yolk and production of enriched, functional food. Although table egg is not the best source of lutein, studies have shown that its bioavailability in human body is higher from lipid matrix of yolk compared with lutein from vegetable sources or food supplements. The egg is highly nutritious food because it contains high-quality proteins with balanced amino acid composition, essential fatty acids, minerals and vitamins necessary for proper functioning of the body and with increased content of lutein it becomes a value-added product. Addition of natural or synthetic sources of lutein in mixtures for laying hens enables the transfer of lutein through hen's metabolism into egg yolk. The increase of lutein content in yolk is noticeable already after one week of feeding the hens with modified mixtures although it takes a longer time for its content to be stabilized. Egg with increased content of lutein in yolk represents quality and accessible source of lutein in human nutrition. Consumption of enriched eggs contributes to increased intake of lutein as well as its accumulation in the human organism.

Key words: *hen's egg, lutein, yolk*

Introduction

Lutein is a plant pigment which together with its stereoisomer zeaxanthin belongs to the xantophyll group of carotenoids. Carotenoids include more than 600 compounds which are divided into two basic groups: xanthophylls (oxygen-containing molecule) and carotenes (pure hydrocarbons, without oxygen in the molecule). Unlike other carotenoids, xanthophylls contain two hydroxyl groups, one at each end of the molecule, which results in their higher polarity in comparison to other carotenoids (Golzar Adabi et al., 2010). Like other carotenoids lutein is soluble in fats and is installed in the intestine in chylomicrons that carry it to the liver. In blood it is transmitted by low (LDL) and high (HDL) density lipoproteins in equal proportions (Parker, 1996).



Picture 1. *Lutein and zeaxanthin*

(http://www.rsc.org/ej/CP/2012/c2cp41583f/c2cp41583f-f1.gif)

The human body cannot synthesize carotenoids so they should be taken with food. Lutein and zeaxanthin are most abundant in dark green leafy vegetables such as spinach, kale, collard greens, lettuce, but also in peppers, tangerines, corn and egg yolk (Sommerburg et al., 1998).

The average content of lutein in egg yolks on the Croatian market is shown in Table 1 (own research, unpublished results).

Table 1. *Lutein content in eggs on the Croatian market*

Lutein content	Free range eggs	Cage eggs (producer 1)	Cage eggs (producer 2)
µg/g yolk	35.1	15.5	12.5
mg/100 g yolk	3.57	1.54	1.7
mg/60 g egg	0.55	0.24	0.20

What makes lutein and zeaxanthin unique compared to other carotenoids in the human organism is their presence in specific eye tissues. They are highly concentrated in yellow spot (macula lutea), a small area of the retina responsible for central vision and visual acuity and the only carotenoids present in this tissue (Landrum and Bone, 2001). Together they make a pigment of the yellow spot. Due to the specific presence in eye tissues they probably have a similar role in humans like in plants, where they act as antioxidants and filters of high-energy blue light (Krinsky, 2002). In recent years there has been more intense research on the role of lutein in maintaining eye health. Lutein intake through food or food supplements increases its content in the serum of humans as well as macular pigment density which is inversely related to the risk of eye diseases, like age-related macular degeneration (Seddon et al., 1994, Bone et al., 2001; Mares-Perlman et al., 2002), a major cause of blindness in people over 65 years, and cataracts (Brown et al., 1999, Chasan-Taber et al., 1999, Gale et al., 2001). Advanced age-related macular degeneration often leads to irreversible blindness for which there is still no effective treatment (Fine et al., 2000). The role of lutein in reducing the risk of developing cardiovascular disease

(Iribarren et al., 1997, Dwyer et al., 2001; Alves-Rodrigues and Thomas, 2002), as well as its ability to protect the skin from damage caused by UV radiation (Chen et al., 2002, Stahl and Sies, 2002) is also being explored. Till now, in poultry industry, lutein was mainly used as a coloring agent for skin, meat and egg yolks, but now the emphasis is placed on increasing the content of lutein in egg yolk and the production of functional foods. Although egg yolk is not the best dietary source of lutein, its bioavailability in the human body is higher in egg yolk compared to vegetable sources or dietary supplements (Chung et al., 2004). It is assumed that the contents of cholesterol and fatty acid composition of egg yolk affect the increase of lutein in human serum (Chung et al., 2004). Therefore, the aim of this paper is to present an overview of the possibilities for enriching hen eggs with lutein, and production of functional foods which could provide multiple benefits for people consuming it.

Enrichment of eggs with lutein

Eggs can be enriched with lutein by the addition of lutein sources in mixtures for hens. Two studies on the effectiveness of the transfer of lutein from hens feeds- into egg conducted Leeson and Caston (2004). In the first study hens were fed diet based on corn and soybean in which lutein was added in proportions of 0, 125, 250, 375, 500, 625, 750 and 1000 ppm. In second study hens were fed with three different mixtures. The first mixture was based on corn and soybean, comparable with the mixture in the first study. The second mixture included corn gluten and alfalfa and added corn oil instead of animal/vegetable fats. The third mixture contained ground flaxseed. Lutein was added in mixtures in proportions of 0, 125, 250 and 500 ppm. Within the first study, with an increase in lutein content in the mixture the content of lutein in eggs was also increased ($P < 0.01$), although the efficiency of transfer of lutein from a mixture into eggs decreased with increasing amounts of lutein in food. The most notable increase of lutein content in eggs occurred already with the initial addition of 125 ppm of lutein in the mixture, while the maximum level of enrichment was achieved by the addition of 500 ppm of lutein. The content of lutein in egg increased from 0.16 mg/60 g of egg (0 ppm) to 1.17 mg/60 g of egg for 125 ppm of lutein in feed, and to 1.49 mg/60 g of egg with the addition of 500 ppm of lutein in feed. The results of another study showed that the addition of corn gluten and alfalfa favored disposal of lutein in eggs, while the inclusion of flax seed in a mixture partially reduced deposition of lutein. Authors believe this is troubling because of the increasing emphasis on the production of eggs at the same time enriched with various functional ingredients. Analysis of eggs showed an increase in the content of lutein from 0.18 mg/60 g of egg (0 ppm) to 1.65 mg/60 g of egg with the addition of 500 ppm of lutein in the group with a mixture based on soya and corn. The inclusion of corn gluten and alfalfa in the mixture increased the lutein levels from 0.29 mg/60 g of egg to 2.04 mg/60 g of egg (500 ppm). In the group of hens where flax seed was added to the mixture, an increase in the content of lutein from 0.24 mg/60 g of egg (0 ppm) to 1.39 mg/60 g of egg (500 ppm) was also observed although the enrichment was lower compared to the two previous groups. The authors conclude that it is possible to increase the content of lutein in eggs 5-8 times above normal values and that such enriched eggs can contribute significantly to the human diet. Leeson et al. (2007) examined the impact of long-term addition of lutein in combination with ground flax seed supplementation on disposal of lutein in eggs and tissues. Laying hens were fed mixtures with 0 or 10% of ground flax seed and three levels of lutein (0, 125 and 250 ppm) for 11 consecutive 28-day period. Addition of lutein to the mixtures significantly increased the content of lutein in eggs ($P < 0.01$). Lutein content in eggs was linearly increased in the group of hens fed with 10% of

linseed (114, 1385 to 1727 μg/egg for 0, 125 and 250 ppm of lutein in feed), whereas in the group without linseed the maximum was observed with the addition of 125 ppm of lutein in feeds (90, 1671 and 1610 μg/egg for 0, 125 and 250 ppm of lutein in feeds), with no further increase in the lutein content in eggs. The authors were able to increase the content of lutein in eggs from 0.10 mg to 1.60 mg/60 g of egg, which represented almost two-fold increase compared to the current lutein intake in the population of North America. Golzar-Adabi et al. (2010) also investigated the effect of lutein supplemented to mixtures for laying hens on the content of lutein in eggs. To the mixtures based on corn and soybean 0, 250, 500 and 750 ppm of lutein was added. The content of lutein in egg yolks increased significantly after 7 days of feeding ($P < 0.01$). The most significant increase was recorded with 250 ppm lutein supplementation, from 0.12 mg/57 g of egg to 1.35 mg/57 g of egg, while the highest content of lutein in egg yolks was observed for 750 ppm lutein added (1.43 mg/57 g egg) to the feed. Results of all the above authors show that feeding hens with lutein enriched mixtures can increase the content of lutein in egg yolk, although the enrichment was slowed with the addition of more than 250-375 ppm of lutein in feed, and it is also necessary to consider the sources of oils/fats in hens feed. Hammershoj et al. (2010) investigated the effect of the addition of three colored (orange, yellow and purple) varieties of carrots in the amount of 70 g/day/hen to the standard feed at the disposal of carotenoids in egg and some indicators of egg quality. Feeding lasted for 28 days. Lutein intake was highest with the addition of purple carrots, reaching a level of 0.8 mg/day/hen. The authors concluded that the use of colored carrot varieties as forage material in feeding hens increases the concentration of total carotenoids in egg yolks by 25-75% compared to eggs layed by hens not fed with carrots. Looking at individual carotenoids in egg yolk it is evident that the content of lutein was increased by 54% and that of β-carotene 100 times. Purple carrot cultivar contained the highest concentration of both lutein and β-carotene and therefore had the most significant impact on the content of carotenoids in egg yolks. Jeon et al. (2012) conducted a trial in which hens were fed mixtures supplemented with green algae Chlorella powder in proportions of 0, 0.5, 1 or 2%. Feeding with experimental mixtures lasted for 4 weeks. Already after first week of feeding authors have observed an increase in the content of lutein in egg yolks in all experimental groups compared to the control. After four weeks of feeding in the group with 2% chlorella powder the highest content of lutein was recorded and it amounted to 27.04 μg/g of egg yolk compared to 13.88 μg/g in the control group. The authors concluded that for the production of lutein-enriched egg it takes at least two to three weeks of feeding with Chlorella powder to ensure the highest possible incorporation of lutein in egg yolk. Jang et al. (2014) fed hens during five weeks with control mixture and mixtures to which a commercial lutein (40 mg/kg) and the crude extract of spinach dissolved in the oils with lecithin, in which the concentration of lutein was also 40 mg/kg, were added. The content of lutein increased in both experimental groups. In group with commercial lutein there was approximately four times more lutein than in the control group. Although there were no statistically significant differences between the experimental groups, in the group with commercial lutein higher content of lutein and less variability in the average content of lutein in egg yolks than in the group with spinach extract was recorded. These results suggest that commercial lutein provides a more efficient and more uniform transmission of lutein in egg yolks than spinach extract in laying hens.

Conclusion

Chicken egg is a foodstuff that provides the highest quality proteins, essential amino and fatty acids, vitamins and minerals in optimal quantities for the human body. Fatty acid

composition of egg yolk as well as the content of vitamins and other fat-soluble substances can be altered through hens nutrition. The content of lutein also. Lutein is a pigment that protects plant cells from oxidation and the harmful effects of solar radiation. Apart from the plant world, lutein shows a protective role in other systems also, such as the human body, which is confirmed by various *in vitro* and *in vivo* studies. It is significant for protection of eyes from developing age-related macular degeneration, and its role in protecting the skin and circulatory system is also explored. Because it is soluble in fats its content in egg yolk may be affected by the addition of lutein sources in mixtures for laying hens. Various authors in their researches showed that it is possible to successfully increase the content of lutein in egg by adding various amounts of natural or synthetic sources of lutein in hens' feed, wherein for synthetic lutein more uniform and prolonged egg yolk enrichment was recorded. Lutein-enriched egg, in addition to the above nutritional quality, has added value in terms of functional activity. Consumption of eggs enriched with lutein increases the content of lutein in human plasma as well as the density of yellow spot pigment, thereby contributing to better protection of the eyes and stronger antioxidant protection of the organism.

References

1. Alves-Rodrigues A and Thomas B 2002. The role of lutein in the prevention of atherosclerosis. J. Am. Coll. Cardiol. 40, 835–836.
2. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE and Twaroska EE 2001. Macular pigment in donor eyes with and without AMD: A Case–Control Study. Invest. Ophthalmol. Vis. Sci., 42, 235-240.
3. Brown L, Rimm EB, Seddon JM, Giovannucci EL., Chasan-Taber L, Spiegelman D, Willett WC and Hankinson SE 1999. A prospective study of carotenoid intake and risk of cataract extraction in US men. Am. J. Clin. Nutr. 70, 517-524.
4. Chasan-Taber L, Willett WC, Seddon JM, Stampfer MJ, Rosner B, Colditz GA, Speizer FE and Hankinson SE 1999. A prospective study of carotenoid and Vitamin A intakes and risk of cataract extraction in US women. Am. J. Clin. Nutr. 70, 509-516.
5. Chen J, Wu A, Pathak MA, Rius-Diaz F, Mihm CM, Goukassian D and Gonzalez S 2002. Dietary lutein and zeaxanthin partially prevent UVB-induced skin careinogenesia in the SKh-1 hairless mouse model (Abstract). In: Society for Investigative Dermatology. Los Angeles, CA.
6. Chung HY, Rasmussen HM and Johnson EJ 2004. Lutein bioavailability is higher from lutein-enriched eggs than from supplements and spinach in men. J Nutr. 134, 1887-1893.
7. Dwyer JH, Navab M, Dwyer KM, Hassan K, Sun P, Shircore A, Hama-Levy S, Hough G, Wang X, Drake T, Merx CN and Fogelman AM 2001. Oxygenated carotenoid lutein and progression of early atherosclerosis: The Los Angeles Atherosclerosis Study. Circulation 103, 2922–2927.
8. Fine SL, Berger JW, Maguire MG and Ho AC 2000. Age-related macular degeneration. N. Engl. J. Med. 342, 483-492
9. Gale CR, Hall NF, Phillips DI and Martyn CN 2001. Plasma antioxidant vitamins and carotenoids and age-related cataract. Ophthalmology 108, 1992-1998.
10. Golzar Adabi SH, Kamali MA, Davoudi J, Cooper RG and Hajbabaei A 2010. Quantification of lutein in egg following feeding hens with a lutein supplement and quantification of lutein in human plasma after consumption of lutein enriched eggs. Arch.Geflügelk. 74, 3, 158-163.

11. Hammershøj M, Kidmose U and Steenfeldt S 2010. Deposition of carotenoids in egg yolk by short-term supplement of coloured carrot (Daucus carota) varieties as forage material for egg-laying hens.J. Sci. Food. Agric. 90, 1163-1171.
12. Hamułka J, Koczara J and Gronek M 2005. Lutein content of selected polish foods and estimation of its intake. Pol. J. Food Nutr. Sci. 14, 2, 201–206.
13. Iribarren C, Folsom A, Jacobs JrD, Gross MD, Belcher JD and Eckfeldt JH 1997. Association of serum vitamin levels, LDL susceptibility to oxidation and autoantibodies against MDA–LDL with carotid atherosclerosis A case–control study. The ARIC Study Investigators. Atherosclerosis Risk in Communities. Arterioscler. Thromb. Vasc. Biol. 17, 1171–1177.
14. Jang I, Ko Y, Kang S, Kim S, Song M, Cho K, Ham J and Sohn S 2014. Effects of Dietary Lutein Sources on Lutein-Enriched Egg Production and Hepatic Antioxidant System in Laying Hens. J. Poult. Sci. 51, 58-65, 2014
15. Jeon JY, Kim KE, Im HJ, Oh ST, Lim SU, Kwon HS, Moon BH, Kim JM, An BK and Kang CW 2012. The Production of Lutein-Enriched Eggs with Dietary Chlorella. Korean J. Food Sci. Ani. Resour. 32, 1, 13-17.
16. Krinsky NI 2002. Possible biologic mechanisms for a protective role of xanthophylls. J. Nutr. 132, 540-542.
17. Krinsky NI, Landrum JT and Bone RA 2003. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu. Rev. Nutr. 23, 171-201.
18. Landrum JT and Bone RA 2001. Lutein, zeaxanthin, and the macular pigment. Arch. Biochem. Biophys. 385, 1, 28-40.
19. Leeson S, Caston L and Namkung H 2007. Effect of dietary lutein and flax on performance, egg composition and liver status of laying hens. Can. J. Anim. Sci. 87, 365-372.
20. Leeson S and Caston L 2004. Enrichment of eggs with lutein. Poult. Sci. 83, 1709-1712.
21. Mares-Perlman JA, Millen AE, Ficek TL and Hankinson SE 2002. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. Overview. J. Nutr. 132, 3, 518-524.
22. Parker RS 1996. Absorption, metabolism, and transport of carotenoids. FASEB J. 10, 5, 542-51.
23. Perry A, Rasmussen H and Johnson EJ 2009. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products.J. Food Comp. Anal. 22, 9-15.
24. Seddon JM, Ajani UA, Sperduto FLD, Hiller R, Blair N, Burton TC, Farber MD, Gragoudas ES, Haller J, Miller DT, Yannuzzi LA and Willett W 1994. Dietary carotenoids, Vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case–Control Study Group. JAMA. 272, 1413-1420.
25. Sommerburg O, Keunen JE, Bird AC and van Kuijk FJ 1998. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. Br J Ophthalmol. 82, 8, 907-10.
26. Stahl W and Sies H 2002. Canotenoids and protection against solar UV radiation. Skin Pharmacol. Appl. Skin Physiol. 15, 291–296.
27. http://www.rsc.org/ej/CP/2012/c2cp41583f/c2cp41583f-f1.gif

Invited paper

TEMPUS CASA PROJECT – A SUSTAINABLE TOOL FOR KNOWLEDGE AND INNOVATION TRANSFER IN ANIMAL SCIENCES

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Abstract

Although regulated by law and other policies knowledge transfer in animal sciences (zootechnics) is rather limited after students' graduation from the Faculty of Agriculture. There is a lack of courses for professional development of teachers from agricultural middle schools, including those teaching subjects in animal sciences. There is as well a need of permanent improvement and upgrading of courses and trainings created for advisors in agricultural advisory services. The TEMPUS CaSA project objective is to contribute to the improvement of agricultural education to meet the needs of Serbian society. CaSA foresees: upgrading quality and availability of vocational agricultural education by strengthening professional and pedagogical competences of educators (University teachers, secondary school teachers, advisors) and creation of the repository for courses and additional contents important for agricultural education. Improvement of agricultural education will be achieved by introducing trainings in active teaching learning (ATL), communication skills, e-learning, together with newest knowledge emerging from research activities incorporated in vocational courses. Creation of the National Repository for Agricultural Education (NaRA), will enable networking of all stakeholders in agricultural education and assure sustainability. In addition, among 13 project partners, the Ministry of education is a compulsory partner for Structural Measures TEMPUS projects. This is important for recognition of the National repository by relevant state authorities. Online courses and teaching material, live stream trainings, results from the research projects, and different data bases will be available in NaRA after project life-time.

Key words: *EU TEMPUS project, agricultural knowledge transfer, innovation, NaRA*

Introduction

Most of the jobs in modern agricultural production permanently need acquiring additional knowledge regardless of the period passed from graduation. Besides research carried out in scientific institutions (Universities, research institutes) occupations that need constant knowledge update are primarily teaching and advising. University teachers, together with teachers in middle agricultural schools and advisors employed in extension (advisory) services are the fundament of agricultural education in all areas of Serbian agriculture, including animal sciences. Knowledge upgrade is compulsory for all the three pillars of Serbian agricultural education: university teachers, secondary school teachers, and advisors. They are thus part of the lifelong learning (LLL) process.

Continuous professional development of agricultural middle school (AMS) teachers is defined by the "Law on basis of education system", article 129 (Official Gazette of the Republic of Serbia, no. 55/2013). According to the law, continuous professional education of teachers is compulsory. It can be provided by different organizations: NGOs, schools, faculties, registered agencies, and Centers for professional development established by local authorities; financed by the Ministry of Education and/or projects funded by EU or other international organization (Popovic, 2013). However, the choice of programs for continuous professional development is made rather inconsistently than according to the established education policy; it is based on the offer of courses, not on the need analysis (Pesikan et al., 2010a). In addition, there is discrepancy between programs and practices in a sense of huge differences between courses in: training type, trainers' competences, training duration, content, and evaluation, as well as monitoring of training effects in classes (Pesikan et al., 2010b).

Knowledge upgrade in advisory service seems better organized: agricultural Farm Advisory System (FAS) in Serbia is entrusted to the Institute for Science Application in Agriculture, Belgrade. The Institute is a coordinating institution in charge of Education of advisors. The legal basis for advisory system is "Law on performing advisory and expert operations in agriculture" (Official Gazette of Republic of Serbia No. 30/10 from 7.5.2010). The Ministry of Agriculture, Forestry and Water Management (MAFWM) issue a Decree on Determining Annual Program on Advisory work Development in Agriculture, for the current year and announces a Competition for the 31 centers (funded by the Government) and 3 private advisory services to apply for the funds provided by the Ministry. According to law provisions MAFWM is in charge for FAS organization, work and performance.

When animal sciences are considered, as an example the accredited Zootechnics curriculum of the University of Belgrade completely lacks pedagogical courses and has only one course for development of some academic skills such as writing and presentation of papers. Also, there is an absolute lack of courses for knowledge refreshment of teachers of middle agricultural schools teaching subjects linked to animal sciences. Table 1 represents an overview of accredited courses for AMS teachers since 2006/07. In the newly published Catalogue for 2014/2015 and 2015/2016 courses offered to teachers of middle agricultural schools are extremely rare: only 3 courses, out of 868 accredited courses could be connected with agricultural practice. In the earlier catalogues from the total number of 1002 courses accredited for professional development of teachers only 12 courses offered were somehow linked to agriculture (7/12 for AMS teachers), while not a single one was devoted to zootechnics, except "Animal welfare and us" a course given by an NGO for animal protection. Among these courses that could be taken by agricultural middle school professors, only 1/12 is offered by the Faculty of Agriculture University of Novi Sad (http://katalog.zuov.rs/).

This actual situation clearly indicates that courses for teachers of secondary school level are an absolute necessity and that animal science professionals must urgently find a way to support people working outside the University, educated by the University, to ensure LLL possibilities to our colleagues responsible for the education of agricultural animal producers and teachers in secondary education level.

When it comes to advisors, in Serbia, from 2010, every year all advisors have education in three areas: knowledge widening, knowledge upgrade, and knowledge application. In addition, specific workshops are organized in all areas of agriculture during the Annual seminar.

Table 1. *Accredited courses for teachers of AMS from 2006/07 to 2014/16*

School year	Number of courses available to AMS teachers	Number of courses available to teachers of zootechnics courses	Accredited courses offered by faculties of Agriculture
2006/07	0	0	0
2007/08	0	0	0
2008/09	15	3	0
2009/10	4	1	1
2010/11	7	2	1
2011/12	6	0	1
2012/14	7	1*	1
2014/16	3	0	0

* part of 1 course

The aim of this contribution is to describe the obtained TEMPUS project and its role in reinforcing knowledge transfers in agricultural, including animal sciences.

Needs of Serbian society - the idea of the TEMPUS project

Starting from the idea that 3 basic pillars of agricultural education: Universities, secondary agricultural schools and advisory services, need mechanisms of LLL implemented, and that such mechanisms are not fully established in Serbia, the Faculty of Agriculture University of Belgrade and the Faculty of Agriculture University of Novi Sad have decided to prepare a TEMPUS project, in the frame of the TEMPUS action HE and society. These two faculties together were participants in a number of previous TEMPUS projects; first was a project that facilitated the reform process according to the principles of Bologna declaration, and their common efforts contributed to reformed agricultural curricula (Poleksic et al., 2004; 2006). During project preparations following challenges for the Serbian agricultural education were identified: a lack of pedagogical education for students of faculties of agriculture during their graduate courses Students that continue their carrier in education, as teachers in secondary schools or university teachers, lack any formal pedagogical education; there is also a need for young university teachers to learn academic skills such as writing project proposals, preparing project budget, presenting project results, writing scientific papers, etc; secondary school teachers and agronomy experts from advisory services need in-service training. In addition, advisors working directly with farmers need some skills development such as modern means of communication and preparing project proposals to be able to apply for funds available for Serbian producers and companies. In conclusion, all the mentioned holders of agricultural education have an obligation to upgrade their professional knowledge, but they also need development of skills their profession requires.

The project idea originated from a general need of the Serbian Society to reinforce agricultural practice, to apply newly generated scientific knowledge, and modern production and processing practices. In addition, agricultural education has to inform and educate the public including raising public awareness about agricultural development, issues such as ecological agriculture, organic production, GMO, and other important issues. The project proposal fits into the Strategy for Education Development of the Republic of Serbia until 2020 (Strategy of Education Development in Serbia to 2020+, 2012). Teachers' education and teachers as key factor of education development are particularly addressed in the Strategy. These should be achieved by: development of in-

service teachers training to improve teachers' competences for encouraging students' creativity, innovation and entrepreneurship; training of all teachers to use ICT in teaching; and implementation of methods of ATL.

Areas of specific interest: development of LLL in the society at large, cooperation with other education levels and development of human resources are national priorities defined that the CaSA project proposal intended to deal with.

The proposal was successful, and a project "Building capacity of Serbian Agricultural Education to link with Society, CaSA" 544072-TEMPUS-1-2013-1-RS-TEMPUS-SMHES officially started in January 2014.

CaSA outline

Building capacity of Serbian Agricultural Education to link with Society is a 3 year (December 2013 – November 2016) national project. CaSA belongs to the TEMPUS sub-program Structural measures and the Action Higher education and society. Its objectives are: to improve quality and availability of vocational agricultural education; to strengthen competences of educators; to create a National Repository for Agricultural Education (NaRA).

Partners contributing to the project are the main national "players" in agricultural education. There are 10 Serbian and 3 EU partner institutions: 5 Agricultural faculties from: Universities of Belgrade, Novi Sad, Kragujevac, State University of Novi Pazar, and private University EDUCONS; Association of Agricultural Middle School in the area of agriculture, food processing and food production; Institute for Science Application in Agriculture responsible for in-service training of agricultural advisors; two NGO training organization (Education Forum and Balkan Security Network); and the Ministry of Education, Science and Technological Development of the Republic of Serbia, compulsory partner for Structural measures projects. According to TEMPUS rules, three EU partner Universities have to be members of the consortium: Banat University of Agricultural Sciences and Veterinary Medicine, Timisoara, Romania; University of Maribor, Slovenia and University Foggia, Italia.

All CaSA project activities are grouped into 11 work packages:

1. Creation of the Repository,
2. Assuring infrastructural support for NaRA functioning /Development of resources,
3. Improvement of competences of university teachers,
4. Modernization of teaching contents,
5. Improvement of competences of Agricultural Middle Schools teachers,
6. Improvement of competences of experts in extension services,
7. Pilot implementation of vocational courses,
8. Quality assurance control of project activities,
9. Dissemination of project results,
10. Exploitation of project results, and
11. Project Management.

Creation of NaRA, National Repository for Agricultural Education will be one of the principal achievements of the project. NaRA will be available as an electronic platform. It

should enable sustainability of the project and association of stakeholders involved in agricultural education and training in Serbia. It is planned that the repository will consist of the following elements: both online courses and information necessary for teaching traditional courses for professional development of agricultural middle schools and experts in extension services; databases of results obtained from research; selected and recorded classes of interactive teaching; selected parts of courses created and/or developed within the project, prepared in the form of online video tutorials and posted together with additional teaching contents; and other relevant contents added to the repository based on authorized decisions of the NaRA Advisory Board. All project participants have jointly prepared and signed the Constituent agreement on project implementation and NaRA management (Poleksic et al., 2014).

CaSA project and knowledge transfer in animal sciences

Similar to other agricultural areas, animal sciences are represented in CaSA primarily by an important number of university teachers that are trained in the frame of the project. From the total number of 60 teachers foreseen to be trained, 15 belong to the area of animal sciences and veterinary medicine, majority of them (12/15) are employed at the faculties in Belgrade and Novi Sad. These young teachers together with the others will create both online and face-to-face courses that, when accredited, will be available from NaRA. These courses will be a valuable addition to the existing selection of courses for in-service training of advisors in animal sciences, and will represent the first officially recognized courses for knowledge improvement in zootechnics for middle school teachers.

So far in CaSA all university teachers were trained in active teaching learning and academic skills. E-learning is foreseen for end 2014, so that in 2015 courses are created, and upon accreditation, implemented in 2016 and further available in the national repository. A NaRA internet domain has been registered, and the Advisory board of the national repository will be established. Parallel with this, a training need analysis (TNA) is on-going, and with the help of EU partners the TNA questionnaire is prepared. Tender procedure for equipment purchase is still ongoing. Dissemination activities (TV, presentations at meetings, discussions, leaflets and posters) are numerous from the beginning of the project as well as regular and extraordinary management activities.

In conclusion, we strongly believe that with the presence of the Ministry of Education and Science as project partner, during project lifetime NaRA will be officially recognized by the two ministries (of education and of agriculture). This will strengthen the position of agriculture and therefore of zootechnics in Serbia and contribute to acknowledgment of modern agricultural practices and knowledge available to all professionals, advisers, teachers, and students. By efficient means of knowledge and innovation transfer, agricultural production and processing, including zootechnics, will be able to play the important role agriculture has to play in Serbian future.

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References

1. http://katalog.zuov.rs/ (accessed April-July 2014)
2. "Law on basis of education system" (Official Gazette of the Republic of Serbia, no. 72/09, 52/11 and 55/13) www.mpn.gov.rs (accessed April-July 2014)
3. "Law on performing advisory and expert operations in agriculture" (Official Gazette of Republic of Serbia No. 30/10 from 7.5.2010)
4. Pesikan A, Antic S and Marinkovic S 2010a. Analysis of the conception of continuous professional development of teachers in Serbia: proclaimed and hidden level (part I) Koncepcija stručnog usavršavanja nastavnika u Srbiji: između proklamovanog i skrivenog modela. Nastava i vaspitanje 2, 278-297.
5. Pesikan A, Antic S and Marinkovic S 2010b. Analysis of the conception of continuous professional development of teachers in Serbia: how far are we from an efficient model? Koncepciija stručnog usavršavanja nastavnika u Srbiji: koliko smo daleko od efikasnog modela? Nastava i vaspitanje 3, 471-48.3
6. Poleksic V, Quarrie S, Pekic S and Pesikan A 2004. Competence building of teachers: Case of the Faculty of Agriculture, University of Belgrade. Proceedings of the 7th European Conference on Higher Agricultural Education (ECHAE), Rethinking Higher Education in the Food Chain and Environment: Profiling the graduate of the future, KVL, Copenhagen, 71-76.
7. Poleksic V, Pekic Quarrie S, Cupina B, Petric D and Drochner W 2006. Curricular reform at Serbian Agricultural Faculties: achievements of the TEMPUS JEP towards meeting the needs for rural development. Proceedings of the 8th European Conference on Higher Agricultural Education (ECHAE): The Public and the Agriculture and Forest Industry 194-197.
8. Poleksic V, Topisirovic G, Pekic Quarrie S, Petric D, Tanaskovic S, Salasan C, Scepanovic D and Quarrie S 2014. From agricultural research and innovation towards improvement of agricultural education. EU Project Collaborations: Challenges for Research Improvements in Agiculture. June 2-4, 2014, Belgrade, Serbia. Book of Abstracts, p.84.
9. Popovic M 2013. "Education and training of teachers in Western Balkans – report: Serbia" Tempus foundation, 2013, p. 45.
10. Strategy of Education Development in Serbia to 2020+ 2012. Belgrade: Ministry of Education and Science of Serbia. ("Official Gazette of the Republic of Serbia", no.107/12.)

Review paper

GETTING IT ACROSS TO YOUR STAKEHOLDERS

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Abstract

Increasingly, research projects are expected to provide impact, and without a convincing impact statement, project proposals are now unlikely to get funding. This impact is not only for others in the scientific community, but also a wide range of stakeholders who are interested in knowing what a project has achieved. Getting it across to stakeholders can be achieved in terms of answers to the questions Why? Who? What? How? When? and Where?

Why - because researchers are spending other people's money and need to be accountable for this. Who - as well as other scientists, non-scientific stakeholder groups include industry and commerce, decision- and policy-makers, consumers and general public, as well as the media. What - in addition to traditional scientific presentations, for non-scientist stakeholders, emphasis should be on any practical applications of the research findings and relevance to perceived societal needs or problems. How - non-scientific stakeholders need a much more varied set of measures, beyond just scientific publications and presentations at conferences/symposia. Website, multimedia and social networks are now essential components of an effective dissemination strategy. When - regular events to describe progress with the research, such as press releases and newsletters should be combined with irregular meetings such as conferences, symposia, trade fairs and other stakeholder group meetings. Where - as well as events held at the researcher's institution, other locations for dissemination activities should be planned locally, nationally and internationally, especially trade and industrial fairs.

The increasing importance of research impact is resulting in many large-scale projects including partners with specific expertise in getting it across to stakeholders.

Key words: dissemination, impact, research results, stakeholders

Introduction

Traditionally research results were disseminated mainly through scientific publications and poster and oral communications at conferences - to other scientists. Thus, stakeholders (anyone with an interest in your research) were generally limited to other scientists. Circumstances have now changed and the scientific community needs to change its approach to communicating its research, to get it across to its stakeholders to ensure effective transfer of knowledge and innovations.

The fate of submitted research proposals is increasingly dependent on the ability of applicants to convince evaluators that their research will have significant impact that goes beyond the immediate scientific research community. A key example of this is the EU's main funding

source for research and innovation, Horizon 2020 (H2020). The importance of impact has been increasing with each new framework programme. Thus "There is a greater emphasis on impact, in particular through each call or topic impact statements... Applicants are asked to explain how their work will contribute to bringing about the described impacts" (Cross, 2014). Compared with FP7, the 'Impact' section of the application form is now the second section of the form instead of the third in FP7, and if two proposals receive the same score for quality of science, it is the 'Impact' section that will determine which proposal gets the higher ranking. Indeed, for 'Innovation Actions' the 'Impact' score is given a weighting of 1.5 to emphasise its importance (Cross, 2014).

Not only is the need for research to have impact on non-research beneficiaries increasing, but the impact of research on the research community itself is evolving with developments in digital technologies, global access to research data, open access publishing, the growth of social media in science and new trends in assessing scientific reputations (such as https://impactstory.org/ and http://www.altmetric.com/). The European Commission has recently described these new trends in scientific research, called 'Science 2.0' (European Commission, 2014). In years to come, these trends will influence the way you communicate with your research scientist stakeholders.

Therefore, for your research to have maximum impact on both your research and non-research stakeholders you need to develop skills in how to get your research output, in its many forms, across to all your various stakeholder groups. Getting it across to your stakeholders can be achieved in terms of answers to the questions Why? Who? What? How? When? and Where?

Why?

Because researchers are spending other people's, usually taxpayers', money to do research. Thus, research scientists need to be accountable for how that money is spent, and what the sponsor gets for that money. What is the impact that the research has had beyond the scientific community? What benefit for industry, policy-makers, the economy and society at large has the research achieved? A key factor for success with project proposals, such as those for H2020, is the expected impact that the research will achieve. Over 38% of the nearly €80 billion to be spent on research and innovation in H2020 (European Commission, 2013a) will go on solving societal challenges. That means research which will have impact to help find answers to practical problems, delivering practical applications and benefit for society. Without that impact on project stakeholders, the proposal won't get funded. Thus, the first calls for H2020 resulted in 16000 proposals (Cross, 2014), an oversubscription rate of nine times, giving a success rate of only 11%. Only those proposals achieving all the expected impacts will be amongst those 11%.

Who?

Stakeholders for the research can be grouped into several categories. Traditionally, the most obvious stakeholders would be other scientists doing similar research. However, the most important stakeholder to be kept in touch with how the research is progressing and the money is being spent is the sponsor of the research, to be provided with regular project reports. Other stakeholder groups could be summarised as a) industry and commerce, b) decision- and policy-makers, c) consumers and general public, and finally d) the media. Stakeholder dissemination strategy needs to be adjusted appropriately for each of these stakeholder groups. Industrial and commercial stakeholders are usually the most important to provide

justification for the research, and these stakeholders need to be developed and cultivated to ensure they retain interest in the research as it progresses. They will be looking for opportunities to convert your research findings and discoveries into new products or services for the market. Decision- and policy-makers, such as central and local governments, will expect your research to contribute to evidence-based decision-making. Consumers and general public will be interested in being informed on the impact of the research in relation to societal challenges, problems or concerns. The media is looking for a good story for its readership, which may not necessarily be the main story from the research. A good quality media reporter will check facts from an interview before any material is broadcast or published. Remember to cater for local, national and international stakeholder groups, which may require a separate focus or emphasis to the research information to be disseminated.

What?

For the non-scientist stakeholders the emphasis should be on any practical applications of the research findings and relevance to perceived societal needs or problems. The level of technical detail should be adjusted for the stakeholder group. Unless the research focuses on methodology, the methods are the least important aspect of the research to be described. The content should focus on justification for the research, the main research findings and their implications in relation to commercial exploitation, policy issues, public interest/safety/concern. The content should be written as far as possible in non-technical terms that would make the science accessible and understood by each stakeholder group. The content should also be presented attractively, and visual images (pictures, diagrams, simple graphics) have more impact than just text.

With 'Science 2.0' in mind, dissemination of research to the scientific community is increasingly going beyond just research publications and presentations at conferences. H2020 requires all H2020-funded research output to be published in open access journals (O'Dea, 2013) to ensure free access of the scientific community to H2020 results. The Commission is also introducing a pilot scheme to open up publicly funded research data 'to make the underlying data needed to validate the results presented in scientific publications and other scientific information available for use by other researchers, innovative industries and citizens' (European Commission 2013b). Already, the growing use of social media by researchers is making a wide range of types of information freely available through the internet. Examples are ResearchGate (https://www.researchgate.net) with nearly 3 million researchers in 2013, Academia.edu (https://www.academia.edu) with over 10 million academics signed up, Figshare (http://figshare.com), a cloud-based data management website and Slideshare (http://www.slideshare.net) for sharing presentations.

How?

This is the most diverse aspect of getting it across to stakeholders. Non-scientific stakeholders need a much more varied set of measures, beyond just scientific publications and presentations at conferences/symposia. A database of different stakeholder groups should be established and a dissemination strategy identified for each stakeholder group. Face-to-face contact is usually more effective than web sites, newsletters, brochures, email reports, provided individuals do not need to travel any distance to attend a dissemination event and are not going to be out of pocket regarding any costs. Thus, regular press releases and conferences, public debates, and individual meetings with key stakeholders should be planned. Dissemination activities requiring the recipient to take the initiative are much less

likely to be effective than those where the recipient is sent information to be kept informed. Thus, a static, unattractive web site describing the research is less likely to have as much impact as an electronic newsletter embedded into the text of an email: email attachments that need to be downloaded before opening are less likely to be read, for example.

A project web site should be designed to be attractive, easy to use, regularly updated and ideally with a range of multimedia sources of information available to cater for different groups of stakeholders. Multimedia approaches to disseminating research are now well developed, such as podcasts, webinars, videos, infographics, blog and discussion boards, particularly to cater for non-expert stakeholders, as well as social media (e.g. Facebook, Twitter, LinkedIn). Announcement of these could be circulated by email to individuals in the stakeholder database, or through the project website with RSS feeds which will ensure that stakeholders who want to be kept up-to-date with project progress are automatically informed of news and updates.

Meetings with decision- and policy-makers should take place at their offices rather than the scientist's. The same is true for industrial and commercial stakeholders. Policy briefings are effective mechanisms for getting research findings and their implications to decision- and policy-maker stakeholders. Information disseminated in English will reach the international scientific stakeholder community, though some institutions specializing in dissemination activities also publish information in several other languages to ensure that non-specialists in other countries can access the research findings.

When?

Press releases to describe progress with the research should be held at regular intervals rather than when there is a particular result to present. A press release or press conference every six months or year, depending on the pace of the research, should be planned, to include representatives of all the media (newspapers, magazines, radio and TV). Try to plan other dissemination activities so that there appears to be always something going on. For example, an electronic newsletter could also be every six months, but in between press releases, six-monthly/yearly policy briefings or annual conferences. The project web site needs to be kept updated every one-two weeks, and will soon attract regular viewers if it becomes clear that information of one sort or another is constantly changing - projects reports, other relevant news items, YouTube video clips, stakeholder group webinars, a regularly updated project blog, as well as its Facebook page and Twitter account, etc! That way stakeholders will feel more involved in the research and take greater interest in its findings, thereby leading to greater impact for the project.

In addition to these regular dissemination activities, conferences, symposia, trade fairs and other irregular meetings of the various stakeholder groups provide opportunities to advertise the project and its results through face-to-face contact.

Where?

In addition to events, such as conferences, symposia, workshop events, demonstrations, open days and press conferences, held at the researcher's institution, other locations for dissemination activities to non-scientific stakeholders should be planned locally, nationally and internationally. For example, trade and industrial fairs are excellent opportunities to interact with industry and commerce and expand the network of contacts. Local authority rooms could be used for consumer stakeholder debates.

Conclusion

As competition for research funds increases, so does the importance of a project's expected impact, not only on others in the scientific community but especially on non-scientist stakeholders. With the large majority of European research now being funded through strong competition for research funds, only those project proposals that can demonstrate, in addition to scientific excellence, an extensive and effective dissemination strategy will get funded.

Increasingly, this is testing the researchers' innovation skills in communication to the limit, so many large-scale projects now include partners with specific expertise in getting it across to stakeholders to increase a proposal's prospects of being funded - specific expertise in answering the questions Why? Who? What? How? When? and Where? After all, research should be done for the stakeholders' benefit.

References

1. Cross A 2014. Horizon 2020 proposal evaluation. UKRO Annual Conference. Bristol 27 June 2014.
2. European Commission 2013. Factsheet: Horizon 2020 budget, available at http://ec.europa.eu/research/horizon2020/pdf/press/fact_sheet_on_horizon2020_budget.pdf
3. European Commission 2014. 'Science 2.0': Science in transition. Public consultation background document of Directorates-General for Research and Innovation and Communications Networks, Content and Technology, available at http://ec.europa.eu/research/consultations/science-2.0/background.pdf
4. O'Dea J 2013. Any questions? A guide to Horizon 2020. Science|Business, 17 July, 2013, available at http://www.sciencebusiness.net/news/76212/Any-questions-A-guide-to-Horizon-2020

Review paper

ACTIVE TEACHING/LEARNING AT FACULTY OF AGRICULTURE – 10 YEARS OF EXPERIENCE

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Abstract

The course in active teaching/learning (ATL) together with the method of lecture assessment by sequential analysis was developed by a pioneering group of experts from the Institute of Psychology. It was developed further and improved by adding research skills, aspects on quality assessment and examination procedures, relevant drama skills and techniques for interactive e-learning. The modules which we developed and implemented at Faculty of Agriculture several times in last 10 years had an impact on the quality of teaching of teachers as well as positive consequences to the student success.

Key words: *ATL, higher education, Faculty of Agriculture, teachers training*

Development of ATL course for university teachers

Reform of higher education, so-called "Bologna process" at European and Serbian universities has brought a dramatic demand for change in the position of students in the teaching process: students becoming partners and even main actors. New curricula, based on achieving a certain number of learning outcomes (both subject-specific and generic) by precisely-defined student workload as ECTS cannot be successfully accomplished with the old concept of university lecturing. Indeed, the current concept of university lecturing results in students lacking many competences important for their professional life such as creative and critical thinking, decision making, problem solving etc. In addition to that an average time for students to complete their first degrees at Belgrade University is nearly eight years, mainly because students do most of their learning on their own after the lecture courses have finished. Our faculties, therefore, are not places for learning: learning is separated from teaching: both in space and in time! Such model of univerity teaching was dominant at our universities according to the EUA experts report on Serbian high education. The lack of any training for university teachers on how to work with students has been seen as a major weekness in applying Bologna process at our universities.

A group of lecturers from the Faculty of Agriculture, Belgrade University recognised the need for improving teaching methods that would actively involve students in the teaching process and this led to a purpose-designed course on improving their teaching and other academic skills. This required a change in their concept of teaching and the role of the student: a shift from teaching to learning changing the focus from themselves as lecturers to students as beneficiaries, which is practically the area of psychology of learning. The original concept of the active teaching/learning (ATL) process was developed at the Institute of Psychology, University of Belgrade (Ivić et al., 2002). Starting from above

mentioned idea on shifting focus from teaching to learning this concept is based on supporting, promoting, enhancing monitoring student activities. The major objective of any ATL teaching unit is to activate as many students in as many relevant activities. In addition to the development of techniques of achieving these objectives in planning the class, method for assessing the quality of the teaching/learning process was developed. This technique, called "sequential analysis" is the unique method of an expert peer reviewing of the class and can, also, be a tool for analysing and improving the teaching/learning process.

The course in ATL together with the method of lecture assessment by sequential analysis was, therefore, developed by a pioneering group of experts from the Institute of Psychology led by Prof Ivan Ivić. In addition to ATL modules, the course contained a set of modules in other academic skills, such as good research practices, curriculum development, quality assessment and relevant drama skills. The modules which we developed and implemented at the Faculty of Agriculture were described in a course leaflet and in the publication "The student at the centre of the teaching process" (Ivić et al., 2008).

The course has been held during the whole school year: twice for 50 teachers of the Faculty of Agriculture in 2003/4 and 2004/5 (sponsored by the Fund for an Open Society and a Tempus Project, respectively), once for 30 teachers of Serbian Faculties of Biology in 2007/8 through another Tempus project. During 2009 teaching staff at Faculty of Agriculture had 2 types of trainings for improving their teaching skills: a) 45 teachers and 30 assistants had training in interactive e-learning held by Miloš Bajčetić and b) 20 teachers had training in principles of writing good textbook given by ATL team. In 2014 through CaSA Tempus project 60 teachers of Serbian faculties of Agriculture have participated all modules of the course.

Present structure of the course

The course has evolved during several years of giving it at different faculties and analysing the results achieved. The major improvement of the course is the introduction of the modules on e-learning methods (for presenting the contents, activating students to learn and assessing the learning outcomes) and on curriculum development and QA. The current version of the course consists of the following group of modules:

1. **Active teaching/learning (ATL)** given by Prof. Ivan Ivić, Dr Ana Pešikan, Mr Slobodanka Antić (Institute of Psychology, University of Belgrade) and Prof. Ratko Jankov (Faculty of Chemistry, University of Belgrade)
2. **Curriculum development, quality assessment and examination** given by Prof. Sofija Pekić Quarrie, Prof. Vesna Poleksić and Prof. Biljana Vucelić Radović (Faculty of Agriculture, University of Belgrade)
3. **Drama skills** given by Prof. Paul Murray (Winchester University, UK, based in Belgrade)
4. **Research skills** developed by Prof. Steve Quarrie (Newcastle University, UK, based in Belgrade)
5. **Interactive e-learning** developed by Dr Miloš Bajčetić (Faculty of Medicine, University of Belgrade)

Picture 1. *ATL training (photo S. Quarrie)*

Pictures 2 and 3. *Drama skills training and Research skills training (photo S. Quarrie)*

Results of implementation of ATL

After several years of implementation of interactive teaching/learning methods at Faculty of Agriculture, it was obvious that improved teachers' competences (Poleksić et al., 2004) resulted in improvement of the student exam performances (Pekić et al., 2005) and student professional competences such as decision making (Pešikan et al., 2005 and 2006) as well as giving them valuable generic skills, for example: how to learn efficiently, how to present knowledge (orally and in writing), how to become critical, how to argue and discuss with others on a specific topic and how to evaluate the teaching process (Pešikan, 2005 and 2006).

It was a challenge to explore the possibility of giving students a chance to experience an assessment of their own learning outcomes by introducing student self-assessment exercises (Pekić Quarrie, 2007). Peer review classes were organised to test their ability to evaluate each other's tests, as well as to evaluate their own tests. Analysis of these evaluations showed a very close association between the teacher's marks and students' marks. After three successful peer review classes we decided to give students a chance to "do the real thing", i.e. to examine each other at the end of the Botany course during the first exam occasion in June 2006 when 10 students took part in the "interactive exam". The large majority of student marks (77%) were the same as the professor's, and the large majority of those marks that were not the same were *lower* than the professor's. Similar results were obtained after the exam in June 2007 with 30 students, indicating a high level of student objectiveness and their critical approach to the evaluation. On both occasions these exams involved only the best/most motivated students as they were held immediately after the course during the first exam occasion and this inevitably required a lot of preparative work during the course. After positive experiences in testing the capacity of good students for peer-reviewing in previous years, we applied similar methodology to all students, especially those with poor knowledge. Three exam exercises, done at the end of 2007/8, has shown their capacity for evaluation and self-evaluation and to analyse their evaluation capacity according to their knowledge, previously evaluated by the professor as illustrated on Fig 1 (Pekić Quarrie et al., 2008).

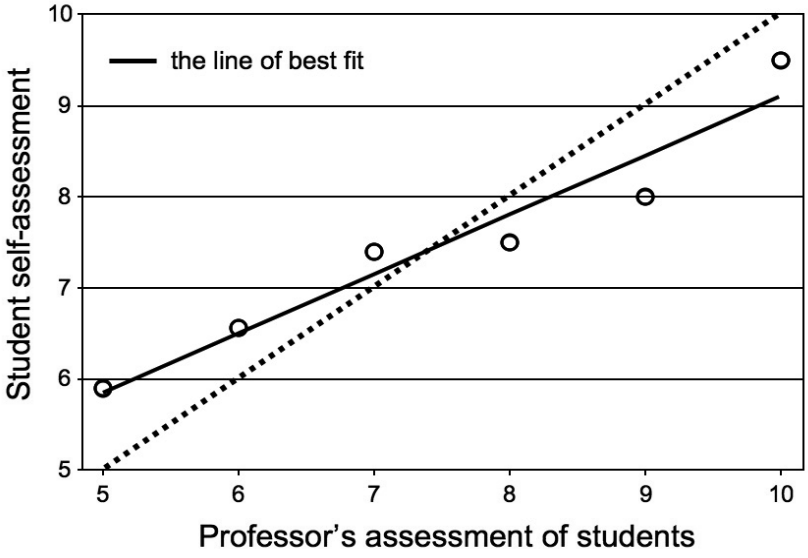

Figure 1. *Student self-assessments as means for each score according to the professor's assessment of each student. The best-fitted line is shown. The dotted line shows equivalence between professor and student scores (from Pekic et al., 2008)*

Results of several years of implementation of the ATL approach have been presented at several conferences on higher education, published in several papers and thus have achieved recognition within both scientific and higher education communities. A research project in ATL at university has recently been accepted for financing by the Ministry of Science and Technological Development, and this will result in further improvement of its methodology.

Conclusion

Based upon all this we strongly believe that courses in the ATL approach together with other academic skills necessary for the development of young university teachers should become a compulsory component of their career development. Such courses will improve all their academic skills, which in turn will results in improved student competences and, consequently, lead to the improvements in the efficiency of the university as a whole. Such lecturer training courses are common place and compulsory in a large number of European Universities. For this to become a sustainable concept in Serbia, we propose the creation of a CENTRE FOR IMPROVING ACADEMIC SKILLS. Such an institution could provide regular training for our academic staff on a long-term basis.

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References

1. Ivić I, Pešikan A and Antić S 2002. ACTIVE LEARNING 2. UNICEF-Instiute for psychology, University of Belgrade.
2. Ivić I, Pešikan A and Antić S (Eds.) 2008. Student in the centre of the teaching process. Ed by Faculty of Agriculture and Education Forum, Belgrade.
3. Pekić S, Poleksić V, Vucelić-Radović B, Quarrie SA and Pešikan A 2005 First steps in improving academic skills at the Faculty of Agriculture, Belgrade University (In Serbian). *In*: Proceedings of Conference Serbian Higher Education on the Road to Europe – Four Years Later. October 2004, Belgrade.Ed. Alternative Academic Educational Network. pp. 121-128.
4. Pekić Quarrie S, Pešikan A, Poleksić V and Quarrie SA 2006 Developing students' professional competences for decision making by active learning methodology. *In*: Proceedings of 8 ECHAE Conference, Prague, September 2006, pp.184-189.
5. Pekić Quarrie S 2007. Student peer review as a tool for efficient achievement of both subject-specific and generic learning outcomes: examples in botany at the Faculty of Agriculture, University of Belgrade. Higher Education 32, 206-214.
6. Pekić Quarrie S, Poleksić V, Antić B, Pešikan A and Quarrie SA 2008. Students as partners: from dream to reality. Student self-assessment exam exercises at Faculty of Agriculture, Belgrade University. *In*: Proceedings of 9 ECHAE Conference, Leida, September 2008.
7. Pešikan A, Poleksić V and Antić S 2005. How to improve competencies of academics? Case of the Faculty of Agriculture, University of Belgrade. Proceedings of the First ISCAR Conference (International Society for Cultural and Activity Research): „Acting in changing worlds: learning, communication, and minds in intercultural activities", Seville, Spain, pp. 510-512 (ISBN: 84-689-3744-4, http://www.iscar.org)
8. Pešikan A 2005. "Profilisanje budućih diplomiranih stručnjaka", Psihologija 38, 3, 239-253.

9. Pešikan A 2006. Developing generic and subject-specific competences of graduates: One or two separate issues?, Brian Dennis (Ed.): Rethinking higher education in the food chain and environment: Profiling graduates for the future. Copenhagen: The Royal Veterinary and Agriculture University (KVL), pp. 99-111

10. Pešikan A, Pekić Quarrie S, Poleksić V and Quarrie SA 2006. Developing students competences for decision making by active learning methodology. Proceedings of 8th ECHAE Conference, Prague, September 2006, pp. 194-197.

11. Poleksić V, Quarrie S, Pekić S and Pešikan A 2004. Competence building of teachers: Case of the Faculty of Agriculture, University of Belgrade. Proceedings of 7th ECHAE Conference, Copenhagen, August 2004, pp. 71-76.

Original paper

HOW TO TRAIN STUDENTS FOR TRANSFER OF KNOWLEDGE: THE ANALYSES OF TEXTBOOKS AND INSTRUCTIONAL MATERIALS FOR STUDENTS OF AGRICULTURE

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Abstract

Transfer of learned knowledge and skills is considered as a fundamental goal of education; without transfer, education would be meaningless. Subject of the study is the analysis of the 16 textbooks and instructional materials intended for students of Master studies "Environmental protection in Agriculture" at the Faculty of Agriculture University of Belgrade. In the study, the following analyses have been made: (1) the analysis of the type and number of *the structural components* of the textbooks; (2) the analyses of the questions, tasks and orders (QTO) in the textbooks, which comprises following analyses: (2.1) *the meaningfulness* of the QTO; (2.2) *the form* of the QTO; (2.3) *the function* of the QTO; and (2.4) *the cognitive processes* that is required by the QTO according to Revised Bloom's taxonomy of educational objectives. Generally speaking, the mechanisms for fostering and facilitating transfer of knowledge and skills in the most of analyzed textbooks are neglected or developed in a small degree, and unevenly distributed among the analyzed textbooks. The most of the materials enable development of just "very near", specific transfer to the situation of the exam in which the students will be exposed to the same type of the QTO like in the initial materials for learning. Except in few of the analyzed materials (three of 16), there is no solid ground for the promotion of transfer.

Key words: *animal sciences, questions, tasks and orders, textbooks, transfer of knowledge*

Introduction

Education system is based on the idea of transfer of knowledge and skills: once developed knowledge or skill can be used in other similar or dissimilar new situations. Without transfer, education would be meaningless. Therefore, through schooling students will learn the selection of important knowledge, skills and abilities by which they would be prepared for their private and professional lives in the future. Hence, educational aims and outcomes of learning/teaching process are based on transfer and they try to make it clear what is purpose of specific type and level of schooling.

For more than a century the problem of transfer of knowledge has been an important topic of research in educational psychology (for review of researches see: Cox, 1997; Singley and

Anderson, 1989; Mayer and Wittrock, 1996; Billing, 2007). Throughout its long research history transfer has been, and still is, a very controversial issue. There is no definition of transfer that all researchers would agree upon. There is a long lasting huge debate on nature of transfer and depending on theoretical lens the same set of empirical data is differently interpreted. One theoretical viewpoint is that transfer can be explained by similarity of two situations, i.e. existing of identical elements in both of them - one situation in which knowledge and skills are acquired and new one in which knowledge and skills have to be applied (Thorndike & Woodworth, 1901; Singley and Anderson, 1989). Other theoretical viewpoint supports the transfer of general skills, that under appropriate conditions students can learn to improve their problem-solving transfer (Salomon & Perkins, 1989; Mayer and Wittrock, 1996), or that "there are multiple manifestations of transfer, ranging from the understanding of domain-specific concepts through the deployment of relatively domain-general reading and argumentation strategies' (Campione, Shapiro and Brown, 1995, p.66). In spite of the conclusion that there is "an obvious need for further inquiry aimed at a better and deeper understanding of the processes underlying transfer and at finding effective research-based and practically applicable ways to facilitate transfer in learners in different educational and training settings" (De Corte, 1999, p.557), the numerous research findings support the idea of transferability of learned knowledge and usefulness of teaching for transfer.

Transfer may be *specific* or 'near' transfer of similar elements in the initial and target task (e.g. within the same type of problem in the same subject domain); and *general* or 'far' transfer of methods, general principles, techniques and attitudes (e.g. between domains). 'Near' or specific transfer implies that the initial and target tasks are closely related, having some similar elements, and 'far' transfer, i.e. general transfer implies transfer of knowledge, skills and attitudes to quite different problem context or content depends on more general, structural similarities (Billing, 2007). These types of transfer are not mutually exclusive, general skills and specific knowledge is complementary in transfer.

The practical significance of transfer of learning is quite obvious in both the cases of formal schooling and of 'knowledge-based economy'. Transfer of learned knowledge and skills is considered as a fundamental goal of education (Marini and Genereux, 1995), what is reflected in Seneca, Ancient Greek author's sentence: `Non scholae, sed vitae discimus" (we do not learn for school but for life). Also, importance of transfer of knowledge between education and workplace settings is clear. Today's business and industry invest enormous amounts of money in in-service training and retraining of personnel and they are strongly interested in acquiring transferable knowledge and skills by their employees to increase rate of return of investment, as well as reduction in spending. Bulk of the literature has been written on training transfer. Training transfer refers to the application, generalization and maintenance of trained skills on the job (Ford & Weissbein, 1997; Volet, 1999; Eraut, 2009; Grossman & Salas, 2011; Volet, 2013). The request of 'employability' has influenced on higher education programs significantly to take into account the competencies, which employers are said to prize most, so-called transferable or generic skills such as higher order thinking processes (problem-solving, critical and creative thinking), communication and team work. Perhaps the other reason is related to students paying fee for studying and "seeking 'value for money' "(Billing, 2007, p. 501).

The review of the literature on transfer shows that transfer of knowledge and procedures learned through instruction unfortunately occur far less often than academics believe (Alexander and Murphy, 1999). There are many research findings on transfer, which implications are important

for the teaching for transfer (or teaching of key skills) in higher education. The main conclusion from the research evidence is that whether transfer occurs is dependent on the conditions. The main conditions that can support or hinder the transfer are as follows (Perkins and Salamon, 1992a,b; Singley and Anderson, 1989; Alexander and Murphy, 1999; Bransford et al., 1999; Bransford & Schwartz,1999; Billing, 2007):

- Transfer is more likely *when the learning environment is designed to encourage cross-situation and cross-domain transfer*. Showing students how knowledge from different subjects or different contents of same subject are mutually connected, or how problems resemble each other is very useful;
- Skills and knowledge must be extended beyond the narrow context. To be widely applicable learning must be guided by generalized principles. *Learning of general principles of reasoning together with self-monitoring practices and potential applications in varied context* supports transfer. Procedures of generalization of higher mental abilities should be in-built in teaching/learning process. The specificity of the context in which principles are learned reduces their transfer;
- *Rote learning of facts discourages transfer of knowledge*. Learning of principles and concepts facilitates transfer to new situations and dissimilar problems because it creates more flexible mental representations;
- Transfer is supported *if students have conceptual knowledge, mental representation of problems* (including how one problem is similar and different from others), and *understanding of the relationships of the components in the overall structure of the problem*;
- Teaching in reasoning and critical thinking is only effective for transfer *when abstract principles and rules are accompanied by examples,* that have to be varied;
- Transfer is fostered *when learning takes place in social context* in which generation of principles and explanations are developed through interaction and so called 'negotiation of meanings';
- *Cooperative methods of learning* improve transfer;
- Transfer is promoted when *the feedback on performance* is given to students;
- Transfer and analogical reasoning are related processes and *transfer is promoted by teaching by analogy* and emphasizing structural similarities between analogues and the underlying goal structure of comparable problems;
- Transfer is fostered if *students are expected to learn to do some performance by themselves* and if they are *aware how to apply skills in different context*. The learner must understand the conditions of application – *when* what has been learned can be used;
- *Learning to use meta-cognitive strategies* is especially important for transfer. Learners are most successful if they are self-aware as learners and are able to monitor and evaluate their learning and develop strategies which keep learning on target. Meta-cognitive skills (e.g. self-monitoring, reflection, self-regulation, executive control strategies, learning-to-learn) are vital particularly in solving complex problems. Meta-cognitive strategies result in more sophisticated conceptions of learning, greater awareness of cognitive strategies, more complex and integrated knowledge structures, and more accessible and usable knowledge.

As we have mentioned previously, transfer of knowledge is encompassed by aims and outcomes of learning, and it has to be promoted by teaching/learning methods and instruction strategies. In

the light of the research evidences on transfer, the effectiveness of instruction and learning resources to foster transfer can be analyzed.

Purpose of this study

In spite of the new technological or 'ICT era' many researches clearly show persistent importance of the textbook in education (see: Johnsen, 1993; Mikk, 2000; Selander, Tholey & Lorentzen, 2002; Pingel, 2010; Ivic, Pesikan, Antic, 2013). Besides the teacher, textbook is the key 'instrument' in teaching/learning process. Textbook is not the exposition of information; its role is to support independent construction of knowledge. The textbook has to create situations for learning and all of its structural components (e.g. core text, illustrations, tasks, questions, summaries, additional resources, critical thinking units, etc) have to be aligned in a harmonious and coherent manner in order to achieve the learning objectives (Ivic, Pesikan and Antic, 2013).

The purpose of this study is to see how the teaching of transfer is supported through the instructional materials for students of animal sciences. To encourage and facilitate transfer of knowledge and skills, the specific measures have to be built-in the textbook. We will take into the account the structural components of textbook (which are intended to empower understanding and acquiring lasting and applicable knowledge) and particularly questions, tasks and orders, which should provoke different mental processes and learning styles, enabling students to practice important knowledge and skills and accomplish desirable outcomes of the course. (Ivić, Pešikan, Antić, 2013).

Subject of study

Subject of the study is the analysis of the 16 textbooks and instructional materials intended for students of Master studies "Environmental protection in Agriculture" at the Faculty of Agriculture University of Belgrade. All textbooks have been written within the frame of the WUS Austria MSDP 004/2009 project, with the aim to help learning process of students. In the study, the following analyses have been made: (1) the analysis of the type and number of *the structural components* of the textbooks; (2) the analyses of the questions, tasks and orders (QTO) in the textbooks, which comprises following analyses: (2.1) *the meaningfulness* of the QTO; (2.2) *the form* of the QTO (e.g. open-ended questions with short answer, essay, multiple choice, matching, etc); (2.3) *the function* of the QTO: supporting the construction of knowledge or evaluation of what has been learnt; and (2.4) *the cognitive processes* that is required by the QTO according to Revised Bloom's taxonomy of educational objectives (RBT - Anderson and Krathwohl, 2001).

The structural components (SKs) of textbook contribute to better understanding of the content; linking content with previous knowledge and students' experience; improving the assimilation of presented material; and improving learning efficiency of students (Ivić, Pešikan and Antić, 2013). Within the textbook there can be different SKs: thematic units or chapters; lessons; questions, tasks and problems (at the end of the lesson or thematic unit); boxes with additional information; boxes with definitions of less known words; illustrations accompanied the text (e.g. drawings, pictures, graphs, schemes, maps, tables, etc); summaries of the lesson or thematic unit; table of content; etc.

QTO are the most important structural component of textbook for activating students in the process of learning. Consideration *of the meaningfulness of QTO* is the first step in the evaluation of the quality of QTO in the textbook. QTO can be meaningless according to several criteria: linguistically incorrect task, unrealistic task (e.g. too difficult or too easy for the student, or there is no prerequisite to enable fulfilling the tasks - no time, no experience, no conditions, etc), intellectually imprecise task (e.g. when student is confused and not know what is required to do in the task), quasi-activating task (e.g. an activity with no purpose or effects for learning is required), and task which suggests the answer (e.g. Is it nice hurting others?) (Ivić, Pešikan and Antić, 2013).

There are *two distinct functions of the QTO*: (a) assessment for learning and assessment as learning, where the role of the QTO is to support the construction of knowledge by creating the situations for learning for students. What the students are doing, they will learn it. Second function of QTO (b) is well-known assessment of learning, i.e. evaluation of what was learnt by students.

Methodology

The method of content analysis has been used in the study. The sample was the convenience sample (Marshall, 1996) involving the 16 textbooks and instructional materials for students of Animal Sciences at the Agriculture Faculty in Belgrade that have been developed and published in the frame of the WUS Austria project and were available for the analyses. In the (1) analysis the unit of analysis was the textbook and in the (2) analyses the unit was individual question, task, or order in the textbook.

The full titles of the analyzed textbooks are not given, just abbreviations, because of discretion. In this study we are not interested in the analysis of the quality of individual textbook than looking for the possibilities for transfer promotion at the wider setting in higher education in Serbia.

Results and discussion

(1) The analysis of the type and number of the structural components of the textbooks

The Table 1 shows that there are 18 different structural components in the analyzed textbooks: Table of contents, Introduction/Foreword, Core text, Important to know, Summary, Illustrations, Questions, tasks and orders, Advanced organizer of thematic unit, Advanced organizer of lesson, Interesting facts, How to learn, Key words, Additional information: website addresses and journals, Goals and outcomes, Dictionary of unknown words and phrases, and Case studies. The range of SKs varied from 5 to 11 per textbook and about 70% of the textbooks have just one third of them (5-7). The number of SKs is an indicator of learner-centered orientation of textbook because the function of the SKs is improving of learning efficacy and fostering transfer.

What types of the SKs are presented in the textbooks? Only three of them (Introduction and/or Foreword, Core text, and Illustrations) are presented in all analyzed materials. But, these components are characteristic for any kind of book. The problem is the absence of the SKs that are specific for the textbook as a special genre of book which have formative-developmental role (Ivić, Pešikan and Antić, 2013). If the textbook does not contain QTO, then it is not a textbook,

and half of the analyzed materials have no QTO at all. The absence of some of the most important textbook's components, and rare appearance of other SKs that are supports to understanding and knowledge construction (such as summary of lesson or thematic unit, dictionary, index of concepts, how to learn unit, case studies, etc.) is significant (but bad) sign in regard of transfer of knowledge.

Table 1. *Structural components of the analyzed textbooks*

						Illustrations			Advanced organizer										
Textbooks	Table of contents	Introduction/Foreword	Core text	Important to know	Summary	Representational (e.g. drawings, photos)	Non-representational (map, diagrams, scheme, table	Questions, tasks and orders (QTA)	of thematic unit	of lesson	Interesting facts	How to learn	Key words	Additional information (websites, journals)	Sequence of integration	Goals and outcomes	Dictionary	Case studies	Σ
1.ZZ	+	+	+			+	+												6
2. EA	+	+	+			+	+	+											6
3. EZ	+	+	+					+					+	+					6
4. PE	+	+	+			+	+	+											6
5.ZR	+	+	+	+		+	+												6
6. ZV	+	+	+			+	+												5
7. ZS	+	+	+	+	+	+	+												6
8. EP	+	+	+			+	+		+		+								7
9. ET	+	+	+			+	+												5
10. GS	+	+	+		+	+	+	+	+						+				9
11. Em	+	+	+									+				+	+	+	7
12.BD	+	+	+			+	+	+	+										7
13. Zz	+	+	+	+		+	+	+											7
14. ZP	+	+	+			+	+	+				+		+					8
15. EG	+	+	+			+	+	+			+								7
16. EM	+	+	+		+	+	+	+	+	+				+					11
Σ	16	16	16	3	3	16	16	9	4	1	2	2	1	3	1	1	1	1	

Hence, the results of first analysis show that there is very few of the structural components, and some of crucial the textbook's components are absent or appear rare and unsystematically,

haphazardly in the analyzed textbooks. That means that they are not designed purposefully with the clear idea to enable and empower students' learning. Generally speaking, the analyzed materials mainly expose the content of the subjects and missed the opportunities to involve students in learning process and support their transfer of knowledge.

(2) The analyses of the questions, tasks and orders (QTO) in the textbooks

(2.1) Analyses of the meaningfulness of QTO

The QTO appears in 9 of 16 textbooks and total number of the QTO is 967 (Table 2). The QTO are not evenly distributed among the textbooks. Almost two-thirds of all QTO (653 or 67.5%) are found in three textbooks. In 4 of 9 textbooks with the QTO, meaningless QTOs exist (unrealistic tasks, quasi-activating, linguistically incorrect and intellectually imprecise tasks). There are not many of them (4.5%), but they shouldn't exist at all in textbooks. The meaningless questions are not only unusable but harmful: consume space in textbooks, de-motivate students for work and hinder transfer.

Table 2. *Number of the meaningful and the meaningless QTO in the textbooks*

Textbooks	*Meaningful QTO*	*Meaningless QTO*	*Σ*
1.ZZ	0	0	0
2. EA	48	3	51
3. EZ	103	0	103
4. PE	47	0	47
5.ZR	0	0	0
6. ZV	0	0	0
7. ZS	0	0	0
8. EP	0	0	0
9. ET	0	0	0
10. GS	30	5	35
11. EM	0	0	0
12.BD	33	29	62
13. Zz	170	3	173
14. ZP	85	0	85
15. EG	374	3	377
16. EM	34	0	34
Σ	**924** **95.5%**	**43** **4.5%**	**967** **100%**

(2.2) Form of QTO

One type of the task form dominates in the textbooks: open-ended question with short answer. Almost all tasks are of this type - 99.6% (see Table 3). Uniformity of the type of tasks is boring for students, is not suitable for different learning styles and cannot stimulate variety of cognitive processes. All these cannot support the transfer of knowledge.

Table 3. *Number of the QTO according to their form*

	Open-ended tasks		*Closed type of tasks*		
*Total number of the QTO in the textbooks****	*Essay*	*Short answer*	*Multiple choice*	*Two-choice answers (yes-no, true-false)*	*Matching*
924 (100%)	-	**920 (99.6%)**	**4 (0.4%)**	-	-

** Note: Only meaningful tasks entered into the analysis*

(2.3) Functions of QTO

Table 4. *Number of the QTO according to their function*

*Total number of the QTO in the textbooks**	*Supporting construction of knowledge*	*Evaluation*
924 (100%)	**85 (9.2%)**	**839 (90.8%)**

** Note: Only meaningful tasks entered into the analysis*

Assessment for learning and assessment as learning are not recognized functions of the QTO in the analyzed textbook, 90% of the tasks checks how much students have learned from the lessons (see Table 4). It indicates that the textbooks are not learner-centered but content-centered what cannot support the transfer of knowledge and skills.

(2.4) Cognitive processes that QTO activate, according to RBT

Textbook must have tasks that involve students in the relevant activities with the content, but number of QTO by itself is not the guarantee of the textbook quality. Mental activities, types of cognitive processes that are initiated by tasks are of crucial importance. Quality of knowledge depends on the quality of cognitive processes that are activated in the process of learning from textbook. In this study, the Revision of Bloom's Taxonomy – RBT (Anderson & Krathwol, 2001) has been used for the analysis of the cognitive processes initiated by the QTO in the textbooks (see Table 5).

According to the findings, the focus of the textbooks is on factual knowledge (essential facts, terminology, details or elements of specific disciplines). The main cognitive processes lying behind the factual knowledge in the textbooks are remembering (46% of all the QTO) and understanding (25.4% of all the QTO). Applying of procedural knowledge (10% of all the QTO), that helps students to do something specific to a discipline or subject of study, is very important particularly in applied sciences like agriculture. However, all the tasks of this type are in one textbook (practicum) intended for training of students in implementation of experimental procedures. In the textbooks is negligibly small percentage of conceptual knowledge, knowledge of classifications, principles, generalizations, theories, models, or structures important for a particular discipline. In the QTO the content of the disciplines is given in the narrow context without learning of general principles, varying the context and using analogical reasoning.

Table 5. *Knowledge dimensions and cognitive processes that the QTO activate in the textbooks, according to RBT*

*Knowledge dimensions***	*Cognitive processes**					
	Remembering	***Understanding***	***Applying***	***Analyzing***	***Evaluating***	***Creating***
Factual Knowledge	**425 (46%)**	**235 (25.4%)**	3 (0.3%)		1 (0.1%)	
Conceptual knowledge	40 (4.3%)	63 (6.8%)	3 (0.3%)	1 (0.1%)	4 (0.4%)	
Procedural knowledge	33 (3.6%)	17 (1.8%)	93 (10.1%)	2 (0.2%)	2 (0.2%)	
Meta-cognitive knowledge						

Note:

**The cognitive processes are defined as:*

• **Remembering**: Retrieving, recognizing, and recalling relevant knowledge from long-term memory.
• **Understanding**: Constructing meaning from oral, written, and graphic messages through interpreting, exemplifying, classifying, summarizing, inferring, comparing, and explaining.
• **Applying**: Carrying out or using a procedure through executing, or implementing.
• **Analyzing**: Breaking material into constituent parts, determining how the parts relate to one another and to an overall structure or purpose through differentiating, organizing, and attributing.
• **Evaluating**: Making judgments based on criteria and standards through checking and critiquing.
• **Creating**: Putting elements together to form a coherent or functional whole; reorganizing elements into a new pattern or structure through generating, planning, or producing (Anderson and Krathwohl, 2001, pp. 67-68)

*** The knowledge dimensions are defined as:*

Factual knowledge is knowledge that is basic to specific disciplines. This dimension refers to essential facts, terminology, details or elements students must know or be familiar with in order to understand a discipline or solve a problem in it.

Conceptual knowledge is knowledge of classifications, principles, generalizations, theories, models, or structures pertinent to a particular disciplinary area.

Procedural knowledge refers to information or knowledge that helps students to do something specific to a discipline, subject, or area of study. It also refers to methods of inquiry, very specific or finite skills, algorithms, techniques, and particular methodologies.

Meta-cognitive knowledge is the awareness of one's own cognition and particular cognitive processes. It is strategic or reflective knowledge about how to go about solving problems, cognitive tasks, to include contextual and conditional knowledge and knowledge of self.

Besides the practicum, there is in fact no applying of knowledge in the textbooks, and no processes of analyzing, evaluating and creating of knowledge. Critical thinking consists of the processes of analysis, evaluation and creation; it is contextual, evaluative and meta-cognitive thinking by its nature. In the textbooks there is no meta-cognitive knowledge in the QTO at all, not even at the level how to learn certain subject. In many European education documents learning to learn is emphasized as one of the key competencies in 21st century (European Communities, 2007; Gordon et al., 2009). 'Learning to learn' is the ability to pursue and persist in learning, to organise one's own learning, including through effective management of time and information, both individually and in groups. This competence includes awareness of one's learning process and needs, identifying available opportunities, and the ability to overcome

obstacles in order to learn successfully. This competence means gaining, processing and assimilating new knowledge and skills as well as seeking and making use of guidance. Learning to learn engages learners to build on prior learning and life experiences in order to use and apply knowledge and skills in a variety of contexts: at home, at work, in education and training' (Gordon et al., 2009, p.45). Development of meta-cognitive knowledge, critical thinking and 'learning to learn' is the task for all disciplines and all subjects at all education levels.

The evidences on transfer emphasize that: learning of principles and concepts facilitates transfer to new situations and dissimilar problems; skills and knowledge must be extended beyond the narrow context; the specificity of the context in which principles are learned reduces their transfer; transfer is supported if students have conceptual knowledge; and learning to use meta-cognitive strategies is especially important for transfer. Bearing in mind this evidences, the results of the analysis of the cognitive processes that is required by the QTO in the analyzed textbooks are discouraging in regard to the transfer.

In spite of such general results of the analysis, it has to be noted that there are three textbooks that are different in comparison with others. They did not fully succeed to make good learning-centered materials, but they have made steps forward to improvement of students' learning. The authors of these textbooks had started to implement some of the active learning ideas and were starting to improve their teaching methods (Pešikan and Antić, 2009), and then first results have appeared. So we come to the issue of the education policy and the system's care for the quality of teaching/learning in higher education and necessity for systematic training of academics about modern conception of teaching/learning and their practical implementation.

Conclusion

Generally speaking, the mechanisms for fostering and facilitating transfer of knowledge and skills in the most of analyzed textbooks and instructive materials intended for the students of Master studies "Environmental protection in Agriculture" at the Faculty of Agriculture in Belgrade are neglected or developed in a small degree, and unevenly distributed among the analyzed textbooks. The most of the materials enable development of just "very near", specific transfer to the situation of the exam in which the students will be exposed to the same type of the QTO like in the initial materials for learning. Except in few of the analyzed materials (three of 16), there is no solid ground for: the development and promotion of transferable, generic skills (higher cognitive processes: problem-solving, critical thinking, communication and team-work); cross-situation and cross-domain transfer; practicing and applying skills and knowledge in wider and different contexts; the development of the conceptual knowledge and the understanding of the overall structure of the phenomenon; the development of meta-cognitive strategies; and for the cooperation with others on the work on the QTO. Staying predominantly at the level of reproducing of the subject's information with understanding is not the good base for the promotion of transfer.

However, two things should be noted. First, the obtained results are in accordance with the results that we have got in the analyses of the textbooks for pre-university education. The main problem of the education system in Serbia is the quality of education (see: Strategy for development of education in Serbia to 2020+, 2012; Ivić and Pešikan, 2012) and higher education has double task, to cope both with the quality of its teaching/learning and the quality of teacher preparation for all education levels. Second, academics in Serbia have no preparation

for their teaching role. They see themselves predominantly as scientists and not as teache promotion is based on their scientific output, and quality of their pedagogical work is ve taken into account. The exception to the rule is the development of Active Learning Pro the Faculty of Agriculture in Belgrade (Pešikan, Poleksić, and Antić, 2005; Pešikan, 200 et al., 2005; Poleksić et al., 2006; Pešikan et al., 2006; Pekić Quarrie, 2007a; Pekic 2007b; Antić, Ivić and Pešikan, 2008; Pešikan, Antić and Quarrie, 2009) and other agr faculties in Serbia (CaSA Tempus Project, 2014). These universities are among the firs recognized the need to improve the quality of teaching/learning. Of course, this requires investment of time and effort and result would not be guaranteed if there is no suppor system to make the changes become viable and compulsory for all higher education inst in Serbia. The findings show that training of the academics in pedagogic skills and learning/teaching methods gives visible effects on improving the quality of teaching/learn transfer of acquired knowledge.

References

1. Alexander PA and Murphy KP 1999. Nurturing the seeds of transfer: a domain perspective. International Journal of Educational Research 31, 561-576
2. Anderson LW & Krathwohl DR (Eds.). 2001.A taxonomy for learning, teaching, and asse revision of Bloom's taxonomy of educational objectives. New York: Pearson Education.
3. Antić S, Ivić I i Pešikan A (ur.) 2008. Student u središtu nastave - Aktivno na Poljoprivrednom fakultetu (Student-centered teaching – Active Learning in the Fa Agriculture in Belgrade). Beograd: Poljoprivredni fakultet i Obrazovni forum.
4. Billing, D 2007. Teaching for transfer of core/key skills in higher education: Cognitiv Higher Education 53(4), 483-516.
5. Bransford JD & Schwartz DL 1999. Rethinking transfer: A simple proposal with implications. Review of research in education, 61-100.
6. Bransford JD , Brown AL and Cocking RR (Eds) 1999. How People Learn: Brain Experience, and School, Expanded edition. Washington D.C.: National Academy Press.
7. Campione JC, Shapiro AM, & Brown AL 1995. Forms of transfer in a community of Flexible learning and understanding. In A. McKeough, J. Lupart, & A. Marini, Teac transfer:Fostering generalization in learning (pp. 35}68). Mahwah, NJ: Lawrence Erlbaum
8. CaSa Tempus Project: TEMPUS project Building capacity of Serbian Agricultural Edu link with Society (acronym CaSA) 2014-2016
9. Cox BD 1997. The rediscovery of the active learner in adaptive contexts : A develo historical analysis of transfer of training. Educational Psychologist 32, 41-55.
10. De Corte E 1999. On the road to transfer: an introduction. International Journal of Edu Research, Volume 31, Issue 7, 555-559.
11. Eraut M 2009. 2.1. Transfer of knowledge between education and workplace settings. Kn values and educational policy: A critical perspective, 65.
12. European Communities 2007. Key Competences for Lifelong Learning - European R Framework. Luxembourg: Office for Official Publications of the European Communities

13. Ford JK & Weissbein DA 1997. Transfer of training: An updated review and analysis. Performance improvement quarterly 10(2), 22-41.

14. Gick ML and Holyoak KJ 1987. The cognitive basis of knowledge transfer. In Cormier, S.M. and Hagman, J.D. (eds.), Transfer of Learning: Contemporary Research and Applications . San Diego: Academic Press Inc, 9–46.

15. Gordon J, Halász G, Krawczyk M, Leney T, Michel A, Pepper D, Putkiewicz E, & Wiśniewski J 2009. Key competences in Europe: opening doors for lifelong learners across the school curriculum and teacher education. CASE Network Reports, (87).

16. Grossman R & Salas E 2011. The transfer of training: What really matters. International Journal of Training and Development 15(2), 103–120

17. Ivic I, & Pesikan A 2012. Education system reforms in an unstable political situation: the case of Serbia in the first decade of the 21st century. CEPS Journal 2(2), 31-53.

18. Ivic I, Pešikan A. & Antic S 2013. Textbook Quality: A Guide to Textbook Standards. Eckart Expertise. V&R Unipress, Gottingen

19. Johnsen EB 1993. Textbooks in the kaleidoscope: A critical survey of literature and research on educational texts. Oslo: Scandinavian University Press.

20. Marini A & Genereux R 1995. The challenge of teaching for transfer. In A. McKeough, J. Lupart, & A. Marini, Teaching for transfer: Fostering generalization in learning. Mahwah, NJ: Lawrence Erlbaum. 1-20.

21. Marshall M 1996. Sampling for qualitative research. Family Practice, Vol.13, No.6, p. 522-525

22. Mayer RC and Wittrock MC 1996. _Problem-solving transfer_, in Berliner, D.C.and Calfee, R.C. (eds.). Handbook of Educational Psychology. New York: Macmillan, pp. 47–62.

23. Mikk J 2000. Textbook: Research and Writing. Baltische Studien zur Erziehungs und Sozialwissenschaft, Band 3 (Baltic Studies for Education and Social Sciences, Volume 3). Peter Lang Publishing, Inc., 275 Seventh Ave., 28th Floor, New York, NY 10001-6708.

24. Perkins DN & Salomon G 1992a. The science and art of transfer. If minds matter: A foreword to the future 1, 201-210.

25. Perkins DN & Salomon G 1992b. Transfer of learning. International encyclopedia of education, 2.

26. Pekic Quarrie Sofija 2007a. Student peer review as a tool for efficiently achieving subject-specific and generic learning outcomes: examples in botany at the Faculty of Agriculture, University of Belgrade. Higher Education in Europe 32, 205-214.

27. Pekić Quarrie S. 2007b. Examples of student peer review at the faculty of agriculture,University of Belgrade to improve quality assurance and reduce corruption. AEGEE Conference on "Bologna process best practices", Niš.

28. Pekić S, Poleksić V, Vucelić-Radović B, Quarrie S i Pešikan A 2005. Prvi koraci u poboljšanju akademskih veština na Poljoprivrednom fakultetu u Beogradu (First steps in academics' competencies improvement at the Faculty of Agriculture in Belgrade). Zbornik radova „Visoko obrazovanje u Srbiji na putu ka Evropi – četiri godine kasnije", UNESCO katedra za menadžment univerziteta - AAOM, Zajednica univerziteta Srbije i Fond za otvoreno društvo iz Beograda, Beograd, 121-129.

29. Pešikan A i Antić S 2009. Implicit theories of academics on the nature of teaching/learning process, 3rd TEPE Conference, Umea, Sweden (Extended summary at http://htk.tlu.ee/tepe/papers/)

30. Pešikan A 2005. Profilisanje budućih diplomiranih stručnjaka, Psihologija, Vol. 38, br. 3, 239-253.

31. Pešikan A, Antić S & Quarrie S 2009. Active Learning – Improving teaching competencies and academic Skills. Final Conference on Higher Education Reform of Biological Sciences (H.E.R.B.S.) in EU Curriculum Development Joint European Project. Faculty of Biology, Belgrade, May 28-30.
32. Pešikan A, Pekić- Quarrie S, Poleksić V and Quarrie S 2006. Developing Students' Professional Competencies for decision making by active learning methodology. In Milan Slavik and Petra Žakova (Eds.): The public and the agriculture and forestry industries: The role of Higher Education in questioning assumptions and matching expectations. Prague: Czech University of Agriculture, 184-190.
33. Pešikan A, Poleksić V and Antić S 2005. How to improve competencies of academics? Case of the Faculty of Agriculture, University of Belgrade. Proceedings of the First ISCAR Conference (International Society for Cultural and Activity Research): „Acting in changing worlds: learning, communication, and minds in intercultural activities". Seville, Spain, 510-517.
34. Pingel F 2010. UNESCO guidebook on textbook research and textbook revision. Unesco.
35. Poleksić V, Quarrie S, Pekić S i Pešikan A 2006. Competence building of teachers: Case of The Faculty of Agriculture University of Belgrade, Brian Dennis (Ed.): Rethinking higher education in the food chain and environment: Profiling graduates for the future. Copenhagen: The Royal Veterinary and Agriculture University (KVL), pp. 71-77
36. Salomon G & Perkins DN 1989. Rocky roads to transfer: Rethinking mechanisms of a neglected phenomenon. Educational Psychologist 24, 113-142.
37. Selander S, Tholey M & Lorentzen S 2002. New educational media and textbooks: The 2nd IARTEM Volume.
38. Singley MK and Anderson JR 1989. The transfer of cognitive skill. Cambridge, MA: Harvard University Press.
39. Strategy for Development of Education in Serbia to 2020+, Ministry of Education, Science and Technological Development of Serbia, 2012.
40. Thorndike EL and Woodworth RS 1901. The influence of improvement in one mental function upon the efficiency of other functions. Psychological Review 8, 247-261.
41. Volet S 1999. Learning across cultures: appropriateness of knowledge transfer. International Journal of Educational Research 31, pp. 625-643.
42. Volet S 2013. Extending, broadening and rethinking existing research on transfer of training. Educational Research Review, Vol. 8, 90-95.

Original paper

MULTIDISCIPLINARY APPROACH IN THE IMPLEMENTATION OF ATL METHODS IN TEACHING OF ANIMAL SCIENCES I. PRESENTATION OF SCENARIOS

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Abstract

One of the problems Serbian HE faces is summing up of knowledge thaught in different courses and its application in real life by professional decision making. In order to give an example of possibilities of interactive teaching methodology and contribute to the transformation of students from knowledge recipients to knowledge co-constructors, a set of scenarios for a multidisciplinary approach in teaching/learning is presented. The set consists of 4 scenarios for knowledge refreshment followed by a final workshop – a debate similar to the real life situation. A multidisciplinary approach to the topic of aquaculture development, water and fish quality in aquaculture is presented in this case. By participating interactive classes and a debate, 4 year students in animal sciences work in groups to exercise solving problems, to make professional decision, and develop other professional and generic skills. This paper presents the result of the process of development o scenarios for such exercise and results of the exercise will be presented in another paper (Part 2).

Key words: *active teaching/learning, case study, knowledge application, teaching/learning scenarios, professional decision making*

Introduction

The primary goal of higher education (HE) is training of professionals able to use acquired knowledge and skills to work efficiently, alone and in a team with other experts, to solve problems and make qualified decisions (Pešikan, 2005; Pešikan et al, 2005; Fry et al., 2008). Real-world professional problems are multidimensional, with more than one solution. Dealing with them needs various competences. Development of procedural and meta-cognitive knowledge (Anderson & Krathwol, 2001) is particularly important when students prepare themselves for real life situation. Unfortunately Serbian HE suffers from a lack of practical work and training of skills, both professional and generic (Pešikan et al, 2006; Ivić, 2008). Moreover, according to the Strategy for education development in Serbian HE there is also a need for wider application of active teaching/learning (ATL) principles, including practical application of the knowledge acquired. Traditional teaching methods predominate in HE in our country. Students are mostly passive subjects that have to understand the knowledge delivered and demonstrate it on exam (Anon., 2012). In order

to prepare students to become competent professionals, independent and responsible, their decision - making capacities have to be developed.

Improvement of the quality of teaching and student activation in classes is possible by introducing new teaching strategy, active learning methodology i.e. learner-centered approach (Lambert & McCombs, 1998; Bransford, Brown and Cocking, 1999; Ivić, Pešikan and Antić, 2001) that contribute to knowledge reinforcement, development of critical thinking and problem analysis from different sides. This includes a shift of student's position from recipient of knowledge to knowledge co-constructor in interaction with the teacher (Moll, 1992; Ivić et al, 2002; Pešikan et al, 2005; Pešikan, 2005; Ivić, 2008; Antić, 2008; Anderson, 2013). Students' capacity for decision-making, problems resolving, argumentation, negotiation, evaluation scientific data and information, critical reasonong etc. could be developed throughout different courses. If they learn in such learning situation, students also have better education outcomes (Johnes, 2006). Student activation obviously demands new approaches for teachers that includes creation of scenarios instead of lessons plans, similar to a theatre scene in which a teacher as a theatre director and students as actors assume completely different roles than in traditional teaching that is primarily delivering of knowledge from teacher to students (Ivić et al, 2002, Antić, 2008). Scenarios are task-directed, interactive, student centered, and usually planned for longer periods than a simple class (Anderson and Pešikan, 2014).The approach in which real-life problems are considered is convenient for student to practice possibilities of different tactics to problem solving and decision - making, but without consequences in real life. In this paper we present a set of teaching scenarios for a multidisciplinary approach to the topics of aquaculture development, water and product (fish) quality in aquaculture.

Materials and methods

In order to place students in a situation of professional decision making, a case was created. To prepare students of the final years of studies for a multidisciplinary approach, four scenarios were prepared to recall classes in which a problem of aquaculture and water and fish quality was tackled from different aspects: ecological, physiological, health and welfare, aquatic animal production and quality. All these aspects have been already studied/learned during classes in Zoology, Physiology of domestic and farmed animals, Aquaculture, and Health protection of domestic and farmed animals. The idea was to organise a workshop during courses thought on bachelor level in final semesters when students would study an immaginary case, approach it from different aspects, and suggest the solution of a problem from the point of view of different stakeholders. A workshop should be organized in the frame of different subjects thought in the 6^{th}, 7^{th} or 8^{th} semester of bachelor study in Animal Sciences (Zootechnics) such as: Professional practice (Internship), Aquaculture, Zoohigiene, Biosafety on farm, and Management of animal production.

The 4 scenarios will be realised during one class each, 45 minutes, in a period of at least 4 weeks. Scenarios are based on 4 assignements students have to accomplish by working in groups and problem solving (by filling a table), as well as reporting results, participating in a discussion and preparation of conclusions.

In addition, students will have about a month to prepare themselves for roles of different stakeholders they have to play in a final workshop (90 minutes). Roles were distributed by drawing roles of stakeholders "from a hat". Students should prepare their roles by

consulting relevant literature. Parts of this preparation are 4 interactive classes held during a semester.

Material prepared for each ATL class: material for random distribution of students in groups, appendices – texts with assignements and tables to be filled in for each class and each group. For each student: a text of the case for the final workshop, descriptions of stakeholders groups and ballots for voting are prepared.

Results and discussion

Table 1 represents a summary of the ATL classes held in this case study.

Table 1. *Summary of scenarios of ATL classes Multidisciplinary approach to aquaculture development, water and fish quality, and a case study*

Professor responsible for the ATL class and scenario author	**Topic of the ATL class**	**Objectives**	**Assignement and lesson flow**	**Appendix/inclosure**
Zorka Dulić, Environmental aspect	Water quality parameters and needs of aquatic organisms	1. to recall abiotic environmental factors thought in Zoology course in the 1st year and to understand the notion of water quality 2. to link knowledge on abiotic factors in aquatic ecosystems with ecological needs of aquatic organisms (fish and invertebrate) 3. to find out and understand which are the most sensitive and the most tolerant groups according to different parameters, to classify them into eurivalent or stenovalent organisms for different parameters	Students have to read and understand the text in the appendix in order to fill in the table 2 by writing values of different water quality parameters suitable for each fish species and daphnids, as well as to align species from the most to the least sensitive. Work in groups to finish the table (15 min.). Reporting of each group (5 min. each). Discussion lead by the professor about the level of sensitivity, species sensitivity, effect of age etc. to the classification in steno- or eurivalent organisms.	Text about water quality and values of parameters: temperature, DO, pH, electroconductivity, hardness, phosphate for different fish species and Daphnia Table 2 to be filled in

Vesna Davidović, Physiological aspect	Influence of different stressors on physiolo-gical and immunolo-gical parameters of stress reactions in farmed fish	1. To remind students what is homeostasis and neurophysiological mechanisms of stress reaction in vertebrates. 2. To remind students the most common stressors in farmed fish. 3. To connect knowledge about stress with physiological and immunological indices of stress response in fish.	Students have to select terms related to 1. Homeostasis, 2. Stressors and 3. Stress indicators. Work in 3 subgroups to separate terms and then group conclude whether they are properly selected (10 min.). They also have to determine rearing conditions that can affect physiological and immunological stress indicators values in fish. Reporting of group representatives (5 min. each). Discussion lead by the professor about generalized neuroendocrine response and fish adaptive mechanisms in maintaining homeostasis during primary and secondary stress response, as well as systemic changes in the tertiary response due to an inability to adapt to the stressors.	• Table 3 in which different terms given in a separate list have to be distributed into 3 columns (homeostasis, stressors, and stress indicators). Among the terms there are words that refer to one of the 3 given categories, but also words that are not connected with any of the 3 categories can be offered. • Diagrams and drawings showing the course of the fish stress response via hypothalamic-pituitary-interrenal axis.
Renata Relić, Health and welfare aspects	Influence of the environ-ment (water) quality on fish welfare	1. To remind the students on the concept of farm animals' welfare 2. According to the given model, the students should note and set aside fish welfare indicators that point to inadequate water quality	Students have to read and understand the text in the appendix in order to fill in the table by writing fish welfare parameters that refer to poor quality water. Work in groups and finishing the table up to 15 min. Reporting of the each group representative in the next 10 min. After the specific list of welfare parameters is formed, professor and students discuss about chosen parameters.	Table 4 with the task "Comparative review in cattle and fish: the needs and welfare parameters according to the farm animals' welfare concept". Cattle welfare parameters in the table are related to the inadequate environmental conditions. Common welfare parameters in fish are listed below the table.

Marko Stanković, Production aspect	Production systems of fish and effects on water and product quality	1. to remember fish culture systems and understand differences between systems 2. to link knowledge about fish culture systems with abiotic environmental factors for aquatic environment 3. to be able to decide which species can be cultured in each system depending on environmental conditions 4. to conclude about product quality in relation to water quality and production system	Students, distributed in groups, have to read and understand the text in the appendix in order to fill in the table 5 by writing names of fish species (carp, trout, African catfish, pangasius, herbivorous fish) into the corresponding table cell, depending on culture system and range of values of water quality parameters (15 min.). After reporting of each group (5 min. each), a discussion led by a professor about fish meat quality compared to water quality. Formulation of conclusions.	Text about fish culture systems in Serbia and in the world. Water quality parameters to be respected for each system and short characteristics of Serbian aquaculture to remind students what was thought during aquaculture classes with special emphasize on possibilities of further development of Serbian aquaculture. Table 5 to be filled in.
Vesna Poleksić, Final workshop	Workshop – Case study Bilateral cooperation Serbia-Vietnam: gunshot on Serbian aquaculture or chance for Serbian economy	1. to develop ability of case analysis, problem solving, and professional decision making, as well as presenting results and explain professional decisions made 2. to develop skills of intra and intergroup cooperation, effective communication, dialogue, discussion and conclusions formulation	After a month of preparation a Final workshop - parliamentary debate is organized. Students play their respective roles of stakeholders during the final workshop. After a short introduction by a professor (5 min.) each stakeholder group prepares a 10 min. presentation of their group recommendation to the government (40 min.). Follows a "parliamentary" debate: questions asked by members of the parliament and stakeholders, moderated by the president. (30 min.). Voting and final decisions and conclusions of students and professors. (5 min.)	**Case study** Details in the text

The 1st ATL class – Teacher Zorka Dulić: Water quality parameters and needs of aquatic organisms

ATL method: students distributed randomly in groups (4 - 6) have to fill the table (Tab 2).

Table 2. *Assignement for the 1st ATL class: using the text given in the appendix fill in the table by ranges of values for each parameter and aline species according to their sensitivity in relation to each environmental factor*

Water quality parameter	Species of aquatic organism							Order of species from the most to the least
	Carp	Trout	African catfish	Herbivorous fish	Salmon	Pangasius	Daphnia	
Temperature (t^oC)								
Dissolved oxygen (mg/L)								
pH								

Ammonia (mg/L)								
Electroconductivity (μS/cm)								
Phosphate (mg/L)								
Hardness (°dH)								

The 2nd ATL class – Teacher Vesna Davidović: Influence of different stressors on physiological and immunological parameters of stress reactions in farmed fish

Table 3. *Assignement for the 2nd ATL class: Distribute the terms offered in 3 categories: isotonia, food quantity, cortisol concentration, isotonic point, hatch density, glucose level, isohydria, manipulative fishing operations, total protein level, total immunoglobulin level, hypocalcaemia, blood sampling, thyroxin concentration, hypothermia, good quality food, parathormone concentration, hypomagnesaemia, control measuring, satisfying water quality, hematuria, air temperature, progesterone concentration, calciuria, predators presence, adrenaline concentration, acidosis, surrounding noise, insulin concentration, isothermia, androsterone concentration. Note that there are some terms that do not belong to any of the 3 categories*

Homeostasis	Stressors	Stress indicators

The 3rd ATL class – Teacher Renata Relić: Influence of the environment (water) quality on the fish welfare

Assignement for the 3rd ATL class: each group fills in the table and explains group decision

Table 4. *Comparative review in cattle and fish: the needs and welfare parameters according to the farm animals' welfare concept*

Nº	CATTLE		FISH	
	Animal needs	Parameters* (examples)	Animal needs	Parameters** (enter into the column)
1	**Adequate living conditions** (i.e. appropriate microclimate and hygiene in the stall, protection from bad weather and predators, comfortable place for resting and movement)	• air temperature too high • poor ventilation • no shelter • easy access of predators • slippery floors • wet bedding	**Adequate environment**	
2	**Proper feeding and watering** (i.e. permanent access to the sufficient quantity of quality food and water, in accordance with the needs)	• insufficient width of the feeding space • insufficient number of the waterers	**Proper feeding**	

3	**Expression of normal behaviour** (i.e. animal housing in facilities that allow sufficient movement and expression of normal behaviour)	•apathy •anxiety •excessive salivation •reluctant movement, rising etc.	**Expression of normal behaviour and possibility to avoid predators**	
4	**Adequate social interactions** (i.e. placement in the appropriate group or separately, in accordance with the type and category)	•aggressive behaviour •submissive behaviour	**Adequate social interactions**	
5	**Protection from disease and injury** (i.e. absence of pain, suffering, illness and injury, the timely provision of professional help)	•body injuries •skin diseases •respiratory diseases •death	**Maintaining good health**	

* Indicators of welfare may be from the environment (environmental characteristics) and from individuals (health, production, biochemical, and behavioural indicators); each indicator consists of measurable properties - parameters (e.g., microclimate in object consists of temperature and humidity, the amount of dust, brightness and other properties whose values can be measured).

** Common welfare parameters of fish are: mortality, body injuries (skin, fins, eyes etc.), presence of diseases and parasites, feed conversion rate, growth, condition factor, swimming behaviour, eating behaviour, fertility, respiration rate, levels of plasma protein, immunoglobulins, glucose, cortisol, enzymes, lysozime and other substances from blood and excretions, phagocyte respiratory burst, values of the water temperature, pH, dissolved oxygen, nitrogen compounds etc.

The 4th ATL class – Teacher Marko Stanković: Production systems of fish and effects on water and product quality

Table 5. *Assignement for the 4 ATL class: Names of following fish species should be put in the table in a corresponding table cell, according to production systems and vales range of environmental factors: Carp, Trout, Salmon, Pangasius, Herbivorous fish, African catfish*

Parameters	Earthen pond	„Riceway“	Cage system
Temperature 20-26^{o}C DO >5 mg/L pH 6.5-8.5 Ammonia <0.5 mg/L			
Temperature 12-16^{o}C DO 7-11 mg/L pH 6.5-8 Ammonia <0.025 mg/L			
Temperature (15-19^{o}C) DO (7-9 mg/L) pH 6.2-7.8 Ammonia (<0.02 mg/L)			
Temperature (22-30^{o}C) DO (2.5-7.5 mg/L) pH 6.5-9.5 Ammonia (0.7-1 mg/L)			

Temperature (20-30^{o}C) DO (>5 mg/L) pH 6.5-8.5 Ammonia (0.2-1 mg/L)			
Temperature (23-28^{o}C) DO (>4 mg/L) pH 6-7 Ammonia (<0.3-2 mg/L)			

Final wrkshop

Approximately 30 students obtain „from a hat", their respective roles of stakeholders that they have to play during the final workshop: Serbian government, importers lobby and producers and weapon traders; Association of Serbian fish producers; Professional public opinion; Consumers' association and media; and Members of Serbian parliament. Depending on the number of students in the generation each stakeholder group is comprised of 4 to 5 students, the 5th stakeholder group of parliament members may be larger. All students receive Appendix 5 – the case, and the list of stakeholders. They have one month to prepare their roles of stakeholders.

Appendix 5 for the Final workshop:

Case study: Bilateral cooperation Serbia-Vietnam: gunshot on Serbian aquaculture or chance for Serbian economy

Serbian government concludes an agreement with the government of Vietnam. Important incomes are expected from export of weapons and possible engagement of Serbian building companies in Vietnam. The government keeps the agreement secret, but part of it are published in media: in the next 5 years together with weapon export and possible building companies engagement, there is import of 10 000 tonnes of pangasius filets per year from Vietnam. Parts of the agreement published are those concerning Serbian export of weapons to Vietnam, and still not precised, building Vietnamese infrastructure that will bring employment to the Serbian industry, and cheap source of protein in Serbian population diet. On the other hand media raise public awareness about the Serbian aquaculture industry that is seriously endangered by pangasius import, which is part of the agreement. The public opinion demands the agreement to be published and a government organizes a public debate in the parliament where all stakeholders will address the assembly. Finally a vote will be organized in order to decide the following: will the parliament ratify the agreement Serbia-Vietnam?

Roles of stakeholders:

1. **Serbian government, importers lobby and producers and weapon traders**. Students have to prepare themselves by studying economic relations between Serbia and Vietnam and/or the rest of the world: types of agreements in the area of agriculture and food production, international regulation, cheap imported fish linked to the large export of weapons and possible engagement of building companies i.e. their workers.

Within this stakeholder group roles can also be divided: prime minister, weapon producers and traders, importers etc…

2. **Association of Serbian fish producers**: Serbian producers are gathered in the Association. Students have to prepare themselves by remembering and studying freshwater aquaculture; carp flesh quality vs. pangasius flesh quality; environmental conditions for carp and pangasius rearing, advantages of carp culture; multifunctional use of carp ponds, part of the Central European tradition. The interactive class held concerning aquaculture will show its importance since its appendix shows the potential of Serbian aquaculture.
3. **Professional public opinion:** Faculty of Agriculture, Faculty of Biology, Faculty of Veterinary Medicine, nutritionists, Chamber of commerce: fish production, water quality, ecology, tourism, quality of water for pangasius culture, effects on meat quality, panga meat vs carp meat quality, fish diseases, traditional carp production in earthen ponds, multifunctional use of carp farms, rural development.
4. **Consumers' association and media**: flesh quality of carp, trout and other species vs. panga meat; levels of protein and lipids, fatty acids composition and quality; healthy diets; management of consumers' perception, marketing, freedom of choice, protection of Serbian fish producers and Serbian economy.
5. **Members of Serbian parliament**: this stakeholder group has to study all aspects mentioned for the other groups in order to prepare for the final debate. This group can be larger than the others, depending on the number of students in the generation; one of them is President of the parliament and moderator of the discussion.

Active teaching/learning will be practiced on 4 introductory ATL classes, in preparation for and during the final workshop. This conception of teaching/learning scenarios gives chance to rise following students' activities: critical reception of information, evaluation of information, argumentation, negotiation, usage of professional language (professional discourse), reasoning about science information in social context, professional decision making, work in a team of professionals, presentation of the decisions taken, answering questions based on their professional knowledge, and solving the problem set in a case. The 4 introductory ATL classes serve to remind the students on topics they had already learnt, together with a 30 days period during which groups of students will prepare themselves for the Final workshop.

Roles of the teachers in such ATL classes and a final workshop will be to: shortly introduce the topics and assignments; follow students' assignment realization; answer the questions; help solving dilemmas; monitor students' inclusion, and stimulate activities in the group; encourage students to ask questions; listen the results presented; ask questions; conclude if the goals are achieved, and lead discussions at the end of each class. At the final workshop when a student plays a role of Parliament president teachers will help drawing final conclusions and formulating the decision.

The final workshop is announced in advance, all the teachers should be present. The case imagined as well as the introductory ATL classes meant to recall what has already been studied during previous courses, should enable students to reflect about and take responsibility for their own learning as suggested by Pešikan (2005), and Fitzmorice (2010). Students are placed in situations similar to real life problems. They practice application of the acquired knowledge. An increase of student motivation for learning and development of their professional and social skills, including transfer of knowledge and its application in new situations is also expected (Pešikan et al, 2006). This exercise is planned for the next school year and results will be presented in another paper (Part 2: Results of the interactive exercise).

Teachers of the Faculty of Agriculture defined following competences required for agricultural professionals: acquire modern knowledge in agricultural sciences; develop agriculture and food technology sector; apply knowledge and professional innovation; knowledge presentation and transfer to non-professionals; food risk-communication; and a number of generic skills (Pešikan, 2005). These competences will be much efficiently attained by creation of a suitable learning environment and the use of active teaching/learning methodology. A multidisciplinary approach in ATL implementation should enable students to develop skills to achieve a wide range of learning objectives. The interactive exercise planned should confirm this consideration.

Conclusions

A multidisciplinary approach proposed in this paper should contribute to better learning by practicing group work, critical reception of information, evaluation of information, argumentation, negotiation, usage of professional language (professional discourse), reasoning about science information in social context, professional decision making, presentation, and defense of the chosen solution as well as training for a democratic debate, respect of opposed opinions and other generic skills that are exercised in a debate foreseen for the final workshop.

We believe that a still dominant teacher-centered classical teaching method cannot fully achieve learning objectives needed for agriculture professionals unlike a musltidisciplinary approach in the implementation of ATL that may enable development of competences required for agricultural sciences.

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References

1. Anderson LW and Krathwohl DR (Eds.) 2001. A taxonomy for learning, teaching, and assessing: A revision of Bloom's taxonomy of educational objectives. New York: Pearson Education.
2. Anderson LW (Ed.) 2013. Teaching for Learning. Thessaloniki: Center for Democracy and Reconciliation
3. Anderson LW and Pesikan A 2014. Tasks, Teaching, and the Quality of Learning for Economically-Disadvantaged Students (in press)
4. Anon. 2012. Strategy of Education Development in Serbia to 2020+ (2012). Belgrade: Ministry of Education and Science of Serbia.
5. Antić S 2008. Od teorije aktivnog učenja ka praktičnoj realizaciji: važni preduslovi, u S. Antić, I. Ivić i A. Pešikan (ur.), Student u središtu nastave – Aktivno učenje na

Poljoprivrednom fakultetu, Beograd: Univerzitet u Beogradu Poljoprivredni fakultet, Obrazovni forum, str.33-113

6. Bransford JD, Brown AL and Cocking RR 1999. How People Learn: Brain, Mind, Experience, and School. Washington, DC: National Academy.
7. Cummins J, Brown K and Sayers D 2007. Literacy, technology and diversity: Teaching for success in changing times. Boston: Pearson
8. Fry H, Ketteridge S and Marshall S (Eds.) 2008. A handbook for teaching and learning in higher education: Enhancing academic practice. Routledge.
9. Fitzmaurice M 2010. Considering teaching in higher education as a practice. Teaching in Higher Education, Vol. 15, No. 1, 45-55
10. Ivić I. 2008. Aktivnosti studenata u nastavnom procesu, u S. Antić, I. Ivić i A. Pešikan (ur.), Student u središtu nastave – Aktivno učenje na Poljoprivrednom fakultetu, Beograd: Univerzitet u Beogradu Poljoprivredni fakultet, Obrazovni forum, str.5-13
11. Ivić I, Pešikan A and Antić S 2002. Active Learning 2. Belgrade: Institute of Psychology and UNICEF
12. Johnes G 2006. Didacticism and educational outcomes. Educational Research and Reviews, Vol. 1 (2), pp.23-28
13. Lambert BL and McCombs NM 1998. How Students Learn: Reforming Schools Through Learner-Centered Education. Washington, DC: American Psychological Association.
14. Moll LC (Ed.) 1992. Vygotsky and education: Instructional implications and applications of sociohistorical psychology. Cambridge University Press.
15. Pešikan A 2005. Profilisanje budućih diplomiranih stručnjaka, Psihologija, Vol. 38, br. 3. 239-253.
16. Pešikan A, Poleksić V and Antić S 2005. How to improve competencies of academics? Case of the Faculty of Agriculture, University of Belgrade. Proceedings of the First ISCAR Conference (International Society for Cultural and Activity Research): „Acting in changing worlds: learning, communication, and minds in intercultural activities“. Seville, Spain, 510-517.
17. Pešikan A, Pekić- Quarrie S, Poleksić V and Quarrie S 2006. Developing Students' Professional Competencies for decision making by active learning methodology. In Milan Slavik and Petra Žakova (Eds.): The public and the agriculture and forestry industries: The role of Higher Education in questioning assumptions and matching expectations. Prague: Czech University of Agriculture, 184-190

Original paper

OPPORTUNITIES AND WEAKNESSES IN PROFESSIONAL DEVELOPMENT OF TEACHERS AT SECONDARY SCHOOLS OF AGRICULTURE IN SERBIA

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Abstract

Recent developments in market economies have showed that education and human resource creation are among the top priorities of national strategies and social, economic, and technological progress policies. The common denominator of educational reforms in many European countries is an attempt to set up a flexible system for professional education and development to respond to changes in labour market demands. In 2012, the Serbian Government adopted *Serbia's Education Strategy until 2020*. This document provides for professional development of teachers and expert assistants at secondary specialist schools. Some of the projected actions involve working out various models of professional development, primarily teacher practice in their respective professions, carried out in companies or institutions. This document focuses on continuing professional development through various forms of formal and informal education.

Success in finding acceptable solutions in food production technology largely depends on educated staff in agriculture and their engagement in transferring their knowledge and technologies to agricultural practice. Secondary school education is most important as it is the education level producing a qualified student who will do a specific job.
The aim of this paper is to address to the need and weaknesses in continuing professional development of teachers at secondary schools of agriculture in Serbia. The weaknesses experienced in practice regarding their development are numerous and often hard to overcome. How to recognise these weaknesses and resolve them is the subject matter of this paper. The paper presents the organizational weaknesses of accredited seminars and their evaluation.

Key words: *professional development, secondary school education, transfer of knowledge in agriculture*

Introduction

In the territory of the Republic of Serbia there are some 70 schools training students in the fields of work in agriculture, and food production and processing. Most often these schools are 'pure' schools of agriculture (about 30 of them), and sometimes these are institutions training students in various fields of work. They are normally combined with the field of work in chemistry, non-metals, and graphic art or personal services, but it usually depends on the labour market demands in a local community.

Within this field of work, students get educated in 20 various occupations, degrees three and four. In the course of their schooling they study general subjects and *specialist subjects* acquiring so necessary theoretical and practical knowledge. These specialist subjects are normally taught by graduated agronomists, graduated veterinarians, and food technologists.

The initial education of teachers, in particular those who teach the so-called *specialist subjects* in secondary specialist schools, does not include (apart from some exceptions) studying didactics, teaching methodology and pedagogy although such knowledge is highly important for planning and conducting the process of teaching adequately. However, although it is necessary that the initial education of teachers should be improved, it must not be interpreted to exclude the need for *continuing* (primarily *voluntary* and *independent*) development of teachers' performance following their employment.

Presently, it is generally held that more and more teachers should be included under continuing development. Professional development in this field arises, among other, as a society's need, namely a need of educational institutions, and as a personal need of teachers themselves. Self-development of teachers should be based on constructivist approach to learning. According to constructivism, a teacher who wishes to develop professionally should construct their knowledge on their own, as well as structure and improve their pattern of thought and change some pattern elements in line with new knowledge.

Rules for professional development and acquiring the vocation of teachers, educators and expert associates list the forms of continuing professional development:

1) continuing professional development programmes conducted through trainings;

2) accredited programmes conducted by university institutions (faculties) as a form of lifelong learning;

3) specialist conventions (congress; seminar, meetings and special days; conference; counselling; symposium; round table; forum;)

4) summer and winter schools;

5) specialist and study tours.

Continuing professional development programmes are approved by the Education Improvement Institute (hereinafter referred to as the Institute). Approved programmes are published in the *Catalogue of continuing professional development programmes for teachers, educators and expert associates* (hereinafter referred to as the Catalogue) for a period of two school years. The Institute calls for a contest for approving continuing professional development programmes every two years.

A continuing professional development programme is materialised through a training that may last at least 8, but no longer than 24 hours. Such training may last no more than eight hours a day. A group can include up to 30 people. A training organizer undertakes to report the holding of a training no later than 10 days before the training starts and after the training they undertake to send via email and in writing all necessary information on the seminar participants, as well as questionnaires filled in. Based on this information, the Institute delivers a final evaluation of the seminar and publishes it on their website. The training organizer then issues a certificate of attendance to all participants who subsequently submit it to their respective institutions.

An hour of attendance at a professional training is valued as one point. A teacher, educator or expert associate undertakes to achieve at least 120 points for five years in various forms of professional development.

Materials and methods

The survey was devised as descriptive science research, using the seminar attendees questionnaire[1] which we obtained from the Institute and which consisted of a dozen questions and a dozen items (a five-level scale evaluation of agreement with specific claims).

The sample was accidental (all participants who attended the entire two-day programme) consisting of 151 teachers at schools of agriculture.

Statistical analysis of the obtained information was done in IBM SPSS Statistics 20. The processing included the calculation and presentation of frequency in number and percentage (frequency analysis), standard deviation (as measures of dispersion i.e. spread of respondent evaluation) and mean. Apart from that, there was a test for existing correlations (Pearson correlation coefficient) between the respondents' answers to a set of questions. Finally, Chi-squared test checked for the existence (and statistical significance) of potential differences in the evaluation of some questions. All results are also presented graphically (histograms) with most graphs given as an appendix to the research (so as not to burden the basic content).

Results and discussion

Starting from the fact that there are few approved programmes in the field of work in agriculture, and food production and processing (merely 7 in the 2010-2011 Catalogue) and from the topicality of organic food production, NASO applied for the contest published by the Institute for approving a professional development programme entitled *Organic Agriculture*. The programme was approved and published in the 2011-2012 Catalogue under no. 309.

That school year, 5 trainings were held and 130 teachers of specialist subjects at 33 schools of agriculture were educated. Most of the participants were teachers of specialist subjects such as graduated agronomists (various courses) and graduated veterinarians. The trainings were organized in different parts of Serbia and co-financed by NASO.[2] They lasted 2 days and were 12 points worth.

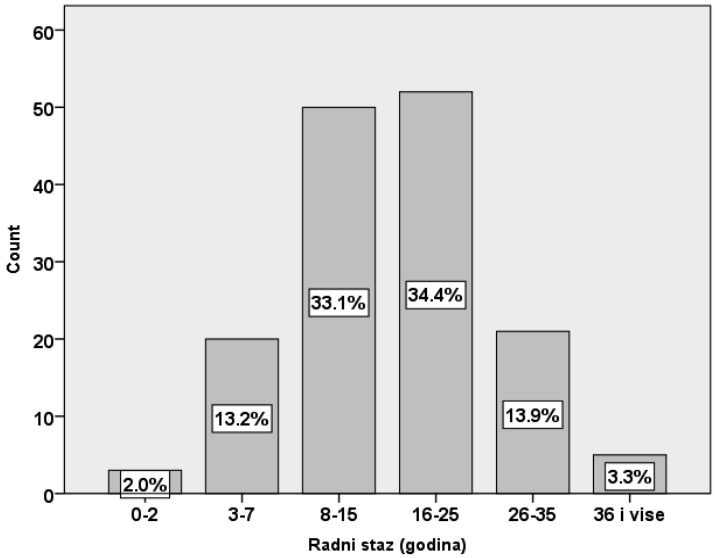
The following year we applied with the same programme upgraded with an organic food processing module. This way the training was also intended for food technologists. The programme was published in the 2012-2013 Catalogue under no. 753. Only one training was held with 21 participants attending who were teachers of specialist subjects in food processing. Table 1 attached an average evaluation of all 6 trainings held.

Starting from the principle of willingness, and based on evaluation lists we were interested in whether the trainings were attended by younger or older colleagues. The lists did not require that they should state their age, but the number of years in service at the institution. We felt that younger colleagues also had fewer years of service (which is not necessarily

[1] Questionnaire filled in by the training participants has been attached as an appendix.
[2] NASO-National Association for Organic Production Development Serbia Organica

true because some colleagues might have had some years of service in other companies). Graph 1 shows average percentage of years of service for the seminar participants.

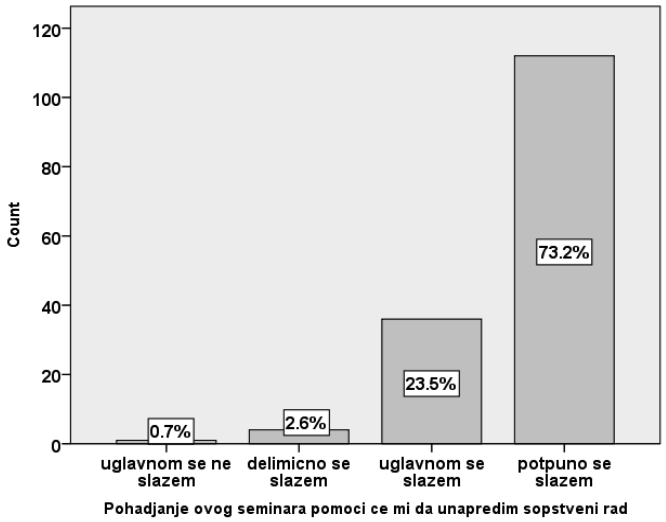
Based on the results obtained we did not confirm our initial belief that the trainings were attended mostly by younger colleagues, but the highest percentage went to the colleagues with longer years of service. It is proof of the desire and willingness of teachers in secondary schools to have continuing trainings and development.



Graph 1. *Average percentage of years of service for the seminar participants*

What we also found interesting was the participants' opinion on applying the knowledge acquired at the training, that is, whether this training would help them improve their own performance.

Most respondents (73.2 %) fully agreed that the seminar would help them improve their performance. Statistical validation that the seminar attendance will improve the respondents' performance may be obtained by Chi-squared test. By applying Chi-squared test we got a *p*-value less than 0.05, confirming the assumption that the respondents felt that the seminar attendance would improve their performance. Graph 2 shows the respondent distribution given their attitude towards the effect of the seminar attendance on improving their performance.



Graph 2. *Respondent distributions given their attitude towards the effect of the seminar attendance on improving their performance*

We were further interested whether there was a correlation between the respondent age and the performance improvement after the seminar attendance, that is, whether younger respondents after the seminar attendance improved their performance after attending the training. In this analysis, years of service were redistributed (the table shows new intervals). Analysing the graph (below), it can be observed that growing years of service result in a dropping number of respondents who fully agreed that the seminar attendance would help them improve their performance. Unfortunately, Chi-squared test of independence did not confirm statistical significance of the correlation between the years of service and the respondent attitude towards performance improvement after the training ($p=0.311>0.05$). Graph 3 shows the distribution of respondents according to the years of service and the attitude towards the effect of the training on improving own performance.

Table 2. *Distribution of respondents according to the years of service and the attitude towards the effect of the training on improving own performance*

Years of service	Attendance at this seminar will help me improve my performance				Total
	I mostly disagree	I partly agree	I mostly agree	I fully agree	
0-7 years	0	0	4	19	23
8-15 years	1	2	8	39	50
16-25 years	0	0	16	36	52
26 years and more	0	2	7	17	26
total	1	4	35	111	151

We had most difficulties in organizing these trainings due to financial problems. Namely, continuing professional development programmes are supposed to be funded from local self-governments' budgets, which is often an obstacle. Due to such problems, it is not only

this training that was never held, but others too. For the trainings in 2012, NASO managed to provide funding, so the participants bore the costs of transport to the place of training. This way we had a solid response from participants and the maximum number of attendees (30) at almost every training. In the 2013-2014 school year, only one training was held and it was funded by the local self-government of the host school, and some teachers bore the costs too.

A particular problem was in communication with some schools. We could not even come in contact with some of them, so their teachers did not attend (one school from the territory of Kosovo and Metohija). Training invitations were sent via email and often were not read, so we did not have an adequate or prompt reply.

Based on the evaluation lists designed at the Institute and their being filled in by participants we have observed the following:

By analysing Table 3 and relevant Graph 4, it can be observed that the respondents in Vojvodina got their seminar information from the catalogue, from the school administration and otherwise, more often from their colleagues in central Serbia. The situation was quite the opposite in other two sources of information. Chi-squared test of independence confirmed that these differences were not accidental ($p=0.000<0.05$). So, there was a statistically significant difference between the frequencies of specific sources of seminar information for these two regions.

Conclusion

Most participants felt that the education was interesting and that it achieved most of set goals. They felt that the participant needs were honoured at an adequate degree for the most part of the education and most participants were active and focused.

Additional comments on holding the seminar mostly referred to the need for organising more training in the field of agriculture. This attitude was confirmed during the trainings and during various meetings held by the community of schools of agriculture. Although these trainings were mandatory as required by the Rules, most teachers of specialist subjects participated willingly. This indicates that there should be more of such trainings in future and that it is something to work on. As for the content of potential trainings, they should be based on the needs of teachers as much as possible; it would be good if educators were willing to be better informed and qualified for the content they present. If at the same time training funding is successfully resolved, including good workshops, the success is inevitable. Indeed, colleagues should be timely informed about seminar itineraries and from our experience trainings should be also promoted because, as we have already witnessed, participants use various sources to get information on trainings.

References

1. Government of the Republic of Serbia, Ministry of Education and Science, Serbia's Education Development Strategy until 2020, discussion draft.
2. Rules for professional development and acquiring the vocation of teachers, educators and expert associates(Off. Gazette of the RS, no. 13/ 2012).
3. Education Improvement Institute, Catalogue of continuing professional development programmes for teachers, educators, expert associates and principals for the 2011-2012 school year.

4. Education Improvement Institute, Catalogue of continuing professional development programmes for teachers, educators and expert associates for the 2012-2013 and 2013-2014 school years.
5. Vratonjic S 2012. Evaluation of internal education of teachers in the process of teaching quality standardization in secondary specialist schools – draft and research.

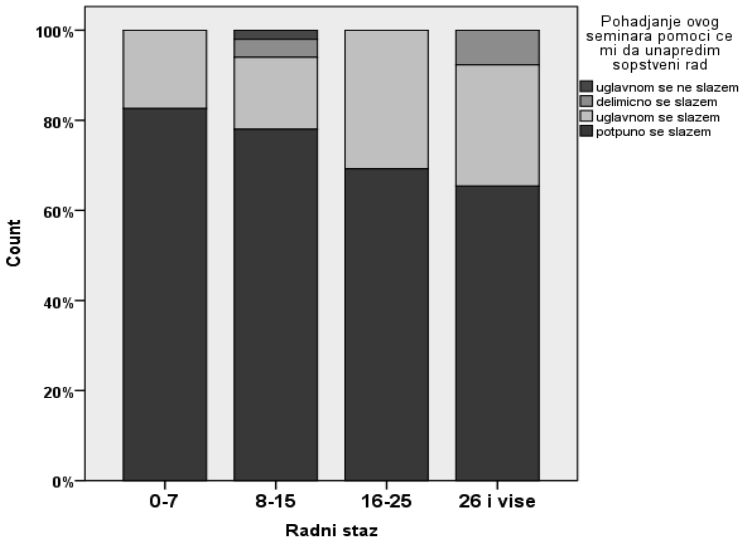
APPENDIX

Table 1. *Average evaluation of all 6 trainings (max. evaluation is 4)*

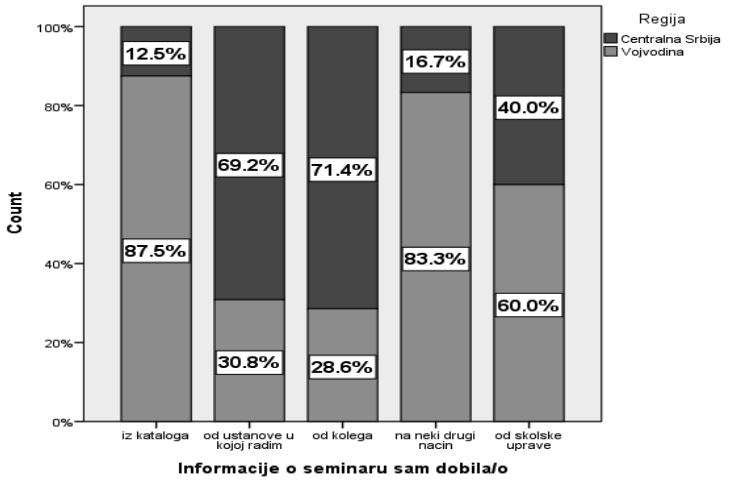
Place of trainings	**Average evaluation**
Požarevac	3.83
Kraljevo	3.94
BanjaVrujci	3.79
Leskovac	3.67
VelikoGradište	3.56
Novi Sad	3.72

Table 3. *Distribution of respondents according to regional affiliation and getting information about trainings*

I have got information about trainings	**Region**		**Total**
	Central Serbia	**Vojvodina**	
From Catalogue	1	7	8
From school in which I work	74	33	107
From colleague	10	4	14
In some other way	2	10	12
From school administration	4	6	10
Total	91	60	151



Graph 3. *Distribution of respondents according to the years of service and the attitude towards the effect of the training on improving own performance*



Graph 4. *Distribution of respondents according to regional affiliation and getting information about trainings*

ЗАВОД ЗА УНАПРЕЂИВАЊЕ ОБРАЗОВАЊА И ВАСПИТАЊА
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УПИТНИК ЗА УЧЕСНИКЕ СЕМИНАРА

1. Пол	Мушки ☐ Женски ☐

2. Године радног стажа у ОВ установама	0-2 ☐	3-7 ☐	8-15 ☐	16-25 ☐	26-35 ☐	36 и више ☐

3. Последњи завршени ниво образовања	средњи ☐	виши ☐	високи ☐
	мастер ☐	специјализација ☐	магистратура ☐
	докторат ☐		

4. Шифра радног места (шифре се налазе на полеђини упитника)					

5. Број програма у каталогу					

6. Поштански број места реализације семинара					

7. Датум почетка семинара									
	дан			месец			година		

8. Семинар је реализован	**а)** у установи **в)** у центру за стручно усавршавање **д)** електронским путем	**б)** у ЗУОВ-у **г)** на неком другом месту

Молимо Вас да уношењем знака X у празно поље изразите свој степен слагања са наведеном тврдњом

	Потпуно се слажем	**Углавном се слажем**	**Делимично се слажем**	**Углавном се не слажем**	**Уопште се не слажем**
1. Дефинисани циљеви семинара су остварени					
2. Теме/садржаји предвиђени програмом су реализовани					
3. Методе, технике и облици рада примењени на семинару обезбеђују учење учесника					
4. У реализацији семинара узимају се у обзир претходна знања и искуства учесника					
5. Семинар је одржан према предвиђеној сатници					
6. Похађање овог семинара помоћи ће ми да унапредим сопствени рад					
7. Начин излагања водитеља је јасан и разумљив					
8. Водитељи семинара дају повратне информације на питања учесника					
9. Услови за рад (простор, техничка подршка) су омогућили успешну реализацију семинара					
10. Целокупна организација је допринела успешној реализацији семинара					
11. Информације о семинару сам добила/о: **а)** из Каталога **б)** од колега **ц)** од установе у којој радим **д)** од центра за стручно усавршавање **е)** од школске управе **ф)** на неки други начин					
Додатна запажања и предлози:					

Ред. бр.	Назив радног места	Шифра
1.	Наставник разредне наставе	1001
2.	Наставник предметне наставе – основна школа	1002
3.	Наставник предметне наставе – гимназија	1003
4.	Наставник општеобразовних предмета – средња стручна школа и уметничка школа	1004
5.	Наставник стручних предмета – средња стручна школа и уметничка школа	1005
6.	Наставник у школи за образовање ученика са сметњама у развоју	1006
7.	Наставник у школи за образовање одраслих	1007
8.	Васпитач у предшколској установи	1008
9.	Медицинска сестра – васпитач	1009
10.	Васпитач у дому ученика	1010
11.	Стручни сарадник у предшколској установи/школи	1011
12.	Сарадник (педагошки асистент и помоћни наставник)	1012
13.	Директор/помоћник директора	1013

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