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SUMMARY

METHANE EMISSIONS FROM DAIRY CATTLE

Gantner, V*.[1], Bogdanović, V.[2], Potočnik, K.[3]

Abstract: Forecasts indicate that the world population will significantly increase till year 2050, placing up an enormous challenge to agriculture sector. Agriculture, especially livestock sector plays an important role in global environmental issues, such as climate change, land degradation, water pollution and biodiversity loss. Regarding the species, cattle are the main contributor to the sector's GHG emissions (65%), but, on the other hand, cattle suffer significant losses due to induced climate change. Under heat stress conditions, lactating cows tend to decrease thteir performances resulting in a significant financial. The reduction of the GHG emission and minimizing the climate change could be achieved by short-term methods that imply increase of production per animal and feeding optimization, and long-term method that imply genetic evaluation and selection based on methane emission variation. In a case of introduction of genetic evaluation of dairy cattle for methane emission as well as selection of cows with lower methane emission intensity into the breeding programs it is necessary to define prediction models for body weight (BW), dry matter intake (DMI) and methane emission (CH4) based on parameters of dairy cattle population in each state. Finally, this will lead to environmentally (and economically) sustainable milk production. Since climate change is a global issue and livestock supply chains are increasingly internationally connected, effective mitigation actions could be achieved only by global approach. Therefore, the future of cattle breeding is in sustainable production system that provide effective production of high quality product with animals selected to high heat tolerance as well as to low GHG emission per kg of product.

Keywords: dairy cattle, methane emission, mitigation, climate change

Introduction

State of Art

Forecasts (US Census Bureau, 2016) indicate that the world population will increase from current 7.2 billion to 9.6 billion till year 2050 (Figure 1). The population growth, combined with the growing incomes and urbanization imposes tremendous challenges to food and agriculture systems. FAO (2011) experts forecasts that the demand for meat and milk in 2050, regarding the levels in 2010, will increase by 73 and 58%, respectively. Expected increase of human population and demands implies an increase in the population of land animals from current 60 billion to 100 billion till year 2050 (Picture 1).

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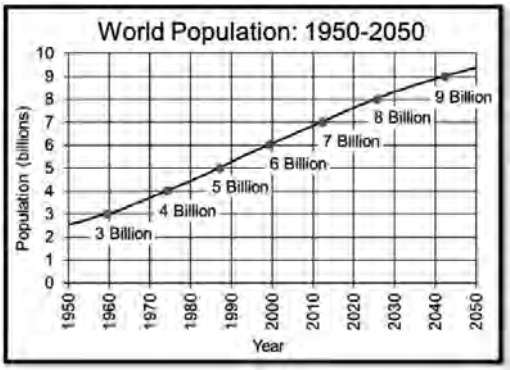
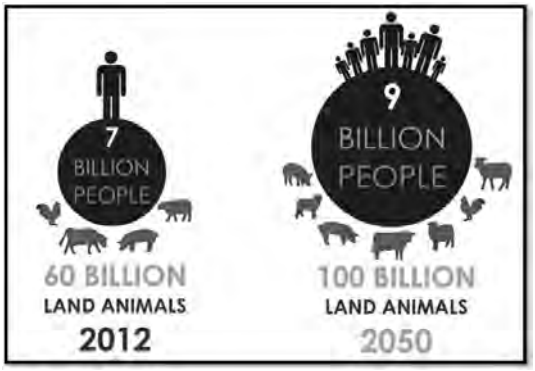


Figure 1 Population growth (US Census Bureau, 2016)



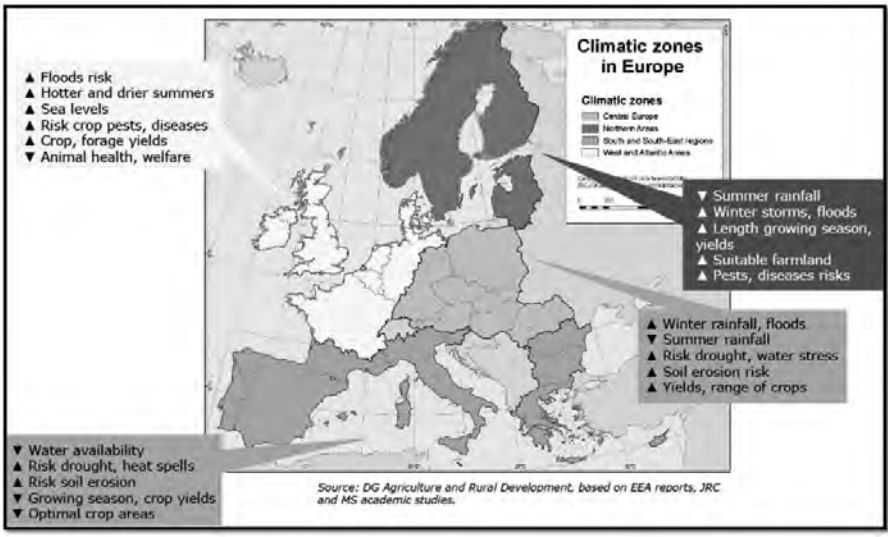
Picture 1 Expected increase of land animals (US Census Bureau, 2016)

At the same time, the natural resources necessary to support increase of global food production will not grow that is necessary production increase must be accommodated within the growing insufficiency of natural resources (land, water and nutrients). Currently, agriculture has significant impact on global environmental issues, such as climate change, land degradation, water pollution and biodiversity loss. FAO 2013. experts stated that climate change is transforming the planet's ecosystems and threatening the well-being of current and future generations, therefore, with purpose to *hold the increase in global temperature below 2°C and to avoid dangerous climate change, global emissions need to be significantly decreased. The latest findings (IPCC, 2018) highlights a number of climate change impacts that could be avoided by limiting global warming to 1.5°C compared to 2°C (lower global sea level rise, …). Furthermore,* limiting global warming to 1.5°C would require rapid, far reaching and unprecedented changes in all aspects of society.

Climate change

It is not questionable whether climate change occurs. The question is: How will farming in the EU be affected by climate change? The changing in rainfall will be a serious problem in many regions. Also, rising temperatures, variability and seasonality as well as extreme events, heatwaves, droughts, storms and floods across the EU are just some of the expected changes (European Commission, DG Agriculture and Rural Development, 2016, http://ec.europa.eu/). Finally, increase of frequency of days with heat stress conditions are also expected.

Due to heat stress conditions, dairy cattle tends to reduce dry matter intake, milk production (West et al. 1999; Casa and Ravelo, 2003) and reproductive performances (Bohmanova et al. 2007; Ravagnolo et al. 2000). Additionally, heat stress affects milk composition, somatic cell counts (SCC) and mastitis frequencies (Bouraoui et al. 2002; Collier et al. 2012; Correa-Calderon et al. 2004; Gantner et al. 2011, 2017a, 2017b; Ravagnolo et al. 2000; St-Pierre et al. 2003; West 2003; Hammami et al. 2013; Smith et al. 2013). The heat stress condition also leads to considerable loss of profit, e.g. between $897 million and $1,500 million per year in the USA (St-Pierre et al. 2003).

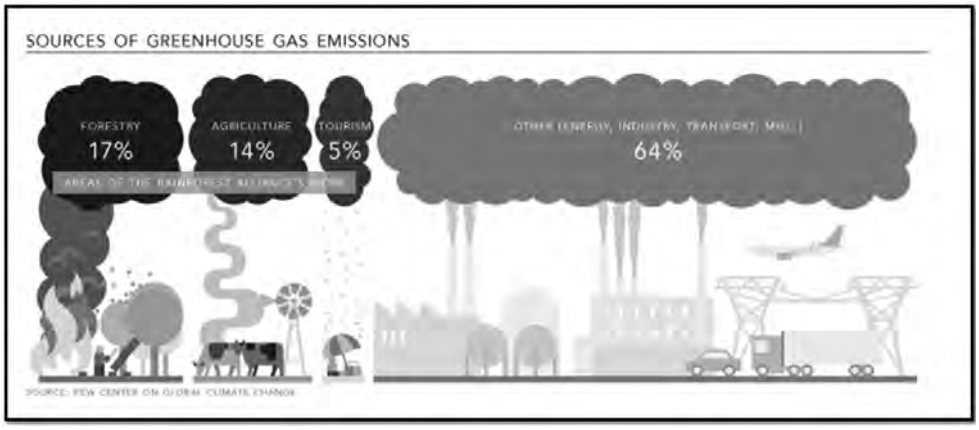


Picture 2 Effect of climate change on European Union (European Commission, DG Agriculture and Rural Development, 2016, http://ec.europa.eu/)

Livestock sector

The livestock sector, within the agriculture, has come into focus because of its significant impact on the environment. The increase of animal-source foods demands caused intensification of the sector. Therefore, the sector becomes increasingly demand-driven, fast growing ultimately resulting the current situation when sector competes for natural resources with other sectors. This increase also induces greater environmental impacts and the livestock sector is often pointed out as being particularly resource-hungry.

The global livestock sector, with total GHG emissions estimated at 7.1 giga tones of CO_2-eq/year (year 2005) which represent 14.5% of all anthropogenic emissions significantly affects the environment (IPCC, 2007). But, on the other hand, it can also deliver a significant share of the necessary mitigation effort (FAO, 2011).



Picture 3 Total anthropogenic emission (FAO, 2013a)

The livestock production supply chains produce (IPCC, 2007): 2 giga tones CO_2-eq of CO_2/year, or 5% of anthropogenic CO_2 emissions, 3.1 giga tones CO2-eq of CH4/year, or 44% of anthropogenic CH_4 emissions, 2 giga tones CO_2-eq of N2O/year, or 53% of anthropogenic N2O emissions, while emissions of hydrofluorocarbons (HFCs) are marginal on a global scale. About 44% of the sector's emissions are in the form of CH4! The remaining part is almost equally shared between N_2O (29%) and CO_2 (27%).

Regarding the species, cattle are the main contributor to the animal production sector's emissions with about 4.6 giga tones CO_2-eq, representing 65% of sector emissions, while pigs, poultry, buffaloes and small ruminants have much lower emission levels that is in interval from 7 – 10% of sector emissions (FAO, 2013a).

Regarding the commodity, beef contribute 2.9 giga tones of CO_2-eq, or 41%, and cattle milk 1.4 giga tones of CO_2-eq, or 20%, of total sector emissions. These commodities are followed by pig meat, with 0.7 giga tones of CO_2-eq, or 9% of emissions, buffalo milk and meat (8%), chicken meat and eggs (8%), and small ruminant milk and meat (6%) products (FAO, 2013a).

GHG emission intensities and mitigation potential

The GHG emission intensities vary greatly among producers (especially for ruminant products, but also for pork and chicken meat and eggs). This variability is caused by: different agro-ecological conditions, farming practices and supply chain management.

This heterogeneity was observed both within and across production systems (FAO, 2013a). Observed variability, i.e. differences between producers with highest emission intensity and those with lowest emission intensity gives opportunity for finding the adequate mitigation option. Generally speaking, the lower productivity is, the higher is GHG emission per kg of product. Higher GHG emissions are mainly consequence of (FAO, 2013b): low feed digestibility that lead to higher enteric and manure emissions, poorer animal husbandry and lower slaughter weights (slow growth rates leading to more emissions per kg of meat produced), as well as higher age at slaughter (longer life leading to more emissions).

Mitigation potential estimates are based on the wide gap in emission intensities that exists on a global and regional scale and within production systems and agro-ecological regions (FAO, 2013a). The estimation for mitigation is around 30% (about 1.8 gigatonnes CO_2-eq).

The reduction of livestock production sector emission could be achieved by reduction of production and consumption, lowering the emission intensity of production, and by combination of mentioned above.

The reduction of the animal production sector emissions by lowering the emission intensity of production could be achieved through application of many technologies and practices (FAO, 2013a). Technical options for the mitigation of GHG emissions along animal production supply chains could divided into the following categories: options related to feed supplements and *feed/feeding management* (for CH_4 only), options for *manure management* which include dietary management, but with a focus on "end-of-pipe" options for the storage, handling and application phases of manure management, as well as animal husbandry options which include *animal and reproductive management* practices and technologies.

The adoption and application of these technics by majority of the world's producers can result in significant reductions in emissions. The mitigation potential varies in interval from 14 to 41%

depending of the selected specie, production system and world's region.

In accordance to FAO (2013a), practices and technologies that reduce GHG emissions can often simultaneously increase productivity, thus contributing to the food security and economic development.

Methane reduction

Methane reduction methods could be classified as short-term, and long-term. Short-term methods imply increase of production per animal and feeding optimization, while long-term method imply genetic evaluation and selection based on methane emission variation. Precondition for genetic evaluation for methane emission is selection of optimal indicators and models for methane emission estimation that are highly accurate and easy applicable in routine animal recording.

The question is: How much methane does a cow actually produce? The gold standard to measure CH4 and GHG emissions is the respiratory chamber (Cassandro et al., 2013). Since this technique is not easily applicable, because is time consuming and costly, it cannot be the method of choice when a large number of measurements are needed (in the case of genetic evaluation). Furthermore, the prediction of CH4 emission can be obtained with several systems that are based on feed intake records, breath analysis and cow characteristics and milk composition records. Some of the possible methods are shown in Table 1.

Method	r	Reference
PME from breath analysis		
Respiratory chamber	0.96	Place *et al.*, 2011
Head hoods	0.96	Place *et al.*, 2011
SF6 tracer technique	0.83	Muñoz *et al.*, 2012
Green feeder	0.89	de Haas *et al.*, 2011
Laser methane detector	0.80	Chagunda and Yan, 2011
FTIR- Fourier Transform Infrared Spectroscopy	0.89	Garnsworthy *et al.*, 2012
PME from milk records		
CH_4 (g/kg DM) = 24.6 (± 1.28) + 8.74 (± 3.581)×C17:0 anteiso − 1.97 (± 0.432) ×trans-10 + 11 C18:1–9.09 (± 1.44)×cis-11C18:1 + 5.07 (± 1.937) ×cis-13C18:1	0.85	Dijkstra *et al.*, 2011
PME from feed intake records		
CH_4 (MJ/d) = 3.23 (± 1.12) + 0.809 (± 0.0862) × DM Intake (kg/d)	0.65	Ellis *et al.*, 2010
CH_4 (Mcal/d) = 0.814 + 0.122* Nitrogen Free Extracts (kg/d) + 0.415 * Hemicellulose (kg/d) + 0.633 * Cellulose (kg/d)	0.72	Moe and Tyrrell, 1979 (cited from Demeyer and Fievez, 2000) Van Es, 1978, IPCC, 2000, 2006
CH_4 (g/d) = feed intake (kg of DM/d) × 18.4 (MJ/kg of DM)/0.05565 (MJ/g) × 0.06 × {1 + [2.38 – level of intake (multiples of maintenance level)] × 0.04}[c]	-	Bannink *et al.*, 2011
CH_4 (g/d) = [grass or grass silage (kg of DM/d) × 21.0 (g/kg of DM) + concentrates (kg of DM/d) × 21.0 (g/kg of DM) + corn silage (kg of DM/d) × 16.8 (g/kg of DM)] × {1 + [2.38 – level of intake (multiples of maintenance level) × 0.04]}[f]	-	

r, correlation with respiratory chambers; [c]18.4 MJ/kg: energy released by each unit of feed DM (Van Es, 1978), 0.05565 MJ/g: energy generated by methane (IPCC, 2006), 0.06 × gross energy intake (GE, MJ/d): methane production level in MJ/d (IPCC, 2000), 2.38 × maintenance feed intake level: energy requirements scaled to an average cow at feed intake level, 0.04: correction factor of 0.04 per unit feed intake level; [f]g/kg of DM: CH_4 production for 1 kg DM of grass, grass silage or concentrate, 21 g/kg of DM: CH_4 production for 1 kg DM corn silage.

Table 1. Methods to predict methane emission (PME) using different indicators (Cassandro et al., 2013)

Our current research indicate that methane emission vary significantly in accordance to used prediction equations. So, if we want to introduce genetic evaluation of dairy cattle for methane emission as well as selection of cows with lower methane emission intensity into the breeding programs it is necessary to define prediction models for body weight (BW), dry matter intake (DMI)

and methane emission (CH4) based on parameters of dairy cattle population in each state. Finally, this will lead to environmentally (and economically) sustainable milk production.

Conclusions

Since climate change is a global issue and livestock supply chains are increasingly internationally connected, effective mitigation actions could be achieved only by global approach. Therefore, the future of cattle breeding is in sustainable production system that provide effective production of high quality meat and milk with animals selected to high heat tolerance as well as to low GHG emission per kg of product.

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THE IMPORTANCE OF THE DEVELOPMENT OF LONG-TERM WILDLIFE MANAGEMENT DATABASES

Csanyi S.*[1]

Abstract: Conservation and sustainable management of wildlife populations require adequate legislation, law enforcement, and a system of evidence-based planning and decision making. A monitoring system of population dynamics/trends, vital statistics, and harvests should be set up to follow up the changes in wildlife populations, especially the changes induced by hunting or other forms of use.

One of the oldest forms of monitoring is game management statistics and long-time records of game management statistics. This kind of data is typically focused on the results of hunting, the capture of game, or data for trophy scoring. In Hungary, the collection of game management statistics started in the late 19th century, and continuous series of data are available since 1960 for the whole country and the 19 counties.

To collect and process the reports of the game management units (GMU) the National Game Management Database (NGMD) was established in 1994. Since that time we store the data in a relational database containing the spring population reports, annual harvest reports, and the trophy scoring data for the five big game species. The database also includes the digital map of the GMUs and various kinds of GIS analyses can be run to study the effect of different environmental factors. The most important uses of the NGMD are as follows: the publication of the annual game management statistics, contribution to the analyses and modeling of management activities (e.g., development of harvesting quotas, risk analysis of ASP, development of management plans for various levels). This long-term database is a unique opportunity to analyze and understand wildlife - environment interactions in many contexts, depending on the availability of another GIS databases (e.g., land use, road systems, habitat characteristics).

Many European countries collect more or less similar data and several of them for over decades. Unfortunately, these databases are not connected, consequently higher level analyses are sporadic and typically depend on personal connections or goodwill. Key players, like the international hunting NGOs, should have been recognized the importance of these databases in depicting the values of field sports, and the contribution of wildlife management to the societies or rural economies. It is also disturbing that many users utilize data published on the internet as open access resources, use them without the knowledge of the data resulting in inconsistent results, insensible conclusions, and incompetent management proposals.

Although there were and are some projects to develop any European wildlife management databases (e.g., Artemis, Enetwild) the data is collected on selective bases, the sources or limited and any results seem to be distant future. Working models and real practices are available, and it would have been time to use them.

Keywords: long-term, wildlife, databases

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AUTOCHTHONOUS PIG BREEDS IN SERBIA-REVIEW OF THE RESEARCH RESULTS CONDUCTED IN THE LAST TEN YEARS

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Abstract: Autochthonous breeds of domestic animals are the breeds which originated in the region of the Republic of Serbia and have an economic, scientific and cultural importance for our country. The results of the researches conducted in the previous ten years mostly treat characterisation of autochthonous swine breeds in regard to body development in different age, determination of the intensity of liveweight gain in different stages of growth and fattening, studies on the reproductive traits, traits of carcass and meat quality, as well as qualitative and organoleptic traits of traditional dried meat products made of meat of autochthonous swine breeds. Numerous studies, from different aspects, engaged in the issue regarding the autochthonous pig breeds in the Republic of Serbia, clearly illustrate the production potential of these breeds indicating that it is essential that they should be preserved. In further scientific activites it seems necessary to direct the research towards genotypisation and molecular and genetic research on autochthonous pig breeds. In addition, it would be essential to deepen the research so as to find the most optimal and sustainable systems of breeding and utilisation of these breeds. Showed researches indicated certain advantages of these breeds regarding specific characteristics of meat and traditional products made of such meat. It is particularly important to precisely define the most optimal systems of keeping and nutrition of these breeds for the purpose of emphasizing their comparative advantages in relation to modern meat-type breeds, particularly regarding specific and recognisable meat quality.

Keywords: pigs, autochthonous breeds, growth, fertility, carcass and meat quality

Importance of animal genetic resources and their preservation

Autochthonous breeds of domestic animals are the breeds which originated in the region of the Republic of Serbia and have an economic, scientific and cultural importance for our country. In their genes these animals contain information about the surrounding environment, they were created during a long-time period under or without the action of man. Their genes represent safety food production in the future since a growing importance will be given to the resistance and adaptability of the breeds.

Autochthonous pig breeds are adapted to specific local environment and their nutrition is based on different natural food sources specific for certain regions. Besides their genetic merit for agrobiodiversity they represent the basis for sustainable local chain of production in pig breeding and are particularly important for regions where arable land and cereal production are limited. From the aspect of scientific and research activities their traits and products are practically unused along with market potential of their products. Today in the Republic of Serbia there are three autochthonous swine breeds: Mangalitsa (Swallow-Belly and White strain), Moravka and Resavka. The most endangered among them is Resavka which is on the verge of extinction.

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The results of the researches conducted in the previous ten years mostly treat characterisation of autochthonous swine breeds in regard to body development in different age, determination of the intensity of liveweight gain in different stages of growth and fattening, studies on the reproductive traits, traits of carcass and meat quality, as well as qualitative and organoleptic traits of traditional dried meat products made of meat of autochthonous swine breeds.

External characteristics, body development and intensity of growth in autochthonous pig breeds

The efficacy of the production of pig meat depends to a great degree on capacity and intensity of liveweight gain, both at various ages and at attaining the finishing weight of an animal. The insight into the regularities and characteristics of growth in pigs and knowledge of the utilisation efficiency of food of different quality are important prerequisites for determination of optimal model and system of utilisation of pigs in order to achieve maximum economic effects of fatenning.

Autochthonous pig breeds belong to the group of late maturing (Mangalitsa) and medium maturing (Moravka and Resavka) breeds. In this respect, regarding the intensity of growth and food utilisation they are much more inferior breeds compared to modern early maturing meat type pigs. Thus, it is particularly important to know thoroughly the traits of growth and body development of autochthonous pig breeds in order to find, in modern conditions, optimal model for their sustainable breeding and therefore their sustainable survival.

Mangalitsa is a late maturing breed. Its head is relatively small, with big ears hanging in front. The auricle is placed high and is elastic to touch. The length of ear is 2/3 of the length of head. The thorax and short trunk are wide, deep and stretch beneath the elbow joint. Backs and loins are flat or slightly rounded, looking from the sides of an animal. A hind part of the body and legs are well developed, wide and muscular. The abdomen is long, cylindrical and somewhat wider in sows than in boars. The limbs are long, wide and muscular. Swallow-Belly strain was developed in the vicinity of Ruma town, in Bođanovci village, while White strain originates from Hungary.

According to Petrović et al. (2007a) Moravka and Resavka breeds were developed in the beginning of the XX century, in the region around the Velika Morava river basin. Primary aim was to improve production traits of a primitive pig breed – Šumadinka and upon the import of Berkshire and Yorkshire noble breeds and later improvement by the English Great Black pig, by unplanned and unsystematic crossing a number of different pig varieties was created among which two became common, the black one, representing the breed called Moravka and the other, coloured strain called Resavka which was more common in the region near Despotovac town in the valley of the river Resava. Moravka strain pigs have body covered with thick or thin black hairs, which are smooth and flat. On lower body parts the hairs are thinner while on the other parts they are coarse and longer. The skin is relatively thin and pigmented (black). The head is long, narrow, slightly sagged in profile. The cheeks are not clearly covered by muscle tissue. The ears are either lopsided or partly lopsided. The neck is of a medium length and has little muscle tissue. The trunk is relatively long, with flat or slightly sagged back line while buttocks are slightly bended towards the bottom part of the body. Along stomach line there are 4-6 pairs of teats. The extremities are of a medium length, thin, soft and poorly covered by muscle tissue.

In the same study the authors compared morphometrical results of Moravka and Resavka presented by the authors engaged in the research on these breeds by the middle of the XX century with

respect to body development of adult animals of these breeds identified in the territory of Kuršumlija municipality. It was reported that in regard to major body dimensions there were no greater differences so that average height of ridge determined in the identified and measured animals was by some 1.4 cm greater compared to average values displayed in older research studies, while an average sow body mass (77.67 kg) was lower by about 16 kg with shorter variation interval. During fattening when pigs were exclusively corn fed and which lasted about six months an average daily liveweight gain on a month basis ranged from 311 to 514 g/day and was considerably lower than liveweight gains obtained in previous studies of older date, while an average daily liveweight gain during a whole lenght of fattening was 385 g/day. Similar results in this respect were presented by Savić et al. (2017) stating that Moravka pig breed, in spite of drastic reduction in the size of population and all consequences ensuing thereof such as negative consequences of sib-mating, etc., preserved its production potential and that in order to improve productivity and sustainability of this breed it should be necessary to work intensively on increasing the population, controlling production capabilities, improving breeding system and establishing and implementing systematic and justified selection.

As a consequence of an increased attention devoted to animal welfare over a few last years the issue of immunocastration as alternative method to surgical castration of male fattening pigs in order to supress so called boar meat taint is of a current interest. The effect of this procedure on the intensity of growth of Mangalitsa breed pigs (Radović et al., 2017b) showed that an average daily liveweight gain upon revaccination was statistically significantly higher in immunocastrated animals compared to surgically castrated animals being 548 g/day versus 392 g/day.

In a comparative study on the intensity of growth in traditional low input fattening of several different genotypes of autochthonous pig breeds and their crossbreds, Radović et al. (2017a) determined that Moravka pigs realised by 101 g higher daily liveweight gain in relation to Swallow-Belly Mangalitsa pigs (368.86 g vs 267.86 g) in the same raising conditions while in crossbreds of these breeds the weight gain was 336.91 g/day. Similar results, but in intensive fattening on complete mixtures adapted to noble breeds, were obtained by Radović et al. (2017c) where Moravka fatteners averagely gained 545 g/day during fattening, while in Mangalitsa the intensity of gain was 480 g/day. Contrary to previous studies, Petrović et al. (2011) determined no statistically significant differences regarding daily liveweight gain between the fatteners of Mangalitsa (307 g/day) and Moravka (316 g/day). Recent studies have indicated somewhat slower gain in Mangalitsa pigs in semi-intensive fattening what is most probable the consequence of different intensity of fattening, differences in finishing body mass as well as differences in genetic structure of Mangalitsa population due to their drastic decrease in the number in the last 60 years (Radović et al., 2017e).

Reproductive traits of autochthonous pig breeds

The importance of pig meat production on a world scale is, besides intensive growth and efficient food utilisation, for the most part, based on a high fertility of this species of domestic animals. Autochthonous pig breeds in the Republic of Serbia belong to the group of primitive (Mangalitsa) and transitional (Moravka and Resavka) breeds what makes their fertility much lower in respect to modern noble breeds which are predominantly used in modern intensive farm production today.

Petrović et al. (2007a) report that the number of liveborn piglets in the litter in identified breeding females of Mangalitsa breed was 7.2 on average with variation interval ranging from 5 to 14

piglets. The results of fertility of this breed monitored during four-year period reported by Radović et al. (2017d) were characterised by an average number of liveborn piglets in litter of 7.31, and the number of raised ones 7.11. In the same research the fertility of Resavka accounted for 7.96 liveborn and 6.96 raised piglets in litter on average. Moravka sows first farrowed in the age of 428 days and Resavka in the age of 441 days. Studied traits did not statistically significantly vary under the effect of the year in contrast to the order of farrowing.

The size of litter in Mangalitsa is smaller compared to previously mentioned breeds. The size of litter is on average 4.60 measured by the number of liveborn piglets, i.e. 4.09 measured by the number of raised piglets (Petrović et al., 2013). The age at first farrowing statistically significantly differed between the two analysed breeding stocks of Mangalitsa, being 615.65 days, i.e. 386.57 days, on average. Average size of litter at weaning did not statistically significantly vary under the effect of the order of farrowing and ranged from 2.92 in the first to 4.25 piglets in the fourth farrowing. Radović et al. (2017d) reported Mangalitsa fertility of average 4.65 liveborn and 4.33 raised piglets, at the age of 556 days at first farrowing. The traits of litter size of Mangalitsa at farrowing and weaning in this research did not statistically significantly vary per years of research while the order of farrowing caused statistically significant differences.

Traits of carcass qualities in autochthonous pig breeds

The knowledge of fatteners carcass quality is of a great importance in the chain of production of pig meat. As it has already been pointed out our autochthonous pig breeds belong to the group of primitive and transitional breeds, and according to production type they belong to the breeds meant for the production of fat (Mangalitsa) and to the breeds of combined production capabilities for combined meat and fat production (Moravka and Resavka). Accordingly, they are characterised by a considerably lower meat yield in relation to modern noble swine breeds of the type for meat production. As modern taste of consumers favours lean meat it was one of the reasons for withdrawing the autochthonous breeds from the production and for drastic decrease in their populations.

The carcass quality traits of Mangalitsa and Moravka are presented in Table 1. The Petrović et al. (2007b) reported that Moravka carcass was on average 11.76 cm (distance from pubic bone to atlas), i.e 6.02 cm (distance from pubic bone to the first rib) longer in relation to Mangalica carcass (106.40:94.64 cm, i.e. 86.64:80.62 cm). Backfat measured at different points in Mangalitsa carcass was thicker (by 13.6 up to 23.6 mm) than in Moravka. This resulted in higher quantity of meat in Moravka carcass compared to Mangalitsa by 3.22 kg (28.71:25.49 kg) and in the content of meat by 5.15 % (30.00:24.85 %).

Table 1. The carcass quality traits of Mangalitsa (MAN) and Moravka (MOR)

Source	1		2		3		4	
	MAN	MOR	MAN	MOR	MAN	MOR	MAN	MOR
Petrović et al. (2007b)	94.64	106.40	80.62	86.64	-	-	24.85	30.00
Petrović et al. (2010)	88.74	95.56	-	-	61.85	63.15	27.18	32.09
Petrović et al. (2011)	-	-	-	-	-	-	31.97	30.87

1- distance from pubic bone to atlas (cm), 2- distance from pubic bone to the first rib (cm), 3- backfat thickness (mm), 4- meat content (%).

Petrović et al. (2010), in a comparative study on the quality of carcasses of Moravka and black Mangalitsa in traditional fattening with minimal nutritional corrections, reached the conclusions similar to those in a previous research. Average length of trunk (distance from pubic bone to atlas) in Moravka was 95.56 cm, and in Mangalitsa 88.74 cm. In contrast to previous research the thickness of backfat at ridge was higher in Moravka by 1.3 cm (63.15:61.85 cm), what made no differences regarding carcass meat percentage which was higher in Moravka compared to Mangalitsa (32.09:27.18 %).

In the research aimed at determining the share of tissue in four main half cuts in fatteners of Swallow-Belly Mangalitsa and Moravka depending on the breed, body mass at slaughter and sex, Petrović et al. (2011) obtained different results in relation to previous two studies particularly in respect to differences regarding carcass meat content in these breeds. Fatteners were divided in two groups which finished the fattening at different finishing masses (first group finished fattening at the mass of 91 up to 100 kg, and second from 101 to 120 kg). Fatteners were kept in fattening pens with a yard and fed complete mixtures for noble breeds fatteners. The groups comprised proportional ratio of animals according to breed and sex. The obtained results suggest that variations in warm and cold halves mass were affected by body mass at slaughter ($P<0.001$). In the four main half cuts the percentage of muscle tissue ranged from 36.02 to 38.59%. Subcutaneous adipose tissue, skin and intramuscular fat accounted for 52.64 to 56.24% of the mass of leg, shoulder blade, back-loin and belly-rib cuts. Moravka fatteners had higher average mass of back-loin part in halves ($P<0.01$) and mass of subcutaneous adipose tissue with skin ($P<0.05$) than Swallow-Belly Mangalitsa. The share of muscle tissue of this cut in total mass of muscle tissue in four half cuts was higher in Moravka pigs (+2.18%, $P<0.01$). Male castrated animals had lower average mass of leg ($P<0.05$), lower mass of muscle tissue in leg ($P<0.01$) and higher share of bone tissue ($P<0.05$) than females. In belly-rib cut, castrates had less intramuscular fat ($P<0.05$) and bone tissue ($P<0.05$) than females. The authors suggest that the system of keeping and nutrition applied in intensive fattening of noble breeds resuled in absence of differences regarding average meat yield in carcasses of Moravka (30.87 %) and Swallow-Belly Mangalitsa (31.97 %) fatteners.

The effect of different system of keeping and nutrition of Swallow-Belly Mangalitsa fatteners on the quality of carcass was analysed in the research reported by Petrović et al. 2012.. One group of fatteners was kept in free range system and fed in the woods and on pasture with minimal addition of corn (up to 0.3 kg/head/day). The other group was kept in enclosed facilities with yard and fed diets containing corn silage, feed meal, soybean flour, corn meal, synthetic lysine, mineral supplements and premixes. In different fattening stages the mixtures containing 15 and 13 % crude protein were used. As regards carcass linear measures statistically significant differences were not determined between the two groups of fatteners, except for backfat thickness in loins measured at medial gluteal muscle. Total mass of all four individual half cuts as well as the mass of muscular tissue did not depend upon the housing system. Fatteners carcass meat yield according to domestic regulations accounted for 29.26 % in animals raised in free range system and 30.19 % in the animals kept in enclosed system. Carcass meat percentage determined according to EU regulations accounted for 34.54 (free range system) and 37.07 % (enclosed system). Very similar result regarding meat yield in Swallow-Belly Mangalitsa fatteners kept in free range system (35.92 %) were reported by Radojković et al. (2017).

The quality of meat and meat products of autochthonous pig breeds

Today we see that consumers' demands regarding the quality of swine halves and meat are more and more rigorous in respect to, first of all, attaining higher yield of muscle tissue of good quality in halves in relation to fat tissue. It is also important that pig meat should have an optimal content of fat tissue with optimal ratio of monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids.

Differences regarding chemical composition, content of fat acids and cholesterol in longgissimus dorsi (*musculus longissimus dorsi* - MLD) between Mangalitsa (white- WM and Swallow-Belly - SBM) and Swedish Landrace (SL), raised in farm conditions, were reported by Parunović et al. (2012a). MLD total fat content was higher in lasasta (10.2 %) and white (14.2 %) Mangalitsa compared to Swedish Landrace. Fatteners MLD in SL contained statistically significantly less cholesterole than in WM (-13.6 mg/100 gr) and SBM (-14.8 mg/100 gr). Higher percentage of saturated fatty acids (+8.5 and +10.1%, $P \leq 0.05$) and polyunsaturated fatty acids (+8.0 and +9.4%, $P \leq 0.05$) was determined in MLD originating from SL fatteners in comparison with both Mangalica strains while a total content of monounsaturated fatty acids was statistically significantly lower in SL fatteners meat. Similar results regarding MLD chemical and fatty acid composition were reported by Parunović et al. 2013.. In this research a statistically significantly higher average content of water ($P \leq 0.001$) and protein ($P \leq 0.05$) was determined, and lower content of fat ($P \leq 0.001$) in MLD samples of SL fatteners compared to WM and SBM. This resulted in statistically significantly ($P \leq 0.001$) higher fat/protein ratio in MLD of WM (0.68) and SBM (0.91) compared to SL (0.20). MLD cholesterol content in both mangalitsa strains was higher in relation to SL (47.1 mg/100 gr), the differences being 15.23 mg (SBM) and 15.84 mg (WM). Higher percentage of SFA and PUFAs, and lower total content of MUFAs was obtained in MLD originating from SL fatteners compared to both Mangalitsa strains. The content of α-linolein acid (*n*-3; C18:3) was significantly ($P \leq 0.001$) higher in SBM in relation to WM and SL, therefore, the *n*-6/*n*-3 ratio of PUFAs was most favourable in SBM compared to the two others. PUFAs / SFA ratio was statistically significantly ($P \leq 0.001$) more unfavourable in both Mangalitsa strains compared to SL (0.20 and 0.15 vs 0.33).

The way of pig housing and nutrition can provoke differences in meat quality and fatty acid profile (Table 2). MLD meat in Mangalitsa fatteners kept in free range system and fed on pasture, acorn and forest plants with minimum supplementary feeding, had higher content of protein, ash and water, lower total fat content and lower pH value (Parunović et al., 2012b). In the same research a higher content of PUFAs was determined, as well as higher concentration of PUFAs n-3 and n-6, with higher PUFAs /SFAs ratio in the meat of pigs raised in free range system (0.183:0.175). The PUFAs n-6/n-3 ratio, which has an important role in decreasing the risk of coronary diseases was substantially more favourable in the meat of pigs kept in a free range system accounting for 9.2 compared to 37.3 in the pigs from conventional breeding. This was contributed to higher concentration of α-linoleic acid (*n*-3; C18:3) which in the meat of pigs from free range system accounted for 0.536 % compared to 0.152 % in the pigs from conventional breeding. All the differences were statistically significant, in contrast to differences regarding average content of cholesterol which accounted for 61.7 mg/100 g (free range) and 63.1 mg/100 g (conventional). Radojković et al. (2017) determined the content of MLD intermuscular fat in SBM fatteners raised in free range system and it accounted for 6.20 % on average. In MLD samples average contents of protein, water, fat and ash were 20.72 %, 70.38 %, 7.68 % and 0.99 %, respectively. Average content of cholesterol was 41.40 mg/100 g.

Share of SFAs in MLD fats was on average 35.62 %, of MUFAs 56.61 % and of PUFAs 6.92 % (Table 2). The PUFAs/SFAs ratio accounted for 0.19, while the PUFAs *n*-6/*n*-3 ratio accounted for 25.07. The authors pointed out that these ratios were not so favourable from the aspect of recommended nutrient ratios for human nutrition.

Table 2. Fatty-acid composition of muscle tissue (MLD) of Mangalisa and Moravka

Reference	Fatty-acid	Mangalitsa	Moravka
Parunović et al. (2012b)	ΣSFA	33.9 con; 35.5 fr	-
	ΣMUFA	57.2 con; 55.5 fr	-
	ΣPUFA	5.93 con; 6.45 fr	-
	†P/S	0.175 con; 0.183 fr	-
Petrović et al. (2014)	ΣSFA	39.45	41.64
	ΣMUFA	56.41	53.78
	ΣPUFA	4.10	4.54
	†P/S	0.10	0.11
Radojković et al. (2017)	ΣSFA	35.62	-
	ΣMUFA	56.61	-
	ΣPUFA	6.92	-
	†P/S	0.19	-
Savić et al. (2017)	ΣSFA	-	42.26♂; 41.21♀
	ΣMUFA	-	53.24♂; 55.07♀
	ΣPUFA	-	4.45♂; 3.69♀
	†P/S	-	0.11♂; 0.09♀

†P/S=(C18:2+C18:3) /(C14:0+C16:0+C18:0; con- conventionally, fr- free range

Petrović et al. (2014) studied a chemical composition and content of fatty acids of MLD in fatteners of both sexes (castrates and gilts) of Swallow-Belly Mangalitsa (SBM) and Moravka (M), kept in enclosed system and fed a complete mixtures for noble breeds. Statistically significant differences were determined in chemical composition between the average content of fat (SBM-5.10 %; M-6.96 %) and protein (SBM- 22.39 %; M- 21.47 %) in the meat of these breeds. Statistically significant differences were obtained also between the average contents of SFAs (SBM- 39.45 %; M- 41.64 %) and MUFAs (SBM- 56.41 %; M- 53.78 %), while regarding the content of PUFAs the obtained differences were not statistically significant (SBM- 4.10 %; M- 4.54 %), as well as regarding calculated ratio of PUFAs/SFAs which accounted for 0.10 in SBM and 0.11 in M (Table 2). The PUFAs *n*-6/*n*-3 ratio was higher than favourable and accounted for 18.7 in SBM and 13.7 in M. In the study on fatty acids profiles under the effect of sex and body mass in MLD of Moravka fatteners Savić et al. (2017) determined the SFAs average content (independent of the sex) of 41.74 %, MUFAs 54.16 % and PUFAs 4.07 %, while the PUFAs/SFAs ratio accounted for 0.10 (Table 2). The authors pointed out that castrated males had higher content of saturated fatty acids C14:0 (1.42 : 1.26; $P<0.05$) and C18:0 (15.07 : 13.38) compared to females. Body mass of fatteners displayed statistically significant effect ($P<0.05$) on the content of some fatty acids so that with the increase of body mass by 1 kg there occurred decrease in total content of PUFAs by 0.038 % and decrease of total content of MUFAs by 0.067 %.

As a measure of technological quality of meat and its suitability for storing and processing the acidity (*pH* value) and colour (CIE L*a*b* system: lightness (L*), intensity of red colour (a*) and intensity of yellow colour (b*) are often determined. In a comparative study on carcass quality and

meat of pigs of differrent genotypes (Swallow-Belly Mangalitsa - SBM, Moravka - M, crossbreds of Moravka and Mangalitsa - MM and crossbreds of Moravka and Duroc - MD) Radović et al. (2017a) determined statistically significant differences between average *pH* values measured in MLD- 45 minutes (pH_1) and 24 hours (pH_2) after slaughter of these studied genotypes. The lowest pH_1 value was determined in MD (5.69), and highest in MM (6.59). The same applies to pH_2 (MD – 5.46 and MM 5.71). The meat of SBM fatteners was darker compared to other genotypes while the most distinguished differences were found in MD genotype (L*- 40.13:55.87). Similar results regarding the acidity and colour of meat were obtained by Stanišić et al. 2013.. They compared the quality of meat between the three breeds (SBM, M and SL). A statistically significant differences were determined ($P<0.05$) only regarding the meat content in MLD ($P<0.05$) which accounted for 7.40 % in SBM, 5.34 % in M, and 2.65 % in SL. The meat acidity in these three breeds did not significanly differ (pH_2: SBM – 5.47, M – 5.48, SL – 5.47), while statistically significant ($P<0.05$) differences were determined regarding meat tenderness after thermic processing (SBM – 5.05; M – 6.29; SL – 6.40) and meat cooking shrinkage (SBM – 29.60 %; M – 39.34 %; SL – 37.09). The SL meat had lighter colour (L*- 55.69) compared to SBM (L*- 38.19) and M (L*- 42.44) and highest intensity of yellow colour (b*- 5.74) in relation to the meat of other two breeds (SBM: b* -2.68; M: b*- 4.16). Stanišić et al. (2016) also determined differences in the meat colour between SBM and SL meat during six-day storage in vacuum packed meat. The lightness of SBM meat at measurings on the first, third and sixth day of storage remained unchanged (L*: 51.38; 50.65; 51.18), while in SL meat it decreased significantly (L*: 61.30; 57.33; 52.24). The authors report that SBM meat from free range system has a considerably higher colour persistency during storage compared to the meat of meat-type breeds what gives it certain advantage from the aspect of placement of this meat on the market as a highly quality pork.

Porcine stress syndrome (PSS) is porcine genetic disease which may cause malignant hyperthermia syndrome. In the research published by Stanišić et al. 2012. the aim was to test *RYR1* gene in 10 Mangalitsa pigs by use of *PCR*-restrictive endonuclease test (*PCR-RFLP*), which represents a simple and reliable technique for *RYR1* gene testing. The extraction of DNA (deoxyribonucleic acid) was conducted by using the root of porcine hair. Test results confirm that *RYR1* genotype in all 10 pigs was negative. On the basis of previously mentioned it can be concluded that Mangalitsa could be one of the genotypes to be used in medicinal and clinical researches.

Production of high quality added value traditional products out of the meat of autochthonous pig breeds represents a potentially highest level of finalisation of this kind of livestock production and possibly one of safer models of their sustainable breeding and survival. Rather demanding market principles which govern liberal economy conditions imply detailed information of the advantages and superiority of some type of product compared to competitive products in order to place them on the market by adjusting more acceptable prices and thus make profit. Parunović et al. (2017) reported results of comparative studies of sensory characteristics and fatty acid profile of traditional cured sausages produced of porcine meat of different pig breeds. The authors analysed three types of kulen (dry pork sausage) and sremska sausage produced with the meat of Swallow-Belly Mangalitsa (SBM), the mixture of meat of Mangalitsa and Moravka in 50:50 % ratio (MM) and Swedish Landrace (SL). Fatteners were kept in semi-free system with unlimited access to green pasture (clover) and cereals supplementation (corn and wheat). The recipe and technological procedure for the production of kulen and sausages were identical, except for the origin of meat. The results of the study showed that in sremska sausage produced with SL meat the cholesterol

content was the highest (64.92 mg/100 g) compared to sausages produced with the meat of SBM (59.65 mg/100 g) and mixture of meat of MM (53.47 mg/100 g). As for kulen the highest content of cholesterol was found in the product made with the meat of MM (66.00 mg/100 g), in relation to SBM (50.16 mg/100 g) and SL (61.48 mg/100 g). On the other hand, sausages produced with SBM meat contained higher level of MUFAs and total content of unsaturated fatty acids as well as lower content of SFAs. The most favourable ratio of PUFAs *n*-6/*n*-3 was found in the sausage produced with SBM meat being 14.38, while in SL it was 35.86, and in MM 37.36. In kulen produced with SBM meat the value of this parameter accounted for 16.96 and it was significantly lower compared to kulen produced with SL meat (25.21) and mixture of meat of MM (36.07). Kulen and sremska sausage produced with the SBM meat were superior in respect to colour, odour, taste and general acceptability of product, evaluated by professional degustators. The authors point out that, with proper combination of meat and adipose tissue of autochthonous breeds, it is possible to make kulen and dry sremska sausage of respectable chemical composition, satisfactory and reasonably healthy content of fatty acids and sensory traits acceptable to demanding customers.

Similar conclusions regarding the quality of traditional dry meat products obtained by the meat of autochthonous and modern breeds were also reported by Parunović et al. (2014). The results of this research showed that kulen made with SBM and M meat had the lowest content of moisture and protein and the highest total quantity of fat. Kulen made by combination of SBM and M meat was more superior in odour, taste, aftertaste and overall acceptability. The highest cholesterol content was found in sausages made of meat of autochthonous pig breeds. However, sausages made of the meat of SBM pigs contained higher levels of MUFAs and total unsaturated fatty acids and lower contents of SFAs. This research showed that swine breed can affect chemical and sensory characteristics of traditional dry fermented sausages.

Conclusion

Numerous studies conducted by the researchers of the Institute of Animal Science of the Faculty of Agriculture of the University of Belgrade which were, from different aspects, engaged in the issue regarding the autochthonous pig breeds in the Republic of Serbia, clearly illustrate the production potential of these breeds indicating that it is essential that they should be preserved. In further scientific activites it seems necessary to direct the research towards genotypisation and molecular and genetic research on autochthonous pig breeds. In addition, it would be essential to deepen the research so as to find the most optimal and sustainable systems of breeding and utilisation of these breeds. Previous researches indicated certain advantages of these breeds regarding specific characteristics of meat and traditional products made of such meat. Ever growing need for food obtained from organic and ecologically acceptable production systems provides more room for utilisation of these breeds. In the future it will be very important to direct the research likewise and to additionaly confirm the efficacy and advantages of raising the autochthonous breeds in these production systems with a special emphasis on confirming their adaptability and resistance. It is particularly important to precisely define the most optimal systems of keeping and nutrition of these breeds for the purpose of emphasizing their comparative advantages in relation to modern meat-type breeds, particularly regarding specific and recognisable meat quality. All these would create conditions for increasing the number of heads in animal populations of these breeds what would also create the conditions for the application of other zootechnical procedures, such as optimal selection and use of

suitable raising methods what would lead to improving their production capacities and additionaly strenghten sustainability of their breeding in the oncoming period.

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PATHWAYS FOR OPTIMISATION OF MILK PRODUCTION AT DAIRY FARM LEVEL

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Abstract: The analysis of possibilities of optimisation of milk production at a farm level was conducted on the basis of a survey carried out within the project entitled "Optimisation of technological procedures and zootechnical resources on farms aimed at improving the sustainability of milk production". The review included 120 farms of different capacities engaged primarily in cattle production. Having in mind all the farm differences and zootechnical conditions included in this analysis, an average annual milk production per hectare of agricultural land was taken as a dependent variable. Basic statistical model for analysis of variance included following factors as fixed effects: farm size, herd size, degree of specialisation of cattle production and cattle breed. Basic statistical model used for analysis of variability of average annual milk production per ha of agricultural land surface was highly significant with determination being over 50%. In all models, herd-size, degree of specialisation and cattle breed (except for the "farm size" factor) expressed significant effect on variability of dependent variable. However, it should be pointed out that the significance of the "farm size" factor varied depending on the model applied. Possibilities for optimisation of milk production in Serbia do exist among which sustainable increase of the farm size in relation to available resources, increase in the herd size, increase of the intensity of production, as well as regular conducting of selection and breeding procedures are particularly distinguished.

Keywords: Farm size, farm management, herd size, production intensity

Introduction

Milk production in Serbia is organised mostly in mixed farm systems, i.e. production systems in which animal and plant productions are both arranged on the same farm. The largest number of cattle farms in Serbia is small-size farms on which up to 10 cows are raised while the number of medium-size and particularly large-size farms is significantly lower. In spite of available natural resources, the fragmentation of land holdings into petty farms leads to considerable heterogeneity regarding their utilisation, and therefore a realised performance (Bogdanović, 2016; Bogdanović et al, 2014; Bogdanović et al, 2012). The existence of a large number of non-commercial, predominantly old aged, poor equipped and poor motivated holdings, with which it is not possible neither to organise more substantial production nor make any long-term production plans, makes this situation even more difficult (Perišić et al., 2006).

On the other hand, a common feature of all farmers engaged in commercial milk production, compared to average farmers engaged in mixed agricultural production, is higher level of their technological knowledge, greater interest in production technological advancements, larger farm surface area, as well as greater number of animals raised on the farm (Bogdanović, 2016). This makes the milk production more efficient and provides more possibilities of its further optimisation and sustainable increase in the intensity of the production.

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Some of the possibilities not only for additional optimisation but also for intensifying milk production are improvements in farm management, the increase of the herd size with forcing the raising of genetically superior animals, as well as various improvements in the field of dairy cows feeding (Huysveld et al., 2017; Golas, 2017; Bogdanović, 2016; Jaklič et al., 2014; Kučević et al., 2011; Perišić et al., 2011; Perišić et al., 2011b; Alvarez et al., 2008; Groot et al., 2006), i.e. constant improvement of all available farm conditions, technological processes and zootechnical resources with simultaneous decrease in production costs (Golas, 2017; Alvarez et al., 2008).

However, whether some program of optimisation of milk production will be accepted by farmers depends not only on specific farm characteristics and zootechnical conditions but also on overall socioeconomic position of the agricultural estate holder, his age and level of education (Ondersteijn et al., 2003), and his readiness to innovate production on the farm (Bogdanović, 2016). Taking all this into consideration, the objective of this paper is to analyze possibilities of additional optimisation of primary milk production in Serbia which would lead not only to its sustainable intensification but also to better and safer market position of farmers in this highly demanding and complex branch of cattle production.

Material and Methods

The analysis of possibilities of optimisation of milk production at a farm level was conducted on the basis of a survey carried out within the project entitled "Optimisation of technological procedures and zootechnical resources on farms aimed at improving the sustainability of milk production" (TR31086). The review included 120 farms of different capacities engaged primarily in cattle production. Research questionnaire was similar to that used by Bogdanović et al., 2014 and Bogdanović et al., 2012, in his research. However, this survey differed from a previous one in that some questions referring to farm management were added with the aim at obtaining more information on milk production.

Since the farms included into this research differed considerably in size, all the farms were divided into following groups: (S) small farms of size up to 20 ha, (M) medium farms of size from 20 to 50 ha, (L) large farms of size from 50 to 100 ha and (XL) extra-large farms of size over 100 ha. Similar grouping was done also regarding the number of cows and heifers raised on the farm, therefore the farms were divided into those (1) which raise up to 10 cows and heifers, (2) farms with 10-20 cows and heifers, (3) farms with 20-50 cows and heifers and (4) farms with more than 50 cows and heifers.

Apart from that, in order to analyse the effect of specialisation of cattle production on the quantity of milk produced, all the farms were divided into 4 groups: (1) mixed farms realising up to 50% total revenue from cattle production, (2) moderately specialised farms realising from 50% to 70% total revenue from cattle production, (3) specialised farms realising from 70% to 85% total revenue from cattle production, and (4) highly specialised farms which realise over 85% total revenue from cattle production.

As regards breed composition, Simmental breed (SIM) is raised on about 75% analysed farms, Holstein-Friesian breed (HF) is represented on about 15% farms, (3) while both breeds (Sim&HF) are raised on 10% of farms.

Having in mind all the farm differences and zootechnical conditions included in this analysis, an average annual milk production per hectare of agricultural land was taken as a dependent variable. This derived trait was calculated on the basis of total annual milk production on farm

(expressed in kg) and size of farm (expressed as the number of hectares of agricultural surface). In this way the comparison of mutually very heterogeneous farms was made possible.

Basic statistical model for analysis of variance included 4 factors analysed as fixed effects, and they were the following: farm size, herd size, degree of specialisation of cattle production and cattle breed. In addition, the effect of a number of other factors was studied as well, which could have certain importance in variability of milk production at the farm level such as characteristics of reproductive and feeding management on farm, contacts with an agricultural advisory service and the age of the holder of an agricultural estate.

The effect of some factors on a variability of average milk production per ha of agricultural land surface was studied by means of GLM procedure of SAS software package 2013..

Results and Discussion

The average values of annual milk production per ha of agricultural land surface and significance of factors included into analysis of variance are shown in Table 1. In all models applied only the factors presented in Table 1 (except for the"farm size" factor) expressed significant effect on variability of studied dependent variable while factors in Table 2 expressed no statistically pronounced significance in any model. However, it should be pointed out that the significance of the "farm size" factor varied depending on the model applied meaning that this factor, in some models, has expressed statistical significance, while in other models it had no important significant effect on variability of studied trait. For this reason this factor was shown in Table 1 together with other factors included into basic model of variance analysis.

Table 1. Means and significance of the model and factors

Model/Factor	**Significance**	**Level of factor**	**Mean (kg)**	**Std. Error (kg)**
Model	R^2=0.51 ***			
Farm size	NS	S	2523.77	221.68
		M	2245.77	330.91
		L	3855.03	1329.52
		XL	4059.58	673.10
Degree of cattle production specialization	***	1	1337.40	167.03
		2	2525.06	379.21
		3	1622.77	255.94
		4	3776.32	306.91
Breed	***	HF	4647.24	506.02
		SIM	2214.41	182.98
		Sim&HF	2364.88	507.47
Herd size	***	1	1817.19	211.52
		2	3009.14	278.39
		3	3785.33	452.21
		4	4908.35	636.68

Basic statistical model used for analysis of variability of average annual milk production per ha of agricultural land surface was highly significant with determination being over 50%, what indicates that 4 basic factors explained more than 50% of total variability in studied trait.

Although the significance of the farm size was not consistent in relation to models applied and in basic model did not exert statistical significance this factor should be analysed because the size of farm, together with applied farm management, can have, in the first place, a significant effect on

expression of cow's production traits (Golas, 2017; Kučević et al, 2011). From the results in Table 1 it can be perceived the tendency of increasing the quantity of milk produced per ha of agricultural land surface with increase of the farm size. However, it is not always the case, particularly if small and/or medium-size farms are in question, what is in accordance with the results of Kučević et al., 2011, suggesting that it is important that every farmer should assess the optimal size of his herd or farm in relation to availability of necessary resources for running the milk production.

Other three factors in basic model expressed a highly significant effect, independently of the model used. It can be seen in Table 1 that the lowest production of milk per ha of agricultural land surface was realised on mixed farms, and the highest production was realised on highly specialised farms. These results indicate that one of the potential directions of optimisation of milk production may be its specialisation, but to a degree providing an optimal utilisation of available farm resources.

In addition, in order to have optimal milk production it seems essential to improve cattle genetic potential for milk production and it seems to be the next direction of production optimisation. In accordance with characteristics of the region (climate conditions, quality of forage crops, breeder's practices, altitude and so on) as a method of improving both Simmental and Holstein-Friesian breed the pure-breeding is being imposed upon with conducting strong selection and application of artificial insemination by the semen of the highly ranking bulls (Perišić et al., 2011a and 2011b).

Finally, the size of a breeding stock, i.e. the number of cows and heifers raised on farm also has a linear and highly expressed effect on quantity of produced milk per ha of land surface. This factor is perhaps the most important source of variability, since it clearly indicates that production of milk increases with the increase of the herd size, what is not always the case when the size of farm is in question. Similar results on the effect of the herd size on production traits were obtained by Kučević et al., 2011 as well.

In addition to previously mentioned, there is an imposed need to make the analysis of the effects and factors in the models which have no more than a slight statistical significance (Table 2) but recognising their effects is necessary in order to determine directions of further optimisation of milk production more precisely.

Table 2. Means of the factors with no statistical significance

Factor	YES		NO	
	Mean (kg)	St.Err. (kg)	Mean (kg)	St.Err. (kg)
Owner born after 1970	3041.01	355.38	2397.30	208.94
Plan to extend the milk production	2637.08	195.16	2204.52	490.60
Regular contact with extension service	2638.95	198.61	2470.97	436.12
Artificial insemination	2760.13	196.63	1363.08	287.86
Using of bulls from AI Centres	2827.08	214.65	1660.53	268.09
Feed production at farm	2639.16	187.75	1499.41	531.68
Using of feed additives	2650.39	204.19	2359.58	414.54

This research also confirms that young farmers are more ready to innovate their production and thus to realise higher milk production per ha of agricultural land surface, what is in accordance with the results obtained by Ondersteijn et al., 2003. The plans to enlarge the production in the

future may be connected with this fact since higher milk production was registered at those farms where breeders have plans to enlarge existing production. On the other hand, a small difference was registered in production results between the breeders who have a regular contact with agricultural advisory service and those who have no such contact what can indicate insufficiently efficient transfer of knowledge towards breeders.

As regards reproductive management used on the farm, it can be perceived that higher milk production is obtained on the farms which conduct artificial insemination with the semen of bulls originating from AI Centres. Although it is understood that this is a regular practice on commercial farms engaged in milk production, it is noticeable that there are breeders who still use natural mating or, in case of AI, they purchase the semen on free market what is directly reflected on lower milk production. Similar tendency can also be perceived with the breeders who themselves produce their own cattle feed and use necessary nutrition supplements which they purchase on free market compared to those breeders who do not produce animal feeds on their farm but depend on a current offer on a free market.

Conclusion

Commercial milk production is extremely demanding nowadays and it is based on constant improving of all available resources which are essential for this production. Possibilities for optimisation of milk production in Serbia do exist wherein a sustainable increase of the farm size in relation to available resources, increase in the herd size, increase of the intensity of production, as well as regular conducting of selection and breeding procedures are particularly distinguished. Besides this, it is necessary to work further on breeders' education in the field of cattle reproduction and feeding management and on more efficient transfer of knowledge towards breeders, starting from high education and research institutions through agricultural advisory service.

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DIFFERENCES IN PERSISTENCY OF HEAT STRESS EFFECT IN DAIRY PRIMIPAROUS COWS DUE TO CATTLE BREED

Gantner, V.*[1], Dokić, D.[2], Gregić, M.[1], Vučković, G.[3], Kuterovac, K.[4]

Abstract: Aiming determination of the persistency of heat stress effect in dairy primiparous cows regarding the breed (Holstein, Simmental) and susceptibility to heat stress test-day records provided by the Croatian Agricultural Agency were analysed. Only cows with detected statistically significant decrease in daily milk yield at set temperature-humidity index (THI) threshold value (65, 70 and 75) were included in the further analyses. The persistency of heat stress effect regarding the daily milk yield and milk contents (fat and protein) was determined as a drop in the subsequent milk recordings (1st and 2nd). Holstein cows had higher drop in daily milk yield compared to Simmentals in both subsequent milk recordings. The analysis of drop in daily milk contents, fat and protein, showed a higher drop in daily fat than protein content with similar values in both breeds. Also, the amount of drop increased in the 2nd compared to the 1st subsequent milk recording in both breeds. The research results indicate that the negative effect of heat stress was more pronounced and more persistent in Holsteins compared to Simmental cows. Also, the negative effect of heat stress was more pronounced and more persistent in cows that were more susceptible to heat stress (heat stressed at the lower THI threshold values). Finally, as cows are less resistant to the heat stress (more susceptible, Holstein breed), the heat stress effect is more severe (the drop is higher and more persistent).

Keywords: heat stress, persistency, primiparous cows, daily milk yield, cattle breed

Introduction

Current dairy cattle production characterizes growing demand for high production per cow. Also, this production occurs in an environment that is changing to cows less comfort zone. In accordance to Battisti and Naylor (2009), by year 2050, most of the world will be exposed to median temperatures in the summer that will be higher than the highest recorded temperatures. Furthermore, Gauly et al. 2013. warned that the heat stress of high-producing dairy cows will cause growing concern among European milk producers. Segnalini et al. 2013. emphasized the need for adequate adaptation strategies development aiming reduction of the negative effects of warming in farm animals in the Mediterranean region. In European Union, GIRA 2012., in the analysis of Regional movements in EU Milk Production, predicts that regions with intensive farming will be replaced by regions with less intensive farming around the Atlantic and with more land suitable for pasture (resulting in lower production costs). According to Hansen 2013., the increase of production makes cows more susceptible to heat stress, which means that heat stress will become an acute problem regardless the climate changes. Modern dairy cows, which are characterized by high levels of pro-

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ductivity, lose the ability to regulate their body temperature at air temperatures as low as 25-29°C. The studies of Bohmanova 2006. and Collier et al. 2006. indicate that heat stress affects high-producing cows much more than low-producing ones. Kadzere et al. (2002) stated that the intensive genetic selection for milk production has changed the thermoregulation physiology of dairy cattle. The high-producing cows have larger frames and larger gastrointestinal tracts that enable them to digest more feed. This creates more metabolic heat and reduces the ability of cows to regulate normal temperature at heat stress conditions. According to Kadzere et al. (2002), the thermoneutrality shifts to lower temperatures due to the increase of milk yield, feed intake and metabolic heat. In dairy cows, dry matter intake, milk production (West et al. 1999; Casa and Ravelo, 2003) and reproductive performances (Bohmanova et al. 2007; Ravagnolo et al. 2000) are reduced due to heat stress. In addition, heat stress affects milk composition, somatic cell counts (SCC) and mastitis frequencies (Bouraoui et al. 2002; Collier et al. 2012; Correa-Calderon et al. 2004; Gantner et al. 2017; Ravagnolo et al. 2000; St-Pierre et al. 2003; West 2003; Hammami et al. 2013; Smith et al. 2013). The heat stress condition also leads to considerable loss of profit, e.g. between $897 million and $1,500 million per year in the USA (St-Pierre et al. 2003). There are many ways to measure heat stress although the temperature-humidity index (THI) is the most common one when it comes to dairy cattle. THI includes ambient temperature and relative humidity and is a useful and simple method of assessing the risk of heat stress (Kibler 1964).

The aim of this research was to determine the persistency of heat stress effect in dairy primiparous cows regarding the breed (Holstein, Simmental) and susceptibility to heat stress (THI = 65 / 70 / 75).

Materials and methods

Data

Individual test-day records of Holstein and dairy Simmental primiparous cows collected during the regular milk recording performed by an alternative milk recording method (AT4/BT4) in the period from January 2005 to December 2012 in Croatia were used for the statistical analysis. Monthly, at each recording, milk yields were measured during the evening or morning milkings. Additionally, at each milk recording, ambient temperature and relative humidity were recorded. Daily temperature-humidity index (THI) was calculated using the equation by Kibler 1964.:

$$THI = 1.8 \times Ta - (1 - RH) \times (Ta - 14.3) + 32$$

Where Ta is average temperature in degrees Celsius and RH is relative humidity as a fraction of the unit. Records with lactation stage in (< 6 days and > 305 days), age at first calving in (< 21 and > 36 months), missing parity, and missing or nonsense Ta and RH value were deleted from the dataset. Only cows with minimum 3 test day per lactation were taken into analysis. Data were provided by the Croatian Agricultural Agency. Variability of daily milk traits (daily milk yield, DMY; daily fat content, DFC; daily protein content, DPC) regarding the breed is presented in Table 1.

Table 1. Descriptive statistics of daily milk traits (milk yield, kg, DMY; fat content, %, DFC; protein content, %, DPC) regarding the breed (Holstein, Simmental)

Breed/Trait	N	DMY, kg	DFC, %	DPC, %
Holstein	293,351	21.18±7.09	4.06±0.91	3.30±0.40
Simmental	273,420	15.58±5.09	4.15±0.83	3.86±0.42

Statistical analysis

The variation in daily milk traits due to heat stress was tested by least square analyses of variance for each given THI value (65, 70 and 75) separately for each breed (Holstein, and Simmental) using the PROC MIXED procedure in SAS (SAS Institute Inc., 2000). Following mixed model was used:

$$y_{ijklmn} = \mu + b_1(d_i / 305) + b_2(d_i / 305)^2 + b_3 \ln(305 / d_i) + b_4 \ln^2(305 / d_i) + S_j + A_k + T_l + e_{ijklm}$$

Where yijklm = estimated daily milk trait;

μ = intercept;

b1, b2, b3, b4 = regression coefficients;

di = days in milk (i = 6 to 305 day);

Sj = fixed effect of calving season class j (j = 1/2005 to 12/2012);

Ak = fixed effect of age at calving class k (k = 21 to 36 month);

Tl = fixed effect of THI class (l = 0 *(normal condition – values under the given threshold)* or l = 1 *(heat stress condition – values equal and above the given threshold)*);

eijkl = residual.

The significance of the differences between the THI classes was tested by Scheffe's method of multiple comparisons. Only cows with detected statistically significant decrease in daily milk yield were included in the further analyses. The daily milk trait measured on the recording day when heat stress occurred was used as the reference level. The drop in daily milk traits was determined in the 1st (test-day milk traits measured within 35 days) and 2nd (test-day milk traits measured between 35 and 70 days) milk recording after heat stress. The persistency of the effect of heat stress as a drop in daily milk traits were analysed separately for each breed (Holstein and Simmental).

Results and discussion

The amount of drop in daily milk traits in subsequent milk recordings (1st and 2nd) after detection of heat stress at different THI values (65, 70 and 75) in regard to the breed (Holstein, Simmental) in is presented in tables 2, 3 and 4.

In the primiparous Holstein cows, that experienced statistically significant ($P < 0.05$) decrease of daily milk yield at THI = 65, in the 1st subsequent milk recording the daily milk yield dropped for 1.45 kg/day, while in the 2nd subsequent milk recording the drop in milk production was even higher (1.91 kg/day). The primiparous Simmentals that were heat stressed at THI = 65 experienced lower drop compared to Holsteins in amount of 1.05 kg/day and 1.13 kg/day in the 1st and 2nd subsequent milk recordings, respectively.

The amount of determined drop in daily milk contents (fat and protein) in the subsequent milk recordings was similar in both breeds. Also, higher drop in the 2nd subsequent milk recording was observed for daily fat and protein content for both breeds.

Table 2. Drop in daily milk traits in the subsequent milk recordings after detection of heat stress regarding the breed (Holstein, Simmental) when THI = 65

Parity	1st milk recording after heat stress			2nd milk recording after heat stress		
	DMY, kg	DFC, %	DPC, %	DMY, kg	DFC, %	DPC, %
Holstein	1.45	0.16	0.06	1.91	0.22	0.08
Simmental	1.05	0.16	0.06	1.13	0.25	0.10

*DMY – daily milk yield, kg; DFC – daily fat content, %; DPC – daily protein content, %

The effect of heat stress in primiparous Holstein and Simmental cows that experience statistically significant ($P < 0.05$) decrease of daily milk yield at THI = 70 (Table 3) and THI = 75 (Table 4) persisted in the 1st and 2nd subsequent milk recordings. Holstein cows had higher and more persistent drop in daily milk yield compared to Simmentals. The analysis of drop in daily milk contents, fat and protein, showed a higher drop in daily fat than protein content with similar values in both breeds. Also, the amount of drop increased in the 2nd compared to the 1st subsequent milk recording in both breeds.

Table 3. Drop in daily milk traits in the subsequent milk recordings after detection of heat stress regarding the breed (Holstein, Simmental) when THI = 70

Parity	1st milk recording after heat stress			2nd milk recording after heat stress		
	DMY, kg	DFC, %	DPC, %	DMY, kg	DFC, %	DPC, %
Holstein	1.23	0.15	0.08	1.65	0.25	0.12
Simmental	0.76	0.16	0.08	0.99	0.29	0.13

*DMY – daily milk yield, kg; DFC – daily fat content, %; DPC – daily protein content, %

Table 4. Drop in daily milk traits in the subsequent milk recordings after detection of heat stress regarding the breed (Holstein, Simmental) when THI = 75

Parity	1st milk recording after heat stress			2nd milk recording after heat stress		
	DMY, kg	DFC, %	DPC, %	DMY, kg	DFC, %	DPC, %
Holstein	0.89	0.12	0.10	1.16	0.26	0.16
Simmental	0.42	0.16	0.09	0.64	0.31	0.17

*DMY – daily milk yield, kg; DFC – daily fat content, %; DPC – daily protein content, %

In accordance to many studies (Kadzere et al. 2002, Bohmanova 2006, Collier et al. 2006, Gantner et al. 2017, Hansen 2013) the THI threshold values depend on a variety of factors, for instance: production level, parity, breed, region. According to Du Preez et al. (1990a, b), heat stress affects milk production and feed intake when THI values exceed 72. Bouraoui et al. (2002) set the threshold at 69, while Bernabucci et al. (2010) and Collier et al. 2012. set in at 68. Vitali et al. (2009) warned of the increased risk of cows' death when THI reaches 80. The results of this analyses showed that the lower is THI threshold value, the higher is the drop in daily milk yield in subsequent milk recordings. Also, the higher drop of daily milk yield was determined in Holsteins with a tendency of drop increase in the 2nd subsequent milk recording. On the other hand, Simmental cows had a lower drop of daily milk yield with the same tendency to drop increase in the 2nd subsequent milk recording. These results indicate that the negative effect of heat stress was more pronounced and

more persistent in Holsteins compared to Simmental cows as well as in cows more susceptible to heat stress (heat stressed at lower the THI threshold values).

Conclusions

The results of this research indicate that the negative effect of heat stress was more pronounced and more persistent in Holsteins compared to Simmental cows. Also, the negative effect of heat stress was more pronounced and more persistent in cows that were more susceptible to heat stress (heat stressed at the lower THI threshold values). Finally, as cows are less resistant to the heat stress (more susceptible, Holstein breed), the heat stress effect is more severe (the drop is higher and more persistent).

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THE EFFECT OF DAIRY CATTLE BREED ON SUBCLINICAL KETOSIS PREVALENCE RISK AND SUBSEQUENT MILK PRODUCTION

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Abstract: Aiming determination of the differences in prevalence of subclinical ketosis and subsequent daily milk yield due to dairy cattle breed more than 4.6 million of test-day records collected in regular milk recording of dairy cattle in Croatia were used. The subclinical ketosis prevalence was indicated by the fat to protein (F/P) ratio, while subclinical ketosis was indicated by the F/P ratio and cows daily production. The effect of subclinical disorders on daily milk traits were tested by Scheffe's method (SAS/STAT). The highest subclinical ketosis prevalence occurred in early lactation (until 15th day of lactation in Holsteins and 25th day in Simmentals) with highest prevalence in first parity cows. Regarding the parity, in both breeds, highest prevalence in the beginning of lactation, occurred in cows in first, then in third parity, with the lowest prevalence in second lactation. Regarding the breed, Holsteins experienced higher subclinical ketosis prevalence compared to Simmentals in all lactation stages and all parities. Furthermore, a significant negative effect of subclinical ketosis on daily milk yield in each parity and both breeds was determined in both subsequent milk controls (A-1, A-2). Finally, it should be emphasized that test-day records could be used as cost effective and non-invasive method for monitoring the herd health enabling farmer early reaction and prevention of development of strong clinical symptoms.

Keywords: subclinical ketosis, prevalence, daily milk yield, cattle breed

Introduction

Efficient dairy farming requires gravidity and parturition each year. The transition period from non-lactating to lactating stage is often stressful for the cow. During this period, due to variety of factors such as dietary changes, negative energy balance, decreased feed intake, weight loss, hypocalcaemia, etc., dairy cows are predisposed to various disorders. Furthermore, cows can experience the environmental stressors (regrouping (Mulligan and Doherty, 2008), or inadequate climatic conditions (Broucek et al., 2007). For instance, LeBlanc (2010) determined that most of the metabolic disorders (up to 50%) of dairy cows occur within the first 2 weeks of lactation. Ketosis presents one of the most common disorder in lactating dairy cows.

Ketosis is a metabolic disorder that can occur both in clinical and subclinical forms where subclinical ketosis is defined as a preclinical stage of ketosis. Clinical ketosis most frequently occurs in high producing cows between the 2nd and 7th week after calving as a consequent of inadequate nutrition and management (Gillund et al., 2001). The prevalence of ketosis could be influenced by

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variety of factors, e.g., breed, parity, season and herd-related factors. Gustafsson and Emanuelson, (1996) as well as Rajala-Schultz and Gröhn, (1998) quoted that clinical ketosis induces economic losses to the dairy farmer through treatment costs, decreased milk production, impaired reproduction efficiency, and increased involuntary culling. Andersson (1988) stated that the subclinical ketosis can be revealed by determining levels of plasma glucose, plasma non-esterified fatty acids (NEFA), as well as milk or urine ketone body concentration.

In order to have efficient dairy farm, dairy cows' health needs to be monitored at the herd level. For that purpose, test day records (TDR) represent an alternative which is much more cost effective and non-invasive when compared to specific diagnostic (Duffiled et al., 1997; Eicher, 2004). TDR include daily milk, fat and protein production, and fat to protein ratio (F/P ratio). Richardt (2004) defined a 1.5 value of F/P ratio as risk level for subclinical ketosis, while Eicher (2004) beside F/P ratio, for indication of metabolic disorders (acidosis, ketosis) also took into account daily milk production.

The aims of this research were to determine the differences in prevalence of subclinical ketosis and subsequent daily milk yield due to dairy cattle breed using monthly test day records in Croatia.

Material and Methods

Data

The individual test day records collected in regular milk recording in period from January 2004 to December 2013 in Croatia were used for analysis. Milk recording was performed according to the alternative milk recording method (AT4/BT4) meaning monthly measurement of milk yield during the evening or morning milking and milk sampling from each lactating cow. Daily milk yield and fat content was projected from partial measurement. Logical control of production data was performed according to ICAR standards 2003.. After logical control data consisted of 1,962,831 test-day records from Holsteins and 2.641.223 test-day records from Simmental cows.

According to parity, four classes were formed: P1, P2, P3, and P4+ that included cows in 4th and higher lactations. Regarding the lactation stage, 16 classes by 5 days were formed: L1 (< 10) … L16 (> 90). Regarding the test date, month-year classes were formed.

The prevalence of subclinical ketosis was indicated by the F/P ratio where F/P ≥ 1.5 was taken as indicator of subclinical ketosis prevalence. The subclinical ketosis prevalence was calculated as frequency of cows indicated with risk in total number of cows in regard to lactation stage and parity classes separately for each breed.

The subclinical ketosis was indicated by the F/P ratio and cow daily production (Eicher, 2004) that is the F/P ≥ 1.5 in cows that yielded between 33 to 50 kg/day was taken as indicator of subclinical ketosis. Only cows with detected subclinical disorder were included in further analyses. Milk yield measured on the test day when subclinical ketosis occurred were used as the reference level. The ketosis index was defined regarding the number of days after the subclinical ketosis indication as follows: D – 0= test-day milk yield collected when subclinical ketosis was indicated, A – 1= within 35 days, and A – 2= between 35 and 70 days.

Statistical analysis

The effect of subclinical ketosis on daily milk yield was analysed separately for each breed (Holstein, Simmental) using the following statistical model:

$$y_{ijklm} = \mu + b_1(d_i/305) + b_2(d_i/305)^2 + b_3 \ln(305/d_i) + b_4 \ln^2(305/d_i) + S_j + R_k + K_l + P_m + e_{ijklmn}$$

Where $_{yijklmn}$ = estimated daily milk trait;
μ = intercept;
b_1, b_2, b_3, b_4 = regression coefficients;
d_i = days in milk (i = 6 to 305 day);
Sj = fixed effect of month-year j (j = 1/2004 to 12/2013);
R_k = fixed effect of region k (k = Croatian counties);
K_l = fixed effect of ketosis index l (l = D – 0, A – 1, A – 2);
P_m = fixed effect of parity class m (P1, P2, P3, and P4+);
e_{ijklmn} = residual.

The significance of the differences between the levels of ketosis index was tested by Scheffe's method of multiple comparisons using the MIXED procedure of SAS (SAS Institute Inc., 2000).

Results and Discussion

Due to negative energy balance in the beginning of lactation, milk fat content tends to increase while milk protein content tends to decrease, what makes F/P ratio good indicator for metabolic disorders detection. According to Heuer et al. (1999), the first test-day record (daily milk yield and F/P ratio) are more reliable predictor of disorders, fertility and milk yield than loss of body condition scoring. Therefore, determination of subclinical ketosis (SCK) prevalence based on F/P ratio during the first 2 weeks of lactation could be beneficial (Iwersen et al., 2009). In this analysis, the subclinical ketosis prevalence indicated by the daily F/P ≥ 1.5 was consider in regard to the lactation stage and parity class separately for each breed (Table 1). In first parity Holsteins, the highest prevalence with indication in 35.03% of cows occurred in first 15 day of lactation, after what continuous declining trend was noticed. The prevalence peak, in first parity Simmentals (in 23.89% of cows), occurred in 25th day of lactation, after what, similar like in Holstein breed, continuous declining trend was noticed. Regarding the parity, in both breeds, highest prevalence in the beginning of lactation, occurred in cows in first, then in third parity, with the lowest prevalence in second lactation. Regarding the breed, Holsteins experienced higher subclinical ketosis prevalence compared to Simmentals in all lactation stages and all parities.

Table 1. The ketosis prevalence (%) in accordance to lactation stage (L1, … L16) and parity class (P1, … P4+) by analysed breed (Holstein, Simmental)

Lactation stage	Holstein / Parity class				Simmental / Parity class			
	P1	P2	P3	P4+	P1	P2	P3	P4+
L1	34.72	27.54	30.57	28.01	18.77	14.62	17.63	14.93
L2	35.03	29.28	33.12	31.10	21.49	18.66	19.88	18.15
L3	33.34	28.48	34.48	32.66	23.00	19.85	22.78	20.31
L4	32.14	29.76	34.82	33.71	23.89	22.61	25.29	22.19
L5	30.02	27.87	32.91	32.83	23.68	22.01	23.73	23.12
L6	28.06	27.04	31.73	31.37	22.94	22.15	24.57	21.50
L7	25.93	25.24	29.26	28.55	22.09	21.55	21.84	21.27
L8	23.91	23.35	28.04	27.74	20.45	20.46	22.21	20.95
L9	22.71	21.89	24.80	25.15	19.06	18.37	20.60	18.54
L10	20.44	20.91	24.50	24.69	18.18	17.39	19.45	18.65
L11	19.73	20.28	22.99	22.83	17.52	16.72	18.82	17.95
L12	16.98	17.81	20.46	20.36	15.22	15.09	16.92	15.71
L13	15.41	16.34	18.82	18.35	13.54	13.98	14.96	13.83
L14	13.94	15.33	17.18	18.05	12.83	12.86	13.89	13.57
L15	13.59	14.86	17.71	17.24	12.16	12.24	13.36	13.14
L16	8.24	9.402	10.39	10.42	8.925	8.42	8.80	8.89

The majority of metabolic disorders cases occurs at the beginning of lactation when physiological demands of the cow are high. Mulligan and Doherty (2008), defined the period 3 week before and 3 week after calving, as critical period associated with a peak incidence of metabolic and nutritional disorders or infectious diseases. These disorders and diseases have been generally associated with increased unplanned culling witch lead to financial loses (Fetrow et al., 2006), by increasing diagnosis and treatment costs, decreasing milk yield and reproductive performance in dairy herd (Suthar et al., 2013). Suthar et al. 2013. stated that prevalence of SCK was high between 2 to 15 days in milk. Furthermore, this high prevalence increased the odds of metritis, clinical ketosis, lameness and displaced abomasum in European dairy herds. Koeck et al. 2013., stated that 87% of ketosis cases in Holstein cows was recorded during the first 30 days of lactation. Results of this research confirm that the highest subclinical ketosis prevalence occurred in early lactation (until 15th day of lactation in Holsteins and 25th day in Simmentals) with highest prevalence in first parity cows. Andersson and Emanuelson (1985) and Duffield et al., (1997) determined that levels of ketosis increase with parity, while Carrier et al. (2004) determined more frequent ketosis prevalence in multiparous cows compared with primiparous cows.

The effect of subclinical ketosis on daily milk yield indicated by the F/P ratio and daily milk yield was evaluated for each parity class and cattle breed (Table 2). All variables included in the statistical model showed to be highly significant ($p<0.01$). A significant negative effect of subclinical ketosis on daily milk yield in each parity and both breeds was determined within 35 days after the detection of subclinical ketosis (A-1). The milk reducing effect continued in subsequent milk controls (A-2).

Table 2. The effect of subclinical ketosis on daily milk yield in accordance to parity by analysed breed (Holstein, Simmental)

Ketosis index	Holstein / Parity class				Simmental / Parity class			
	P1	P2	P3	P4+	P1	P2	P3	P4+
D-0	35.10^{A}	35.55^{A}	36.41^{A}	35.15^{A}	35.80^{A}	35.71^{A}	36.12^{A}	35.27^{A}
A-1	31.52^{B}	33.57^{B}	34.44^{B}	31.00^{B}	28.91^{B}	30.99^{B}	30.97^{B}	27.57^{B}
A-2	30.90^{C}	32.71^{C}	33.49^{C}	30.35^{C}	28.10^{B}	29.60^{C}	29.55^{C}	27.02^{C}

*values within the same column marked with different letter differ statistically highly significant ($p < 0.01$)

Toni et al. (2011) found that cows with F/P ratio in interval (< 1; > 2) showed a consistently lower milk production. Furthermore, McArt et al. 2012. determined that cows with subclinical ketosis, compared to non-ketotic cows, in the first 30 days of lactation, produce 3.4 – 6% less milk daily. In accordince to Rajala-Schultz et al. (1999) the average total loss per cow due to ketosis prevalence was over 300 kg/lactation.

Conclusions

The transition period is the most critical period for high producing cows associated with a peak metabolic disorders prevalence. Generally, metabolic disorders prevalence has been associated with high financial losses due to increased treatment costs, decreased milk production, impaired reproduction efficiency, and increased involuntary culling. The results of this research indicate highly significant decline of daily milk yield as a result of subclinical ketosis detected based on fat/protein ratio and daily milk yield. This indicate that F/P ratio could be good indicator for subclinical ketosis prevalence, while F/P ratio associated with daily milk yield could be accurate indicator for subclinical ketosis detection. Finally, test-day records could be used as cost effective and non-invasive method for monitoring the herd health enabling the farmer early reaction and prevention of development of strong clinical symptoms.

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PRELIMINARY DATA ON THE INFLUENCE OF TEMPERATURE ON THE MYOGENIC GROWTH FACTORS AND THEIR IDENTIFICATION IN KOI CARP *(CYPRINUS CARPIO HAEMATOPTERUS)*

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Abstract: We show the variations due to the influence of temperature on the growth and myogenic growth factors in koi carp (Cyprinus carpio haematopterus). The study was performed on samples from 24 different fish which were separated into two groups, large and small fish, raised in three different temperatures (5, 25 and 30^{0}C). Protein profiles, RNA/DNA ratios and the relative transcription of genes in white muscles were estimated. Protein profiling using SDS-PAGE showed concentration differences in proteins located at the same zone in the gels, present in some of the large fish raised at 5^{0}C and 25^{0}C, which points to the influence of the fish size and temperature on the muscle protein profile of the koi carp. These types of variations were not recognized in samples from fish raised at 3^{0}C. The average values for the R/D ratio in large fish adapted to 25C was the highest (P = 0.027). The third aspect of this study was to identify some genes that are involved in the development of fish muscles. Reverse transcription of isolated mRNA from white muscles and amplification of cDNA with in house designed primers were done. We were discovering the activity of twelve genes that are involved in muscle growth as follow: cystathionine-□-synthase, myogenin, □-homocysteine-methyl-transferase, myoD1, methionine-synthase, methionine-tetra hydrofolate reductase, myf6, myf5, myoD2 and myostatin. As a marker gene for technique accuracy was used leptin. In the white muscle samples of the small fish we identified the activity of myogenin, myf6, MyoD2 and myostatin genes, while large fish we discovered the activity of myogenin, myf6, MyoD2 and myf5 genes.

Keywords: fish, muscle, growth, genes, proteins, RNA, DNA

CORRELATIONS OF SOME BIOCHEMICAL AND HEMATOLOGICAL, PARAMETERS WITH POLYMORPHISMS IN AS1-CASEIN AND B-LACTOGLOBULIN GENES IN ROMANOV SHEEP BREED

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Abstract: Alfa S1-casein (aS1-CAS) and beta lactoglobulin (β-LGB) genes are common marker genes in sheep breeding. The aim of this study is to investigate the association of the polymorphisms in mentioned marker genes with the appropriate biochemical and hematological parameters in Romanov sheep breed in Siberia. Blood samples from fifty unrelated females and males (20+20) were included in the research. The genotyping of aS1-CAS and β-LGB was performed with PCR – RFLP technique using Rsa I and Mbo II enzymes, respectively. aS1-CAS) gene was almost uniformly present in estimated samples which showed 0.98 frequency of non A allele. β-LGB gene showed high level of variability among the estimated samples of Romanov sheep where the frequency of allele A was 0.62, while the frequency of allele B was 0.38. The genotypes for β-LGB are present in the following frequencies: AA – 0.490, AB – 0.255 and BB 0.255, respectively. The results of genotyping were correlated with the following biochemical parameters: total proteins, albumins, globulins, urea, uric acid, alkaline phosphatase, cholesterol, LDL, HDL, triglycerides, glucose, amylase, LDH, AAT, lipase, creatinine, Ca, P, Ca/P, Cl, Mg, K and extracted DNA, and listed hematological parameters: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, MPV, PDW and PCT. Concerning the correlations, Spearman coefficient of correlation was calculated and it was suitable for the both variants linear and nonlinear relationship between parameters. The significant ones from biochemical parameters are: albumin, LDL, glucose, Ca, Mg and especially amylase and urea. In terms of hematological parameters, the significant ones for the marker genes are: hemoglobin, MCV, MCH, and especially for PDW and PCT. The idea is that correlation profile is unique inside the groups of animals with different genotypes. It means that metabolism is not completely the same in animals with different genotypes.

Keywords: biochemistry, hematology, aS1-casein, β-lactoglobulin, correlation

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THE HEAT STRESS IN GOAT IN ANDALUSIAN GOATS: ADVANCES IN THE KNOWLEDGE OF THE GENETICS BASIS (A BRIEF REVIEW)

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Abstract: Andalusia (South of Spain) is one region in which there are different indigenous goat breeds perfectly adapted to the environment. The heat stress was studied in the Murciano-Granadina, Payoya and Florida breeds because their distribution is in dried parts of Andalusía with a wide variation in temperature and humidity. Three phenotypes related with heat stress were detected in goats: sensible, robust and resistant, showing a genotype-environment interaction. There were identified some genes related to the resistance to the heat stress using «beadchips» with 55,000 SNPs: the casein kappa gene (CSN3), the acetyl-coenzyme A carboxylase alpha gene (ACACA), the malic enzyme I gene (MEI) and genes from the HSP family such as HSPI («heat shock factor binding protein I») responsible for inhibiting the function of the caloric stress protein «heat shock» 27 (HSP27). It was observed also a negative correlation between heat stress resistance and some of the selection criteria currently used such as milk production and cheese extract. This antogonism obliges us to find new methodologies applicable to the breeding genetic program of these breeds that allows to improve the resistance to heat stress without affecting the production levels. These new strategies go in the way of the determination of biomarkers in milk and blood detectable with other methodologies such as the use of analysis of middle infrared spectra or transcriptomics.

Keywords: heat stress, genetics, goat

Introduction

Climate change has triggered all the alarms in animal production and can significantly affect those regions where goats are produced and which are already particularly heated. In Spain, particularly in Andalusia, goats are found almost 65% of the days of lactation outside a comfortable environment in relation to the average temperature. The high temperatures produce a stress on the goats that makes their milk production decrease and therefore cause economic losses not to be ignored. In Spain the concern about the increase of CO2 in the atmosphere, already highlighted by the European Environment Agency (EEA, 2008) and other international organizations such as the World Health Organization (WHO) is increasing every day. These organisations have concluded that together with periods of intense rain we will experience an increase in drought in our continent (with temperature increases between 1 and 5.5ºC) particularly in the southern and southeastern areas. In these areas it is estimated that the reduction of humidity in the soil will be around 20% which clearly highlights their vulnerability. In this sense, several papers have been published evaluating these increases in Spain. De Castro et al. (2005) calculated that in Andalusia rainfall decrease could lead to a decrease of more than 20% of water supply in the swamps of the Guadalquivir basin. The change will therefore affect both intensive and extensive production systems, increasing costs in the former and decreasing resources, response capacity and the increase in diseases and pests in the latter. Therefore, the probability that the animals in these areas are subjected to heat stress

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(heat stress), i.e. that the animals live above their thermal comfort threshold, is very high and, as a consequence, production will be seriously diminished if measures are not taken to alleviate these situations such as the implementation of a new diet or the modification of their environment through the use of forced ventilation, with the consequent cost. But there is also another strategy consisting of detecting the genetic variability of animals for sensitivity/resistance to heat stress in the different breeds of production animals, particularly in the domestic goat (Capra hircus, L).

Aims

In Andalusia (South of Spain) there are different indigenous goat breeds perfectly adapted to the environment. The breeds in which our group of research is carrying out studies about breeding genetic of resistant to heat stress are Murciano-Granadina, Payoya and Florida. The main objective of this brief review is to review our experience in the study of the effect of heat stress on milk production, the reaction to stress and its genetic basis.

Heat stress and its effects on milk production

In 2010 Bernabucci et al. observed that in animals the heat stress originates when the total caloric charge, i.e. the sum of the generated internal heat and the environment, exceeded the dissipation capacity in them. In the USA it was observed in cattle that this excess negatively affects milk production in different breeds (St-Pierre, 2003), estimating this loss between 68 and 2072 kg/cow/year in the states of Wyoming and Louisiana respectively. In other ruminant species the studies carried out are scarce. However, it is generally known that goats are more heat tolerant than cattle due to their morphology and physiology at the time of heat dissipation (Bernabucci et al. 2010) although it has also been observed that heat reduces the productivity of this species (Silanikove, 2000). In studies carried out by our research group it has been observed that in the Andalusian breeds studied (Murciano-Granadina, Payoya, Florida and Malagueña), breeds located in areas of high thermal stress (temperature and humidity) during a large part of the year, the annual production of fat plus proteins has been reduced between 1.9 and 3.1% in the first two (Menéndez-Buxadera et al. 2012) and of 1% in fat and 0.9% in protein in the Florida breed (Carabaño et al. 2013).

Heat stress responses

The first response we can expect is a decrease in feeding. Obviously this response along with the need for more energy to dissipate heat can cause a state of lack of food and a negative energy balance with a clear influence on production and reproductive yields and of course on the healthy state of animals. These negative effects have been observed repeatedly in cattle (Rhoads et al, 2010; Wheelock edt al. 2010). In goats it is understood that being more resistant than other ruminants due to its smaller metabolic size, i.e. greater surface area in relation to its weight and its greater capacity to retain water, the effects observed have not been so remarkable. This fact could be observed in an experience in the Murciano-Granadina breed (Hamzaoui et al, 2013) in which a group was subjected to extreme conditions of temperature and humidity (37ºC during the day and 30.5ºC at night), decreasing its feeding by 40% initially, recovering later and reducing this percentage to only 14% in relation to goats in thermoneutral conditions. The results showed that the concentration

of protein was only reduced by 12.5% and fat by 12%. The results concluded that these animals suffered thermal stress that led to physiological changes that were later annulled in a process of readaptation to environmental conditions.

Genetic bases of the reactions to heat stress

In the studied goat breeds it was possible to identify animals with three types of responses to thermic stress: animals sensitive to the increase of temperature and humidity with a decrease of their genetic value; animals called robust that maintained it in spite of the conditions and tolerant animals that increased their genetic value when the temperature increased. This different response demonstrates that there is a clear genotype-environment interaction. In recent years a genotyping study of all animals has been carried out using the so-called «beadchips» containing thousands of single nucleotide polymorphic markers (SNPs) with the aim of analysing the correlation between the complete genome (Genome-wide association study, GWAS) and characteristics of economic interest and introducing genomic selection. In our case, up to 55,000 SNPs are used. The aim is to detect if there is any genomic region whose gene variants are associated and/or originate the variability observed in the phenotypes. In the Florida goat breed, several SNPs have been associated with greater or lesser heat stress. Some of them are related to milk production (Zidi et al, 2014), as the casein kappa gene (CSN3), the acetyl-coenzyme A carboxylase alpha gene (ACACA), the malic enzyme I gene (MEI) and genes from the HSP family such as HSPI («heat shock factor binding protein I») responsible for inhibiting the function of the heat stress protein «heat shock» 27 (HSP27) (Xi et al, 2007). In the Murciano-Granadina and Payoya goat breeds we are currently validating these results, not having to be identical to those observed in the Florida breed, because the action of these genes does not have to be the same action in all breeds, since it depends on multiple factors such as the structure and genetic composition of the populations, the possible existence of different gene linkages in the different breeds, etc. On the other hand the application of this methodology to the breeding genetic in goats has produced some first results that demonstrate a negative correlation between resistance to heat stress and some of the selection criteria currently used as milk production and cheese extract. This antagonism greatly complicates the use of this methodology (SNPs) because there is a risk that the breeding towards milk production will be stopped by this antagonism between the two selection criteria. It is therefore necessary to design an index that attempts to integrate these two criteria without prejudice to either of them.

Animal genetic selection has undergone an extraordinary development in recent years with the incorporation of genomic information into the genetic evaluations of animals, i.e. the so-called genomic selection has been initiated. Our group is implementing it in goats (Molina et al., 2018). In the case of heat stress, this type of selection can separate the productive from the non-productive component of heat tolerance. For this purpose, the latest generation of transcriptomics technology (RNA sequencing) is used to detect genes or genomic regions responsible for heat stress tolerance. In addition, the development of methodologies that highlight the physiological consequences of heat stress, translated into changes in the levels of certain substances (biomarkers) in both blood and milk (Tian et al., 2016), and that can be measured, for example, through analysis of medium infrared spectra (MIR), can jointly contribute to finding tools that allow to search ways to improve resistance to heat stress without damaging production levels.

Conclusions:

The Murciano-Granadina, Payoya and Florida goat breeds analysed have a natural resistance to heat stress and are perfectly adapted to the Andalusian environment. The work carried out on these breeds using SNPs markers (simple nucleotide polymorphism) has confirmed the relationship with heat stress and genes related with the HSP family (heat shock factor) as well as with casein kappa (CSN3), acetyl-coenzyme A carboxylase alpha (ACACA) and the malic enzyme I (MEI) in the Florida breed. The other breeds are currently being analyzed in this moment. Antaganism has been again detected between selection by resistance to heat stress and production characteristics established in the current selection programs (milk production and cheese extract). So, it is essential to design a new index that attempts to integrate the two criteria to avoid this antagonism.

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INFLUENCE OF MATING SEASON AND NATURAL GONADOTROPIC HORMONE APPLICATION IN ANESTRUS SEASON ON SHEEP FERTILITY AND LAMB BODY WEIGHT AT BIRTH

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Abstract: Sheep fertility results depending on mating season, hormonal estrus induction and synchronization method in anestrus season, as well as their influence on lamb body weight at birth for improved Sjenička Pramenka breed, are shown in this research work.

The experiment was performed on 282 ewes on two farms. On first farm there were 112 ewes and they were mated in the season without hormonal treatment. During anestrus period hormonal treatment was performed on 170 ewes with aim to synchronize and induce estrus (second farm). Sheep received polyurethane sponges impregnated with 60 mg medroxyprogesterone acetate (MAP). After 12 days sponges were removed and each sheep received 500 IU PMSG. Two days after applying PMSG according to previously set plan sheep were mated.

On the second farm where hormonal treatment was applied fertility was higher by 39.84%, and that can be explained with the fact that sheep were treated with natural gonadotropic hormone gave birth to higher number of twins, which was in one group higher by 18%, and lower number of singles by 38%. In first group there were 75% singles and 25% twins, while in second group 37% of singles were born, 43% of twins, 17 % of triplets and 3% of quadruplets were born.

Average lamb body weight at birth on the first farm was 3.85 kg, and on the second farm 3.37 kg, therefore, determined difference between farms was minimal and statistically insignificant ($P<0.05$). Difference in body weight for lambs at birth depending on birth type singles-twins for both farms was existent and statistically very significant ($P>0.001$).

Research has shown that satisfying results were achieved during estrus synchronization in anestrus season compared to normal mating season when no hormonal therapy was applied for fully grown improved Sjenička Pramenka sheep.

Keywords: improved Sjenička Pramenka, mating season, medroxyprogesterone acetate (MAP), PMSG, sheep fertility, lamb body weight at birth.

Introduction

Sheep have specific reproductive rhythm. Mating season starts end of summer and lasts until the end of autumn. Mating season is tightly connected to the length of photoperiod (Mekić et al., 2012). Melatonin secretion, which is produced by pineal gland, increases as the nights become longer. Increased melatonin secretion in sheep body leads to activation of hypothalamus – hypophysis – ovaria axis which sets up and maintains sexual activity. Depending of breed and geographic location, anestrus period varies from 3 to 8 months (Stojković et al., 1998).

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Complex neuro – endocrine activity in sheep body during estrus period is under immediate influence of photoperiod length during the year, which is manifested through mating season (estrus) and non-mating season (anestrus) (Nenadić et al., 1993).

Modern scientific approach enables control over sheep reproduction process with more or less success. Most intensive sheep mating activity is during October and November when fertility is at its peak (Mekić et al., 2011).

Estrus can appear naturally even out of mating season only in very small number of sheep breeds, which means that fertility intensity is very small and lamb production as well as mutton production is exclusively seasonal. (Mekić et al., 2009).

Today there are several ways to induce out of season mating and lambing, among which most efficient are: hormonal method, ram effect, flashing method and artificial control of daylight photoperiod (Mekić and Stojković, 2002).

Widely applied and with best results are treatments with synthetic progestogens and natural gonadotropins. Ovulation and estrus control can be performed throughout the year. During mating season progestogen which is given to the sheep acts as artificial corpus luteum and blocks follicle maturing. For sheep in anestrus period it could be induced ovulation via hypothalamus – hypophysis – ovaria axis and normal luteinization after sponge removal. Application of natural gonadotropic hormones in the end of progestogen treatment intensifies estrus, increases ovulation percent and induces faster synchronization of all ovulations which leads to higher fertility.

Hormonally synchronized sheep give significantly higher number of lambs and have shorter service period than sheep which are in natural cycle. This has positive effect to total number of lambs during the year.

Numerous researches have shown significant reduction in fertility for synchronized sheep in first estrus after synchronization, no matter if it was performed in our out of season. In the season this flaw is rectified with spontaneous estrus appearance in next cycles. After synchronized estrus out of mating season, small number of sheep achieves estrus spontaneously.

Using synthetic hormones such are progestogen PMSG, GnRH stimulates the hypothalamus – hypophysis – gonads connection (ovarium), with aim to activate reproductive process out of mating season. (Intervet, 2002; Miller et al., 1998; Ozbej and Tatli, 2001).

Aim of this research is wish to contribute to enlightening of differences in sexual reactions of sheep mated in the season (without applied hormone therapy) and sheep who were treated with hormone therapy, induced synchronization and estrus in anestrus season, therefore, to see their influence on lamb body weight at birth.

Materials and Methods

Research was performed on two sheep farms near Valjevo. Experiment included 282 Sjenička Pramenka ewes. First group of 112 sheep was mated in the season without hormonal therapy, while second group of 170 sheep were treated with hormonal therapy out of mating season.

All sheep from second group were treated with progestogen plus PMSG. Induction and estrus synchronization was performed in the anestrus season using polyurethane sponges soaked with 60mg of medroxyprogesterone acetate (MAP) which stayed inside of sheep vagina for 12 days.

After 12 days sponges were removed and each ewe was injected with 500IU of PMSG. After 48h after application of gonadotropic hormone sheep were mated according to previously set order. Mating was performed in controlled environment, in the single boxes.

Obtained experiment results were processed using standard statistical analysis. Comparison of significance between arithmetic means of two treatments was done using T-test. Differences between groups and birth type of newborn lambs were determined using variance analysis and LSD test.

Results and Discussion

Reproductive parameters: reproduction represents most important limiting factor in production increase and animal husbandry production profitability increase as a whole. In general practice with estrus control using hormonal treatments sheep fertility can be significantly increased, lambing can be planned and early fertilization has positive influence on increase of productive life per sheep.

From the data given in table 1 it can be seen that for first group of 112 sheep 128 lambs were born, while in second group out of 170 sheep 262 lambs were born.

Table 1. Sheep reproductive parameters depending on season and hormonal therapy.

No	Parameters	Treatment				Total
		I		II		
	Number of sheep that had lambs	112		170		282
	Total number of lambs	128		262		390
	Birth type: Singles	96	75%	97	37%	193
	Twins	32	25%	112	43%	144
	Triplets	-	-	45	17%	45
	Quadruplets	-	-	8	3%	8
	Sheep fertility, %	114.28%		154.12%		

Second group of sheep had higher fertility compared to the first group by 39.84%. Higher sheep fertility of second group can be explained by the fact that second group of sheep had higher number of twins born by 18.00%. In second group 17.00% of triplets and 3.00% quadruplets were born, while in first group there were no triplets and quadruplets.

Considering presented data it could be concluded that during estrus induction and synchronization use hormonal therapy and 500IU of PMSG dose in anestrus season lead to increase of the number of ovulated egg cells on ovaries, which resulted in birth of higher number of lambs per sheep by 39.84% compared to sheep that were seasonally mated without hormonal therapy.

When hormonal therapy is used next to the fertilization level it is important to obtain as higher as possible number of twins. Twins are important because it is very hard for sheep to bring up more than two lambs. Ratio between singles, twins, triplets and quadruplets for second group was 37:43:17:3%, while for first group there were no triplets and quadruplets and ratio between twins and singles was 25:75%. Therefore, in the first group there were more singles by 38% compared to the second group, at the same time second group had more twins by 18% compared to the first group (table 1).

Lamb body weight at birth

Data for average lamb body weight at birth were analyzed compared to fertilization season and type of birth, results are shown in table 2.

Table 2. Average values and variability of lamb body weight at birth, kg

Farm	Birth type	Parameters					Variations	
		n	$\overline{x}$	$S_{\overline{x}}$	Sd	CV(%)	Min.	Max.
I	Singles	96	4.32	0.04	0.44	10.19	3.30	5.30
	Twins	32	3.39	0.05	0.29	8.55	2.70	4.40
	S + Twl.	128	3.85	0.05	0.58	15.06	2.70	5.30
II	Singles	97	4.49	0.05	0.52	11.58	3.50	5.50
	Twins	112	3.59	0.04	0.42	11.70	2.40	4.50
	Triplets	45	2.83	0.05	0.32	11.31	2.20	3.40
	Quadruplets	8	2.59	0.12	0.33	12.74	2.20	3.00
	S+Twl.+Tr+Q	262	3.37	0.05	0.77	22.85	2.20	5.50

From the data in table 2 we can conclude that determined difference in body weight at birth between singles at farm 2 and twins from farm 1 of 1.10 kg (32.45%) was statistically very significant (P>0.001), while difference between singles at farm 2 was higher than singles at farm 1 by 0.17 kg (3.79%) however, that difference was not statistically justified (P<0.05). Determined differences between singles in group one and singles in group two of 0.93 kg (21.53%) and singles from group one and twins from group two of 0.73 kg (16.90%) were statistically very significant (P>0.001).

Birth type of newborn lambs was also significant. At the first farm twins had lower body weight at birth compared to singles by 0.93 kg (21.53%), while that difference at second warm was 0.90 kg (20.04%) in the favor of singles. Mentioned differences between birth types at both farms in favor of singles were statistically very significant (P>0.001).

From the obtained data it could be concluded that body weight of lambs at birth were within the expected limits, that lambs at second farm had slightly higher body weight compared to lambs at first farm, however reasons for those differences should be searched in breeding conditions.

If we compare results of this research with research by Mekić et al., (2017) for same genotype, where determined fertility for synchronized sheep in anestrus period was 184.90% we can conclude that fertility was higher than in new research, most probable reason for those results is the sheep physical condition in different research years.

In the research by Stojković et al., (1993) for Ile-de-France breed after treatment with FGA average fertility was 161.06%. Higher fertility for Ile-de-France breed of 177.42% was determined by Mekić et al., 2003., after treatment with FGA + MMSG. For improved Sjenička Pramenka treated with FGA+500IU of PMSG determined fertility was 122.83%, and for ewes treated with FGA+750IU PMSG fertility was 146.00% (Mekić et al., 2012). It is confirmed in research by Mutavelić et al., (1989) that higher dose of PMSG, (sheep treated with 750IU of PMSG compared to control group treated with 500IU of PMSG), has beneficial influence on sheep fertility increase. Mekić et al., (2004) treated Sjenička Pramenka sheep with FGA + 1000IU of PMSG and achieved fertility of 192% which is significantly better result compared to our present results.

Fertility rate depends on numerous factors such are: genetics, seasonal changes, age, environment conditions, diet, illnesses, female hormonal status, sperm quality, hormonal dose (Webb et al., 1994; Levis et al., 1996; Beck et al., 1996; Husein et al., 1996 and 1998.; Yavuzer, 2005; Mekić et al., 2012).

This research has shown that estrus induction and synchronization can be successfully performed using hormonal method in anestrus season for the sheep of improved Sjenička Pramenka. Beside to estrus induction out of mating season, for the sheep that were hormonally treated higher

fertility of 39.84% was achieved compared to sheep that were mated in the season without using hormonal therapy.

Conclusion

Based on conducted research on estrus induction and synchronization for improved Sjenička Pramenka sheep using polyurethane sponges soaked in 60mg of medroxyprogesterone acetate (MAP) and 500IU of gonadotropic hormone PMSG in anestrus period on sheep sexual reactions and lamb body weight at birth compared to same parameters for sheep that were mated in the season without hormonal therapy, following conclusions can be made:

1. Average sheep fertility for sheep that were induced and synchronized was 154.12%, while for seasonally mated sheep without hormonal therapy it was 114.28%. In second group of sheep higher fertility by 39.84% was achieved compared to first group.
2. Above mentioned difference in fertility of 39.84% can be explained with the fact that for second group of sheep 18.00% more of twins were born.
3. In the second group of sheep 17% of triplets and 3% of quadruplets were born while in first group there were no triplets or quadruplets.
4. Determined differences in lamb body weight at birth between groups were existent, however they were not statistically significant ($P<0.05$).
5. Singles at first farm and at second farm had higher body weight compared to twins on both farms, determined differences were statistically very significant ($P>0.001$).

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EFFECT OF CRYOPRESERVATION ON SPERM PARAMETERS OF RAMS FROM LACAUNE BREED

Andreeva M.*[1], Metodiev N.[2], Stefanov R.[1]

Abstract: The aim of the study was to determine the sustainability of the ejaculates of rams from Lacaune breed by examining the effect of low temperatures on sperm parameters before and after cryopreservation.The experiment was carried out with seven 2-year-old rams from Lacaune breed, placed under the same conditions of growing, feeding and breeding use. From each ram we received 2 consecutive ejaculates. The received ejaculates were examined by computer assisted sperm analyzer (CASA) in terms of changes in kinetic parameters such as VCL, VSL and VAP. After the sperm assessment, the ejaculates were frozen by Cassu's pasay technology, and after thawing the samples were analyzed for the same parameters by CASA. After freezing and subsequent thawing, the amount of sperm with progressive movement is greatly reduced (from 323.36 to 35.5 mill/ml) on the contrary of the amount of non-progressive moving (from 2970.86 to 1443.57 mill/ml) and especially of the static sperm (from 6.71 to 1796.92 mill/ml). These processes also lead to a decrease in VCL (from 102.44 to 21.57 μm / s), VSL (from 27.83 to 10.48 μm / s), VAP (53.99 to 14.36 μm /s). Lacaune rams are not susceptible to long term freezing due to the damaging effect of low temperatures on their sperm cells.

Keywords: ram, spermatozoa, cryopreservation, sperm parameters

Introduction

Artificial insemination is a widespread procedure in breeding management of many farm animals. The great benefits of its application (economic, health, genetic, etc.) determine the need for fundamental and applied research on various factors and parameters that determine the quality of the mature adult genes acquired and the magnitude of this procedure as a whole.

The main indicators of sperm are very variable and depend on different factors. Robayo et al. (2008), found that cryopreservation can significantly affect the quality of frozen and thawed sperm by reducing the characteristics of sperm parameters, such as mean velocity (VAP), linear velocity (VCL) and straight-line velocity (VSL). The presence of certain additives in the reductions of glutathione (GSH) and catalase (CAT) influences the kinematics and membrane functionality of the semen during the storage of liquids, cooled to 5°C for more than 24 hours (Boland et al., 1985). Other authors established a correlation between the kinematic parameters of ram sperm and their migratory efficacy in the cervix (Abadjieva et al., 2014). Continuous linear velocity, VAP, rectilinear velocity, and linearity are mutually related to migratory efficacy in the sheep's cervix. Seed fluid incubation may also have an effect on its kinematics and acrosomatic integrity in ram semen (Kistanova et al., 2007). There is a significant effect of incubation on the mobility characteristics and the number of sperm with normal acrosome. However, they did not detect a significant change in the

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rate of linearity, curvilinear velocity, mean passage, lateral displacement of the head, and incidence of tail sperm strokes during incubation. Different dilution media (Bag et al., 2004; Gil et al., 2003; Gholami et al., 2012; Singh et al., 2013; Soltanpour and Moghaddam, 2013; Mostafapor and Ardebili, 2014; Jiménez-Rabadán et al., 2015) also affect the characteristics of the sperm.

The aim of the study was to determine the sustainability of the ejaculates by examining the effect of low temperatures on their sperm parameters (motility, concentration, kinematics) before and after cryopreservation.

Materials and Methods

The experiment was carried out with seven 2-year-old rams from Lacaune breed, placed under the same conditions of growing, feeding and breeding use. The animals had normal sexual performance, aged 2 years and were fed with meadow hay ad libitum, carrots (1 kg per ram per day) and concentrated mix (bought from forage company with 12% crude protein) in dose 1000g per ram per day. From each ram we received 2 consecutive ejaculates.

SPERM ANALYSIS. The sperm analysis was performed in a specialized laboratory of the Institute of Biology and Immunology of Reproduction. The assessment of semen quality parameters and various kinematic parameters of motile spermatozoa were carried out by Sperm Class Analyzer (SCA, Microptic, Spain). The extended semen (diluted with colloid diluent 6AG) was loaded into a Leja 20 chambers (Leja Products B.V., Nieuw-Vennep, The Netherlands) and examined using a microscope with warmed stage (Nikon, Tokyo, Japan). The analyses of sperm motility patterns were performed with SCA operating system. In addition to the overall percentage of motile spermatozoa, the software of SCA also measured the concentration and velocity of movement.

The following velocity values were recorded:

- the curvilinear velocity (VCL, mm/s - the average path velocity of the sperm head along its actual trajectory);
- the straight-line velocity (VSL, mm/s - the average path velocity of the sperm head along a straight line from its first to its last position);
- the average path velocity (VAP, mm/s - the average velocity of the sperm head along its average trajectory);

TECHNIQUES FOR CRYOPRESERVATION OF SPERMATOZOA. The cryopreservation was performed at the Institute of Biology and Reproduction Immunology. Cryopreservation of semen straws was done by the method of Cassou 1964.. After assessment, the ejaculates normozoospermia were diluted with colloid diluent (6AG), containing cryoprotectant glycerine and then equilibrated. After equilibration the semen were divided in plastic straws (tubes, 133mm long, 2mm in diameter and volume of 0, 25 cm3). One end of the straw was closed with powder of polyvinyl alcohol, placed between two layers cotton-paper filter, and the other end was sealed. The cryopreservation of the straws was conducted on the vapor of nitrogen, and after that they were placed in containers with liquid nitrogen (-196°C) for long term preservation. Before their usage, the straws were thawed by pulling them off the container with liquid nitrogen and placing them directly in horizontal position on water bath, with temperature of + 34°C.

STATISTICAL ANALYSIS**.** All data were calculated by statistical program IBM SPSS 19. The differences of the values of motility of the spermatozoids (static, non-progressive and progressive) and their kinematic parameters were compared before and after thawing. The values of the studied

parameters were compared with Paired T-test. The significance of the differences between groups was evaluated by t-criterion of Student.

Results and Discussion

Figure 1 shows the sperm motility expressed in millions per ml. The different motility of the spermatozoids was compared (static, non-progressive and progressive) before freezing and after thawing.

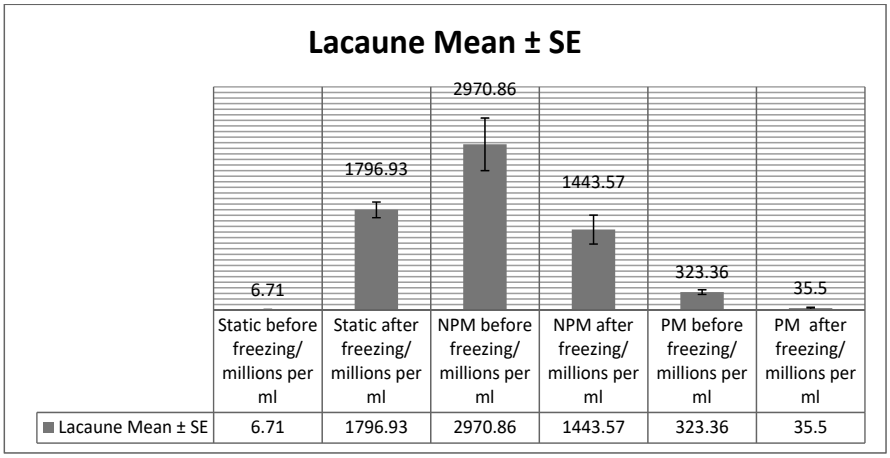

Fig. 1 Number of static, nonprogresive and progresive moving spermatozoa before freezing and after thawing for the Lacaune breed.

From the graphical values, it is seen that the number of static sperm is higher after defrosting, and the number of progressive and non-progressive moving sharply decreases after thawing. After freezing and subsequent thawing, the amount of sperm with progressive movement are redusing from 323.36 to 35.5 mill/ml. Greatly reduced and the amount of non-progressive moving from 2970.86 to 1443.57 mill/ml) and especially static from 6.71 to 1796.92 mill/ml.

Figure 2 show the values of VSL, VCL and VAP the before freezing and after thawing of Lacaune sheep.

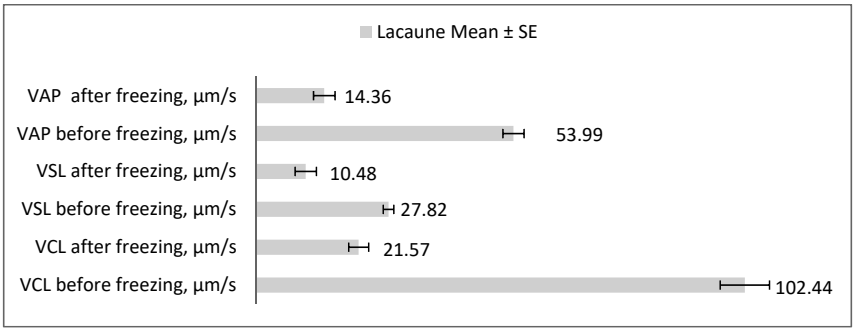

Fig. 2 Values of VAP, VSL and VCL before freezing and after thawing of Lacaune breed.

From the values in the graph, it appears that after freezing the values of VAP, VSL and VCL are significantly reduced for instance decrease in VCL from 102.44 to 21.57 μm / s, VSL from 27.83 to 10.48 μm / s, VAP 53.99 to 14.36 μm / s.

Regarding the motility indicator in Lacaune breed, it is observed that after thawing of the ejaculates there is a decrease in the quantity of progressively moving sperm cells. At the same time, highly increase in static and non-progressive moving sperm cells was observed in this breed after thawing. This shows that the sperm of Lakon breeds suffer much by the effects of low temperatures.

Motility is considered to be one of the most important characteristics of sperm assessment and depends of many factors. Some authors find that in the same breeders the ejaculates obtained during the breeding and at the end of the breeding season shows different data regarding motility. (Mostafapor and Ardebili, 2014) The reason for this according to these and other authors (Perumal et al., 2014) is the different activity level of mitochondrial function of the male gametes. The dependence between mitochondrial activity in bioenergy and motility of human sperm is also investigated and by Piomboni et al.2012..

Similar to our results, Kubovičová and colleagues were also received. When examining the VAP, VSL, VCL and VCL speed parameters of the Lacaune breed, they also found a high reduction in speed parameters.

Lacaune rams are not susceptible to long term freezing due to the damaging effect of low temperatures on their sperm cells.

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EXPOSURE OF BROWN HARE (*LEPUS EUROPAEUS P.*) POPULATION TO HARMFUL EFFECT OF LEAD (PB) AND CADMIUM (CD) IN THE FOOD CHAIN DUE TO ANTHROPOGENIC FACTORS

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Abstract: Numerous anthropogenic impacts result in the reduction of some species of game, which inhabit different areas with intensive agricultural production, i.e. agrobiotope. One of these effects is pollution of the environment by heavy metals, i.e. lead and cadmium. An animal species that can be a good bio-monitor in order to define the presence of these heavy metals in the food chain is the hare *Lepus europaeus P*. This paper compares the results of several authors on the presence of heavy metals in the liver in order to determine the significance of anthropogenic effects on the hare. The paper describes the conditions in the environments where hares were exposed to intensive agricultural production, industrial pollution and pollution caused as a result of ore mining. According to the results shown, high concentrations of lead and cadmium can have detrimental effects on the reproductive performance and the number of hare according to (Halecki et al., 2017), especially when it comes to the proximity of the ore exploitation site. From the aspect of industrial pollution, higher levels of presence of heavy metals are evident, but the effects are also dependent on other factors that are not anthropogenic, such as the natural presence of heavy metals in the soil.

Keywords: brown hare, lead, cadmium, anthropogenic factors

Introduction

The hare *Lepus europaeus P*. as a game of steppe plains, is of a high level of distribution especially in Europe. Many natural influences have negative effects on the population of this game, such as: climate factors (Beuković et al., 2013a; Awadi et al., 2018), parasites and diseases (Kornas et al., 2014; Chiar et al., 2016), as well as the increasing number of numerous predators (Chiar et al., 2016; Humel et al., 2017). When it comes to anthropogenic effects, their impact is increasingly important for the populations of hare (Marbotin et al., 1998; Arvil et al., 2014). Anthropogenic factors influence the availability of food (Popović et al.), which is most often associated with loss of natural habitat (Batáry et al., 2011; Pavliska et al., 2018), and intensive agricultural production in terms of increasing arable areas (Smith et al ., 2005), as well as more and more frequent traffic (Heigl et al., 2016). Therefore, measures of sustainable management of hare in the hunting ground (Beuković et al., 2013b, Popović et al., 2014) become very important for the population of hare in agrobiotope. A hare, as a game that has quite well adapted to agrobiotope, can serve as a good bio monitor to detect this bad anthropogenic effect (Beuković et al., 2015). The hare is not migratory species, and therefore it can be a good biomonitor, because if they have enough food they stay where they were born. They also have a fast reproductive cycle that makes them extremely plastic in terms of both natural and anthropogenic influences.

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Sources of contamination

High doses of lead and cadmium may have a lethal and sub-lethal outcome (Iwegbue, 2008; Danieli et al., 2012), regardless of the mechanism of anthropogenic contamination. In the natural environment, cadmium is in the ground, while lead accumulates on the surface of the soil. From the soil, they easily cross into the plant and then into the chain of herbivore species (Kastori, 1995). Cadmium is positioned most often in the above-ground parts of plants, and the level of cadmium in the plant is in high correlation with the level in the soil (Petrović and Kastori, 1994).

In an agrobiotope, if there are no industrial sources of pollution or exploitation of ore, the use of phosphorus fertilizers in intensive agricultural production can be one of the ways of soil contamination. The presence of heavy metals depends to a great extent on the origin of the crude phosphate (Kastori, 1995; Lin et al., 2010). Relatively high concentrations of cadmium ions are found in the raw phosphates from which phosphorus fertilizers are produced, because the phosphate ores contain from 1 to 110 ppm of it (Ubavić et al., 1993).

Determined levels of lead and cadmium in brown hare due to various anthropogenic effects

Determining the presence of lead and cadmium in liver of hare (n 150) in the hunting grounds of Vojvodina, where the habitat of hare is agrobiotope in the presence of intensive agricultural production, Beuković et al., (2015) have found average levels of 165.6 ppb (Pb) and 231.2 (Cd).

The authors mentioned that the values ranged from 0 (without detection) to the maximum value for Pb 900 ppb and Cd 957 ppb. The authors compared the obtained values with the maximum allowed values of 500 ppb defined by the EC (2001) and by MAFW RS (2014). It was found that only in two hunting areas there was established high liver contamination with lead, while observing the entire population in 5% of hare, the value is higher than 500 ppb (Beuković et al., 2014). Analyzing the presence of cadmium in the liver of the hare, Beukovic et al (2016) state that higher contamination was observed in relation to lead, pointing out that in one hunting area 25% of the analyzed population had more than 500 ppb values. In a study by Demirbas et al. (2017), analyses of liver and kidney muscle samples, fifteen adult hare during the hunting season 2013/14 was performed in Kırıkkale, Turkey. In addition to intensive agricultural production, the mentioned area also has industrial complexes in the area where sampling was carried out. The authors state that in 5 of 15 individuals in the liver, and in all 15 individuals in the muscular tissue, a value higher than 500ppb was established, while the cadmium values were below the maximum allowed (Demirbas et al., 2017). Recorded levels of cadmium in livers of hare were 890 ppb and lead 2.190 ppb (Demirbas et al., 2017). Analyzing the correlation association, it was found that there is a strong significant and positive correlation between the concentration of Pb and Cd in the liver and muscles (Demirbas et al. 2017).

In a research of Halecki et al. (2017), three sites with intensive agricultural production were analyzed for the presence of heavy metals (Pb and Cd) in the liver, kidneys and muscle tissue. The research was performed on three different sites with different anthropogenic influences that conditioned the presence of heavy metals in the tissue of hare. The first location was near the lead and zinc mines with more than a decade of exploitation, near Olkusz, Poland.

The second location was exposed to the most industrial pollution, with lower contamination of heavy metals, in the vicinity of Wawrzenczyce, Poland. And at the third location there was no

anthropogenic influence on sources of contamination with lead and cadmium, the environment of Dabrowa Tarnowska, about 70 km east of Krakow. The study was conducted on n= 223 animals. Mean value of lead in the liver for the year 2006/07 were; for locality 1 – 1,830 ppb / 1,550 ppb; locality 2 - 890 ppb / 840 ppb; locality 3 – 1,110 ppb / 830 ppb (Table 1.). When it comes to mean values of cadmium in the liver of the hare for the year 2006/07 on the locality 1, the following values were recorded 1,990 ppb / 1,860 ppb; locality 2 – 1,120ppb / 1,270 ppb and locality 3 – 1,300 ppb / 1,210 ppb (Haleck et al. 2017).

Table 1. Presence of lead and cadmium in the liver of the brown hare, depending on the type of anthropogenic effects

	Serbia 2013/14 (Beuković et al., 2014)		Turkey 2013/14 (Demirbas et al., 2017)		Poland 2006/07 (Halecki et al., 2017)	
	Cd (ppb)	Pb (ppb)	Cd (ppb)	Pb (ppb)	Cd (ppb)	Pb (ppb)
Intensive agriculture	231.2	165.6	-	-	1,300.0 1,210.0	1,110.0 830.0
The presence of industrial pollution and intensive agriculture	-	-	803.0	2,190.0	1,120.0 1,270.0	890.0 840.0
Vicinity of mines	-	-	-	-	1,990.0 1,860.0	1,830.0 1,550.0

Analyzing the results of Beuković et al. (2014); Demirbas et al. and Haleci et al. (2017), which are shown in Table 1, a comparison of the pollution intensity was performed, depending on the type of anthropogenic activity. It should be noted that the methodology in all three studies is basically the same, with certain methodological variations that do not have significant differences. It can be noted that the presence of heavy metals (lead and cadmium) largely depends on the natural properties of the soil. That can be seen as a direct comparison of results in an intensive agriculture area (Beuković et al., 2014; Halecki et al., 2017). A multiple higher concentration of heavy metals in the hunting grounds of Poland compared to the hunting grounds of Serbia in conditions of intensive agricultural production was noted. When comparing results in intensive agricultural production with exposure to industrial pollution (Demirbas et al., Halecki et al., 2017), it can be noticed that the level of cadmium was higher in Poland and the lead level was higher in Turkey. However, in comparison to all the mentioned anthropogenic impacts, the exploitation of ore is the largest pollutant of the environment, as can be seen from the contamination of the liver of the hare, (Haleci et al., 2017), where the highest contamination of both cadmium and lead was recorded.

Conclusion

Based on the analyzed results of contamination of the environment with heavy metals due to anthropogenic factors, it can be concluded that there are significant influences in terms of increasing the level of heavy metals. High concentrations of lead and cadmium can have detrimental effects on the reproductive performance and the number of hare according to (Halecki et al., 2017), especially when it comes to the proximity of the site where the exploitation of the ore is done. From the aspect of industrial pollution, higher levels of presence of heavy metals are evident, but the effects are also dependent on other factors that are not anthropogenic, such as the natural presence of heavy metals in the soil. Intensive agriculture has the least impact on the occurrence of elevated levels of cadmium and lead in relation to other anthropogenic effects, primarily because the natural

factor is the natural contamination of the soil. Some studies mention possible contamination with phosphate fertilizers, which cannot be clearly linked to the effects of declining numbers or reduced reproductive activity of hare, primarily due to the effects of other factors.

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THE PESTICIDE IN BROWN HARE ADIPOSE TISSUE AS CONSEQUENCE OF ANTHROPOGENIC FACTORS

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Abstract: The impact of pesticides on brown hare population in agro biotope under intensive conditions of agricultural production was analysed in this review paper. The use of pesticides represents one of anthropogenic impacts which if not applied in an adequate way may have drastic negative consequences when it reaches animal food chain. The most often visible examples of inadequate application of pesticides can be seen in mass poisoning when both herbivore and carnivore species fed on their residues perish. In the same way, another significant problem which is not easily visible are low doses of pesticides which can be found in brown hare tissue and which can, in the long run, lead to decrease in brown hare density but without visible mass deaths what most often causes a decrease in reproductive potential in conjunction with other natural and anthropogenic factors.

Keywords: brown hare, pesticide, anthropogenic factors, agro biotope

Introduction

As a consequence of intensive agricultural production in a form of anthropogenic effect, Lazić et al. 2013. report about accumulating atrazine residues and its degradation products in the environment what is particularly dangerous for underground waters. During the application of pesticides, their largest part falls on the ground which afterwards by washing off penetrates into a deeper soil layers and eventually into underground waters (Pucarević et al., 2003). Further fate of pesticides in agro biotope, depends primarily on the time both of semi-decay and decay and of physical and chemical characteristics of the traits of soil and waters with which they come into contact. A negative effect of pesticides on environment is particularly expressed in the example of underground waters and soils (Fenol et al., 2014; Sharma et al., 2014; Kolpin et al., 1995). The connection of underground waters with large river flows also represents one of the essential factors in circulation of contaminants in agro biotope. Đinović-Stojanović et al., 2013. reported that a presence of organochlorine pesticides and polychlorinated bifenil in the fish from the river Danube (Batajnica, Serbia) pointed to the need of continuous monitoring of organochlorine and some other pollutants as well. Organochlorine pesticides and polychlorinated bifenils got into environment as a consequence of their use in intensive agriculture (Nie et al., 2012; Meng et al., 2013), but although they were removed from the use in early eighties of the last century their residues can still be found in different parts of environment such as water, air and soil (Loganathan and Kannan, 1994; Castro-Jimenez et

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al., 2011; Barakat et al., 2013). The example of glyphosate clearly illustrates their persistency and mobility what can have a significant impact on agro biotope although its use is forbidden. Sojanović et al. (2017) report following quantities of pesticides in localities of Subotica (0.0834 mg /kg), Sremski Karlovci (0.1486 mg /kg) and Erdut (0.1956 mg /kg) (Sojanović et al., 2017). Even in protected zones such as the Nature Park "*Stara Tisa*", from where water resource is used for irrigation of arable land, the water analysis determined the presence of following pesticides residues although the levels were below critical values: terbuthylazinedesethyl, terbuthylazine, clothianidin, clomazone (Bursić et al., 2017c). The presence of pesticides in arable crops is in direct relationship with anthropogenic impact which is a main cause of further contamination of animal species. The most frequently detected pesticides in cabbage samples were clothianidin, pyrimethanil, metalaxyl, azoxistrobin and thiamethoxam. In onion samples, the most abounded pesticides were metalaxyl-M, dimetomorph, spinosad and spirotetramat. The detections were below the MRLs. These results indicate that the producers have started to apply GAP, but not in all the segments since the detection of pesticides which are not registered for use in the Republic of Serbia were applied (Bursić et al., 2017a).

Mass poisoning of different game species as a direct anthropogenic effect

By analysing the anthropogenic effects which are reflected through a large spectrum of effects one of the most visible and most dangerous seems to be a mass death of game species. Numerous studies over the previous period have researched mass poisoning of fauna and game species testifying about the danger of anthropogenic impact of man on wild animal species (Antoniou et al. 2005; Berny, 2007; Martínez-Haro et al., 2008). The most common causes leading to mass deaths of wild game due to application of pesticides are first of all, the use of forbidden (banned) pesticides, then overdosing and overuse as well as an inadequate way of use. The most frequent active substances of pesticides which caused mass deaths were insecticides of the order: organophosphorus compounds (forat) and methylcarbamate compounds (carbofuran), but also the importance of rodenticides should be pointed out (zinc-phosphide and bromadiolone) (Beuković et al., 2015). Mass deaths occur not only in wild game which directly consume treated vegetable food in food chain, but animals also often get killed due to secondary poisoning of mammalian predator species (Berny et al. 1997; Antoniou et al. 1996) and birds (Elliott et al. 1996; Soler Rodríguez et al. 2006; Berny et al. 2008).

Berny and Gaillet, (2008) point out the importance of inadequate placement of bait containing insecticides (aldicarb, mevinphos, and partly carbofuran) which caused 33% of secondary poisoning of red kites (*Milvus Milvus*) in France. On the example of imidacloprid more cases of negative effects can be perceived. It is an insecticide which reacts on contact and gastrointestinally and primarily has an effect on: leaf louse, thrips tabaci, scutiform moth, leaves miners of citrus fruits, potato beetle and pests in the soil. It can be applied by means of irrigation system and it remains in soil for a long time. Its negative action and mortality rate in partridges were reported by Berny et al. (1999). By analysing a mass poisoning of wild birds in Spain, Martínez-Haro et al. (2008) reported that poisoning in the period from 1990 to 2005 caused many deaths of predatory bird species the causes identified as aldicarb and carbofuran (27.59% each) to methomyl (8.62%) and strychnine (6.9%) what is in compliance with the research (Soler Rodríguez et al. 2006). Nevertheless, taking all into consideration, the mammals, especially carnivore species, are also, to a great degree, endangered in secondary contamination, therefore, according to Lamarque et al. (1999) in France the poisoning of

carnivore accounts for 39% in which fox alone accounts for 23.6% in all recorded deaths caused by poisoning. When ungulates are in question it is thought that the per cent of their poisoning is about 25% although it has been confirmed in only 2.1 % cases (Edwards et al. 2000).

Anthropogenic effect on brown hare population due to contamination by pesticides from agro-biotope

A brown hare is highly adaptive species which successfully accommodated to the conditions of intensive agricultural production. When leverets are in question, they are on the second place per frequency of poisoning among mammals. Edwards et al. 2000. report that in hares poisoning accounts for 26.2 cases and in rabbit 9.2%. The loss of biodiversity in hare population is another example of anthropogenic impact (Mezei et al., 2018.). The authors in mentioned paper point out that in different localities there exists statistically significant difference in presence of pesticides in brown hare adipose tissue. The authors point out the locality of Bačka Palanka with high presence of pesticides in relation to other localities what may be one of the causes of biodiversity loss (Mezei et al., 2018; Bursić et al., 2018.). By analysing the presence of pesticides in kidneys, out of 30 pesticides the 8 were detected in which in all samples of hares a low contamination was recorded except for two samples where residues of oxamil, carbendazim and cymoxanil (Bursić et al., 2017b) were determined in concentrations higher than allowed by EU Regulation (EC 2005). By analysing the results in one hunting ground in the territory of Bačka, an intensive agricultural production is pointed out as one of the causes of the occurrence of pesticides residues wherein a significant correlation of age and level of pesticides in brown hare adipose tissue has been found (Beuković et al., 2017). Observing the results in previously mentioned hunting ground the most frequent were residues of following pesticides: cyprodinil, difenconazo, metaxil M, tebuconazol and thiamethoxam (Beuković et al., 2017). According to the research of Šovljanski et al. 2006. it is thought that insecticides used in sugar beet treatment are extremely toxic while fungicides and herbicides are of lower toxicity what is again a significant example of anthropogenic impact on hare population which use sugar beet in their nutrition. It should be pointed out that intensification of agriculture dramatically reduced diversity of wild plant species in agricultural areas what is by many authors considered to be very important for decreasing the density of hare population in Europe. The problem of food deficiency in summer after gathering winter crops is particularly pointed out (Beuković et al., 2011) in conjunction with treating the stubble fields by total herbicides. Because of mentioned anthropogenic effects the weeds which are at that time essential green food for large monocultures are destroyed while pesticides are introduced into food chain. A solution to these problems may be development of "green corridors" between the fields under monocultures and the organic vegetable production (Beuković et al., 2011)

Conclusion

On the basis of this review it can be clearly seen that in many studies it has been found out that previously mentioned groups of pesticides are to a significant degree present in the tissue of brown hare and other animal species, particularly in regions under intensive agricultural production. Yet this issue is given special attention to only in situations when poisoning is massive and huge, i.e. when protected predatory species get killed. A comparatively disturbing can, first of all, be a latent

presence of pesticides in food chain which in conjunction with other factors may have serious consequences on reproductive status of hare in agro biotope. What should certainly alleviate current situation regarding the use of pesticides in intensive agricultural production may be better control of forbidden pesticides and pesticides used in a prohibited and irregular way.

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HEALTH STATUS MONITORING OF THE EUROPEAN BROWN HARE (*LEPUS EUROPAEUS*) FOR THE PURPOSE OF ASSESSEMENT OF ITS POPULATION SIZE IN SERBIA

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Abstract: In the last couple of decades the case of declining of the European brown hare (*Lepus europaeus*) populations was noted in whole Europe including Serbia. Numerous causes for this can be referred to anthropogenic impacts - landscape changes (agricultural intensification, the use of natural hare habitats by livestock for the purpose of pastures), use of pesticides, machinery and traffic, as well as home range size. Other important causes are inadequate reproduction and the level of survival due to predation (incline in golden jackal population), hunting (especially illegal hunting), variations in climate and diseases, as one of the most important cause of European brown hare population declining. As in other animals, they are etiologically divided to: viral, bacterial, fungal, parasitic and other diseases. During hunting season in 2017 (October and November), a study on 46 hares (whole carcasses or eviscerated organs) from the four locations near Belgrade was conveyed. A post-mortem examination and histopathological investigations were performed and, additionally, routine bacteriology and parasitology of the gastrointestinal system were carried out. The results point to the significance of continuous health status monitoring and discovering the causes and frequency of certain conditions in free-ranging European brown hare populations. In this review paper, some health problems in hares are discussed in more details, taking into account the findings obtained in this preliminary study.

Keywords: health status, Lepus europaeus, monitoring

Introduction

The European brown hare (*Lepus europaeus*) is considered as one of the most important game animal species in Europe. Unfortunately, in the last couple of decades the case of declining of the European brown hare (*Lepus europaeus*) populations was noted in whole Europe including Serbia, and this problem is affecting all regions of Europe simultaneously (Frölich et al., 2003; Strandgaard and Asferg, 1980; Tapper and Parsons, 1984; Hutchings and Harris, 1996; Marboutin et al., 2003). Although there are numerous hypothesized causal factors for this decline, it can be referred to health status, or the presence of anthropogenic impacts, different diseases, and the predation (Olesen et al., 2006; Marinković et al., 2018).

During hunting season in 2017 (October and November), a study on 46 hares (whole carcasses or eviscerated organs) from the four locations near Belgrade was conveyed. A post-mortem examination and histopathological investigations were performed and, additionally, routine bacteriology

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and parasitology of the gastrointestinal system were carried out. The results of this recently conveyed preliminary study point to the significance of continuous health status monitoring and discovering the causes and frequency of certain conditions in free-ranging European brown hare populations (Marinković et al., 2018). The aim of this paper is to review possible causes of the decline of the European brown hare (*Lepus europaeus*) population, as well to emphasize the importance of the health status monitoring, especially pathomorphological examination of the hunted animals, and animals found dead in nature.

Factors affecting decline of the European brown hare population

Numerous factors can be referred to the anthropogenic impacts - landscape changes, use of pesticides, machinery and traffic, as well as home range size. One of the important anthropogenic factor are the landscape changes which can inflict the population number of hares. This especially refers to agricultural intensification and use of natural hare habitats as pastures for livestock or for crop production. European brown hare, if available, prefers weeds and wild grasses. However, in areas of agricultural intensification these foods are reduced and crop species, especially monocultures, are increasingly used as a food source. Together with intensive crop production goes the intensive use of pesticides, which are very dangerous and can harm the European brown hare population. The effect of pesticides can be dual. On one side incidents of poisoning have occurred (Chlewski, 1976; Rimkus and Wolf, 1987; Edwards et al., 2000), but on the other side, the use of herbicides is reducing the abundance of weed in cereal fields which decrease the quality of summer diet for the hares. Also, after summer harvest and ploughing fields remain without vegetation, and this is potential lack of summer food for the hares at the time of main breeding and lactating season.

Besides mentioned facts, machinery used for crop harvesting operation is a source of mortality in leverets. Also, "road kills" can additionally decrease the number of hares in the areas where the network of traffic roads is present (Olesen et al., 2006; Posautz et al., 2015; Kornaś et al., 2014).

Other important causes are inadequate reproduction and the level of survival due to predation (incline in golden jackal population), hunting (especially poaching), variations in climate and diseases, as one of the most important cause of European brown hare population declining. The red fox (*Vulpes vulpes*) is considered to be the most important predator for the European brown hare. Oral vaccination of foxes against rabies led to rising of its population in Europe which could be one of the important factors for the declining of hare population (Posautz et al., 2015; Olesen et al., 2006; Reynolds and Tapper, 1995; Panek, 2009; Goszczynski and Waseilewski, 1992). Other predators than the red fox, golden jackal (whose population is in constant incline during past decade), crows, ravens, common buzzards, goshawks, owls, domestic dog and cat also prey on hares, especially leverets (Olesen et al., 2006; Penezić and Ćirović, 2015; Markov and Lanszki, 2012).

Heavy hunting pressure and poaching can jeopardize hare population. Besides that climate influence, particularly climate change can have negative impact on the hare population especially on the leverets (Olesen et al., 2006).

Diseases are considered as one of the natural factors of hare mortality and have greatly influenced the decline of this species in past few decades. As in other animals, diseases in European brown hare can be divided etiologically to: viral, bacterial, fungal, parasitic and other diseases (Frölich et al., 2003; Marinković et al., 2018; Wibbelt and Frölich, 2005).

Among the viral diseases, virus which is the causative agent of European brown hare syndrome (EBHS) is one of the most important. It is classified as a *Calicivirus,* a small (30 to 35 nm) icosahedral, non-enveloped virus (EBHSV) (Ohlinger and Thiel; 1991; Gavier-Widen, 2012). This disease is reported in many European countries (Frölich et al., 2001; Frölich and Lavazza, 2008; Wibbelt and Frölich, 2005) and it is characterized by acute hepatitis and hemorrhages of various internal organs (Poli et al., 1991; Fuchs and Weissenböck, 1992). Interesting fact is that high incidence of degenerative and inflammatory hepatic and renal lesions were noted in liver and kidney tissues originating from the hares from Serbia. So, further investigations are necessary to establish the etiology of these changes (Marinković et al., 2018).

Also, a group of *Leporipoxvirus* are considered as important etiological agents for the hare population, and these two related strains myxoma and fibroma virus can cause fibromatosis as a rare incident in hares or myxomatosis. Fibromatosis is a benign, self-limiting disease with localized fibroblastic nodules within the subcutis (Wibbelt and Frölich, 2005; Grilli et al., 2003; Fenner, 1994). Although myxomatosis is a disease commonly observed in European wild rabbits (*Oryctolagus cuniculus*), hares can also be infected and disease is characterized by multiple firm subcutaneous nodular masses at the head, back and limbs. Virus is transmitted via hematophagous insects or by close contact between hares and affected rabbits or their excretions (Wibbelt and Frölich, 2005; Barlow et al., 2014). Hares are anecdotally infected with papilloma virus usually through cutaneous lesions or by hematophagous insects when focal pedunculated nodular masses – warts can develop (Wibbelt and Frölich, 2005).

Among bacterial diseases, several are important for the health status of the European brown hares: pseudotuberculosis, pasteurellosis, brucellosis, tularemia and staphylococcosis (Wibbelt and Frölich, 2005). Pasteurellosis is considered to be the most important hare disease and sometimes up to 80% of a population might be killed by this infection which is the most common during the winter season, and early spring when sudden, widespread epidemics can occur. This disease is caused by small non-spore forming, gram-negative coccobacillus *Pasteurella multocida* subsp. *multocida*, commonly present in the upper respiratory system. Stress, usually, sudden drop of temperature, especially during the night, inadequate immunological status, food shortage, etc. can induce the multiplication of these bacteria, and the development of disease. The disease can occur either in peracute septicemic haemorrhagic form or acute to chronic with fibrino-purulent pleuro-pneumonia and fibrinous pericarditis.

Other important bacterial disease of lagomorphs and rodents is pseudotuberculosis caused by *Yersinia pseudotuberculosis,* a small rod-shaped gram-negative bacterium, and it is considered to be of one of the most important lethal infections in hare with population losses of up to 50%. This disease can also occur in birds and other animals as well as in humans. Rodents and wild birds are considered to act as a natural reservoir for this microorganism. The disease is common during cold and humid part of the year, but beside weather factors, stress, inadequate food, opportunistic infections are also important factors for the onset of this disease. Common clinical manifestations are dyspnoea and diarrhea, and characteristic morphological lesions are multifocal caeseous granulomatous nodules within spleen, liver, intestine and mesenteric lymph nodes (Wibbelt and Frölich, 2005; Fratini et al., 2017; Frölich et al., 2003).

Staphylococcosis in hares is caused by *Staphylococcus aureus* and characterized by multiple abscessatration of the skin, subcutis, and sometimes internal organs and joints, in rare cases as an acute septicaemia which can cause the exitus lethalis without specific morphological changes (Wibbelt and Frölich, 2005).

Two important bacteria can cause disease in hares which also have zoonotic potential: *Francisella tularensis* and *Brucella sp.,* although previously mentioned *Yersinia pseudotuberculosis* also have that kind of potential. Brucellosis is caused by *Brucella suis biovar 2,* gram-negative, non-sporing, small rods. It is believed that wild boars and hares are reservoir species for this agent, but this bacteria is also capable of infecting domestic pigs and other wild or domestic animals and as mentioned humans as well. The most likely source of infection are aborted fetuses. Infection can occur orally, via the reproductive system, the conjunctiva and percutaneously, but the venereal transmission seems to be the dominant way of infection in hares. The course of the disease in hares can be acute or chronic, and the infection in hares is either latent or involves the development of granulomatous nodules or abscesses in the reproductive system – uterus and testes, regional lymph nodes, liver, spleen, lung and other tissues (Thorne, 2001; Gyuranecz et al., 2011; Frölich et al., 2003)

Tularaemia is caused by *Francisella tularensis*, a highly infective gram-negative, non-motile, non-sporing, aerobic coccobacillus with zoonotic potential which occurs naturally in lagomorphs and rodents, with ticks and other arthropods the main vectors, but infections of many other mammals and birds. Infection occurs directly through close contact or indirectly via contaminated food or insect vectors. In the European brown hare in most cases, clinical signs appears as short apathy followed by fatal septicemia. Numerous necrosis are found within liver, spleen and lymph nodes (Gyuranecz et al., 2010; Hestvik et al., 2017; Tomaso et al., 2017; Frölich et al., 2003).

Several other microorganisms can also play important role for the health status of European brown hare, and some of them are also zoonosis and also found in Serbia as well: *Bacillus anthracis, Borrelia burgdorferi* (Talleklint and Jaenson, 1994; Jaenson and Talleklint, 1996), *Campylobacter sp., Leptospira sp., Listeria monocytogenes* (Mišić and Marinković, 2002) and *Salmonella sp.*

The most diseases, especially parasitic diseases are density dependent. These diseases can play important role in the health status or can cause mortality in the hare population and in that manner have negative influence on the abundance of this species in nature. Generally, parasitic diseases can be caused by protozoa, nematodes, cestodes, trematodes and acarines and insects.

Coccidiosis is one of the most important protozoal infections in hares. It can occur in the intestinal and hepatic form. Intestinal coccidiosis is caused by variety of species of *Eimeria* such as *E. europea*, *E. hungarica*, *E. robertsoni*, *E. semisculpta*, *E. septentrionalis*, *E. stefanskii* and *E. townsendii.* These coccidia invade cells within epithelial lining of the intestines causing severe catarrhal enteritis and gaseous distension of the gut. Sometimes Often small yellowish nodules within the mucous membranes can be noted. Hepatic coccidiosis is caused by *E. stiedai* which invade epithelial cells of the bile ducts causing hyperplastic lesions of the bile ducts. As in rabbits transmission is feco-oral, and up to 60-100% of the animals in population can be infected with coccidia (Kornaś et al., 2014; Chroust, 1984).

Toxoplasma gondii is protozoal organism causing Toxoplasmosis, disease which in most cases is acute fatal disease in hares. Infection occurs by ingestion of food or water contaminated with oocysts from cat feces. Common pathomorphological changes are interstitial pneumonia, multifocal areas of hepatocellular necrosis, encephalitis, and necrosis of lymphoid follicles of the lymph nodes (Jokelainen et al., 2011; Sedlak et al, 2000, Frölich et al., 2003).

Hares can be infected with both trematodes - *Fasciola hepatica* and *Dicrocoelium dendriticum* and these parasites can cause cholangitis with extensive bile duct hyperplasia with proliferation of mucous glands, and sometimes extensive hepatic cirrhosis (Cuervo et al., 2015; Diakou et al., 2014; Chroust et al., 2012; Sergi et al., 2018). These parasites were also noted in the hare population in Serbia (Marinković et al., 2018).

Gastro-intestinal nematodes can be important due to its pathogenic influence on the process of digestion, and also as a cause of serious anaemia in hares. Several nematode species are important for hares - *Graphidium strigosum,* gastric parasite, *Trichostrongylus retortaeformis*, small intestine parasite, causing catarrhal enteritis and *Trichuris leporis* commonly found on the caecal mucosa, causing necrotic lesions within the gut wall due to its toxic metabolites. *Passalurus ambiguus* can be found both in small and large intestine. The juvenile stages are rather found in the mucosa of the small intestine and the cecum, while the adult worms are located in the anterior part of the cecum and the large intestine (Diakou et al., 2014; Chroust et al., 2012; Sergi et al., 2018; Dubinský et al., 2010).

Lungworms *Protostrongylus sp.* can cause severe infection followed by dyspnoea and seromucosal nasal discharge due to catarrhal pneumonia and pleuritis. This parasitic infection has negative influence to the immunity of the animal, and as a consequence of parasitism hares are more prone to secondary bacterial infection in contrast to clinically healthy hares (Diakou et al., 2014; Chroust et al., 2012; Sergi et al., 2018; Dubinský et al., 2010; Frölich et al., 2003).

Hares also can be hosts for numerous Cestodes - *Paranoplocephala wimerosa*, *Andrya cuniculi, Andrya rhopalocephala, Cittotaenia denticulata, Mosgovoyia pectinata, Mosgovoyia ctenoides.* Most of them can cause catarrhal enteritis with malabsorption, degenerative changes on the liver (*Taenia pisiformis* larvae), and some of them are important zoonosis - *Ecchinococcus granulosus* and *Ecchinococcus multilocularis* (Diakou et al., 2014; Chroust et al., 2012; Sergi et al., 2018; Dubinský et al., 2010; Chaignat et al., 2015). Besides mentioned parasites hares can be infected with numerous other parasites such as lice *(Haemodipsus setoni), Sarcoptes scabiei* (Restani et al. 1985*), Sarcocystis sp.* and many other.

Conclusion

There is a multicausal etiology for the decline of the population of the European brown hares (*Lepus europeus*), and different diseases have a great importance in this declining. Constant health status monitoring is important for the assessment of the population size and it is necessary to intensify and to facilitate its implementation in Serbia. The pathomorphological examination of the hare carcasses, followed with microbiological and parasitological examinations, plays a crucial role in this process.

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PRODUCTION LOSSES AND MORTALITY OF PHEASANTS DEPENDING ON CULTIVATION TECHNOLOGY IN VOLIERS, NUTRITION AND HUNTING CONDITIONS

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Abstract: The paper presents an overview of domestic and foreign studies of the influence of various factors on the production losses and mortality of pheasants in the parent flock, as well as young pheasants in the course of nurturing and after settling in the hunting ground. Production losses in the parent flock depend on the genetic structure of the birds, the size of the group, the relationship of the sexes, the arrangement of space in the pheasantry, the nutrition, the age of the females and the duration of the laying season. The state of physical condition and mortality of young pheasants in nursing affect: temperature, ventilation, nutrition, lighting, hygiene... The losses of young pheasants after settling in the hunting ground occur because of diseases and parasites, the lack of food (and water), and most of all because of predators. Compared to the number of eggs in the breeding flock, the number of young birds surviving to winter is only 7-10 per female pheasant. Due to such a small number and high production costs, maximum control of all procedures in the technology of cultivation of pheasant, nutrition and in settlement of the hunting grounds, as well as the division of jobs between the pheasantries in order to their specialization is necessary.

Keywords: pheasants, nutrition, losses, mortality, hunting, predators.

Introduction

Due to inadequate natural reproduction of pheasant and high hunting pressure, each year in Serbia a large number of young pheasants is reared in voliers, with the aim of settling them in hunting grounds at a certain age (Đorđević et al., 2011a). However, such a procedure is very expensive, not only because of direct costs (facilities and equipment, parent flock, feed, medicine, energy, work force...), but also because of a large percentage of losses, starting with the breeding flock (mortality of adult individuals, variability in bearing capacity, percentage of fertilization and percentage of incubated eggs), through the mortality of young pheasants in voliers, up to particularly large losses after settling in hunting grounds (diseases and parasites, adverse weather, lack of food and predators).

Deeming et al. (2011) state that the survival of artificially raised pheasants is much lower than pheasants born and raised in hunting grounds, primarily because of predators. Contrary to this, the lack of food is of minor importance and can be partially compensated by additional feeding of settled pheasants or by their adequate physical condition (physical body reserves) during settling (Đorđević et al., 2011b; 2012a). This problem occurs only in the second half of the year due to high temperatures and harvesting of large areas under monocultures, when there is less food and shelter. Due to the mentioned problems, more active hunting on the polygons is becoming more and more important today.

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Production losses in breeding flocks and during incubation of eggs

In Serbia, there are over twenty registered pheasants farms, some of which have a rounded production process (production of eggs in the parent flock, incubation of eggs and cultivation of pheasants chickens), and others only have pheasant chickens (originating from other pheasant farms). The losses of this species of game in the pheasant farms are mostly indirect, and they relate to a smaller number of laid, fertilized and hatched eggs.

The breeding technology of the breeding flock aims to produce as many eggs as possible per female pheasant and the higher the fertility of each egg (Đorđević et al., 2012c; 2013a).

These characteristics depend on the genetic structure of the breeding flock, the size of the group, the relationship of the sexes, the arrangement of the space on the pheasant farm, the diet, the age of the female pheasants and the duration of the bearing season (Esen et al., 2010). The number of laid eggs, their weight and fertility are particularly influenced by nutrition (Nowaczewski and Kontecka, 2005). Nutrition affects the number and size of the laid eggs directly through the necessary nutrients which are provided in the meal, or indirectly through the physical reserves which were provided in the earlier period. Considering the multiple bearing capacity of the female pheasants in the voliers, their needs are considerably higher than the needs of pheasants in nature. Urošević (2005) stated that under our conditions the average bearing capacity of female pheasants in the period April-June amounts up to 41-45 eggs, although according to Bojović 2012., under the conditions of the extended day, it can be up to 140 eggs. Such intensity and productivity require a good knowledge of the needs of the female pheasants. These needs were thoroughly examined and specified in some norms (Đorđević et al., 2014; 2015b). Apart from that, Bojović 2012. found a higher laying capacity in female pheasants that had a lower percentage of protein in the meal. The author's explanation is that the laying capacity, in addition to nutrition, is influenced by a large number of factors that vary from farm to farm. One of these factors is the way of keeping the individual birds. Kontecka et al. (2014) examined the effect of holding layers in a cage or a volier on the number of eggs laid. The cages were 1.5 m in size (lenght) × 1.5 m (width) × 0.5 m (height). In every cage there was a harem with nine females and one male. The density of the settlement was 4.4 birds /m^2. In each of the voliers there were 50 cocks and 400 laying hens were placed on an area of 900 m^2. The ratio of the sexes was 1: 8 and the density of the population of 0.5 birds /m^2. The established production results were better in the experiment with the cage system of holding (Table 1).

Ovoscoping of the eggs before placing them in the incubator is an important measure of elimination of non fertilized eggs, which increases the number of hatched eggs. However, on some of our pheasant farms, the classification of eggs is performed only according to their mass and shape, which has a negative influence on the results. It is accepted as satisfactory if the number of eggs hatched is around 85% of fertilized eggs. In an experiment, Popović et al. 2013. found 53.73 to 72.08% of eggs hatched, which is a consequence of the lack of ovoscoping of the eggs. Biological quality of eggs, incubator regimen and human involvement are of the significant importance for the results of incubation. Biological quality refers to the fertilization of eggs, the manner and length of keeping and the proper selection of eggs for the plantations. The fertilization is influenced by the structure of the main flock, the manner of holding, and the relationship of the sexes. The influence of nutrition on the biological quality of eggs is in correlation with the content of vitamins and minerals (Suchy et al., 2008). Fertilization of eggs in nature can be up to 96%, but due to large losses (up to 85%), the real increase in one female pheasant is very low. Cold and rainy periods, the

presence of unknown persons and other harassment of the breeding flock may have unfavorable influence on the ferilization (Popović and Stanković, 2009). Fertility is lower at the beginning and at the end of the laying season.

Table 1. Performance and hatchability results (%) of pheasants kept in different housing systems (Kontecka et al., 2014)

Trait	Housing system			
	cage		aviary	
	mean	SEM	mean	SEM
Laying rate	59.59*	2.59	27.23*	1.88
Hatching eggs	90.08	1.36	86.43	1.19
Small eggs	5.49*	0.43	6.50*	0.54
Cracked eggs	4.43*	0.24	7.07*	0.24
Dead embryos until day 8 of incubattion	2.67*	0.31	4.74*	0.55
Dead embryos until day 8 of incubattion, unhatched and crippled chicks	18.18	0.73	18.44	1.24
Hatchability from set eggs	61.74	3.79	63.02	1.69
Hatchability from fertilized eggs	79.06	0.87	79.98	1.45

Losses during breeding of young pheasants

Small pheasants are a very sensitive category of this type of game, in which significant losses occur due to inadequate temperature, ventilation, nutrition, lighting, hygiene (Đorđević et al., 2013b). The first phase of breeding of pheasants (12-15 days of age) is carried out in the battery holding system, after which it passes to the floor system (boxes or breeding cottages). After day 30, preparations for settlement in the hunting ground begins (Kokoszynski et al., 2008). In order to maximize the adaptation to the conditions of the hunting grounds, young birds are encouraged to search for food, as well as to stay away from the predators. Grain food is given on the ground during that period and the perches are installed. A characteristic of small birds to stay on trees during the night, acquired during the stay in voliers, is very important for the survival from predators (Whiteside et al., 2016). Apart from that, installation of perches in voliers influences the anatomic development of birds. In order to maximally prepare young pheasants for settlement in the hunting area, Santilli and Bagliacca (2017) compared a group of young pheasants raised in cottages (2.5×3.5 m) with perches installed at 30, 60 i 120 cm above the ground, with the group without perches in the objects. Perches contributed significantly to less breakage of feathers (2.1:2.3; $p<0.05$), increase of greater body mass, and to length and width of tarsus (Table 2).

One of the important factors for the technology of cultivation of young pheasant is the density of population. Đorđević et al. (2010) were investigating the influence of two protein levels and two density of population on the mortality of young pheasants (Table 3). During the first stage of rearing, the young pheasants were kept in cages on four floors, area 6.4 m^2 after treatment , and in the second stage of rearing on the floor, surface of 18 m^2, with an outlet of 70 m^2 per treatment. The experiment did not determine the significant influence of the investigated factors on the percentage of mortality, individually or as their interaction. In addition, mortality was far below the results of some previous research. For example, Braastad (1986) stated that mortality for 16 weeks, including death of birds caused by pecking, was from 2.0 to 8.7%. When comparing the production results in

two pheasant farms in Serbia, Popović and Stanković (2009) found the mortality of young pheasants up to day 40 in the first pheasant farm of 8.33 to 13.39%, and on the second pheasant farm of 3.60 to 4.74%. Otherwise, mortality could be increased due to overpopulation of voliers and the onset of cannibalism. Overpopulation occurs on pheasants farms with big parent flock and which deal with incubation of eggs and with production of young pheasants. At the peak of bearing capacity, a great number of young pheasants is present. Since there is not enough room for all of them, cannibalism occurs.

Table 2. Morphological measures collected at the 5th week of age (n=30 each subgroup) (Santilli and Bagliacca, 2016)

	Body weight, g	Tarsus length, mm	Max tarsus thickness, mm	Min tarsus thickness, mm
No perches				
Male	384.5a	60.97ab	6.65a	4.86a
Female	305.8b	57.72c	6.17b	4.55b
With perches				
Male	391.8a	62.09a	6.81a	4.83a
Female	321.9b	59.12bc	6.33b	4.60b
No perches	345.2	59.35	6.41	4.71
With perches	356.9	60.61	6.57	4.72
Standard error of mean	2.88	0.33	0.04	0.03
F values of tested effects				
Perches	4.12*	3.65*	3.73*	0.04ns
Sex	166.6***	22.2***	33.7***	25.8***
Interaction sex×perches	0.58 ns	0.04 ns	0.02 ns	0.64 ns

Table 3. Influence of protein levels and density on young pheasants mortality, % (Đorđević et al., 2010)

Proteins	Density	Period		
		0-15. days	15-42. days	0-42. days
26% SP up to 4 weeks of age and 20% SP from 4 up 6 weeks of age	450 birds/group	1.45^{ns}	1.00^{ns}	2.44^{ns}
	550 birds/group	0.73^{ns}	0.55^{ns}	1.27^{ns}
30% SP up to 4 weeks of age and 24% SP from 4 up 6 weeks of age	450 birds/group	2.78^{ns}	0.44^{ns}	3.00^{ns}
	550 birds/group	1.55^{ns}	0.91^{ns}	2.43^{ns}

Appropriate physical fitness obtained during the stay on pheasant farms is very important for the survival of young pheasants in the hunting grounds untill they learn to search for food. That is the reason why the feeding of young birds in voliers is done in adequate mixtures of concentrate, in accordance with normatives. Pekeč 2003. found out that the diet of pheasants with higher protein content leads to a significantly higher body weight (457.07:373.85 g). In addition, their gradual settlement (to the hunting area) from the shelter is practiced, where they receive food and water for some time, until they fully adjust to the conditions in the hunting ground.

Losses of young pheasants after settling in hunting grounds

After the spring period of the abundance of food of plant and animal origin in the hunting ground, due to the drought and the harvest of monocultures on large surfaces during the second

half of summer, there is less and less food and water for young pheasants that were recently inhabited. According to research in Ireland, about 70% of young birds aged 12 weeks die or die due to lack of food, parasitic infections and predators, which represents a huge loss and cost for hunting grounds (Popović et al., 2009). Especially large losses are made by carnivores, primarily foxes (Lanszki, 2005). It is believed that the poor physical condition of artificially raised pheasants inhabiting hunting grounds just fits the fox. Due to such high mortality, it is necessary to continue for some time with the feeding of 3-4 pounds of grain feed per 100 young birds after the release of young pheasants to the hunting area, and to implement the measures of control of the number of predators (Đorđević et al., 2012b, 2015a).

Winter feeding is most often the only way of feeding pheasants in the hunting grounds of Serbia. The aim of winter feeding is to reduce not only the losses of game, but also the preservation of its mass and fitness. Pheasants are extremely resistant birds and rarely die of starvation. However, in search of food, in cold weather and deep snow, they become much easier a predator prey (Lanszki, 2005). In order to achieve better results of additional nutrition, it is necessary to begin to accustom pheasants to feeds immediately after harvest. Additional feed consists of grains and possibly pelleted foods with a diameter of 3-5 mm, as well as juicy foods in days without frost. The daily amount of grain food is about 40-60 g per bird during winter. Plots under corn are effectively used during winter time (Gabbert et al., 2001), which also serve them both for food and shelter. Food is given to arranged or just improvised places.

Conclusion

Taking into account the laying capacity of female pheasants from some of our experiments (40-45), then the percentage of eggs which are put into incubator after classification (85-95), percentage of hatched eggs (55-70), percent of mortality of young pheasants in voliers (3-10) and the percentage of mortality after settlement into hunting grounds (up to 70), we come to the number of 7-10 young pheasants per female that would live until the next winter. Such a small percentage significantly increases the costs of pheasant production for hunting grounds. Therefore, it is necessary to have a maximal control of all steps in the technology of breeding of young pheasants, their feeding and the control of predators, in order to reduce production losses and mortality. In addition, it is recommended that "division" of jobs be carried out among the phases of the work, so that they are separately engaged either in keeping the parent flock and producing one day-old chicks, or just by raising young pheasants and preparing for settling in hunting grounds. By specializing in a particular type of work, pheasant farms could work more efficiently, with fewer production losses and mortality.

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THE ROLE AND SIGNIFICANCE OF PHEASANTS AS A POTENTIAL RESERVOIR OF THE WEST NILE VIRUS

Milutin Đorđević[1], Branislav Pešić[2]

Abstract: Pheasants on pheasant farms are grown in semi-open facilities and as such they are exposed to contact with different vector of disease that has the character of zoonosis. Having in mind that the presence of West Nile virus in the mosquito population was first detected in 2012, there is a real risk of transmission of infection from the mosquitoes to the birds, among which are pheasants, which represent the reservoir of infection. Capturing of the mosquito population in the economic backyard of the phesant farms and detection of viruses in them justifies the need, in the next phase of the study, to control pheasants, as potential reservoirs for the presence of the virus. Controlling oropharyngeal swabs and taking blood samples to control antibody titers will determine the status of individuals and the potential risks of transmission of this virus. The above research would serve as the basis for assessing the level of health risk by the local population.

Keywords: pheasant, mosquitoes, West Nile virus, reservoir, pheasant farm

Introduction

The West Nile virus (*Flaviviridae*, *Flavivirus*) is the cause of the zoonotic arbovirus (arthropod-borne virus) infection widely spread throughout the world, which has a significant impact on both human and animal health (Beck et al. 2013). The virus was first isolated in 1937 from the blood of a febrile woman in the West Nile district of Uganda and hence the name of the virus (Smithburn et al. 1940). In nature, this virus is maintained in an enzootic cycle that includes ornithophilic mosquito species as transmissive vectors and some birds as reservoirs. Although all members of the Flaviviridae family have a similar genetic code, share conserved non-structural proteins and produce mono-polyproteins, they differ in cis-acting elements that control viral RNA replication and translation. The Flavivirus genome contains a 3'-5 'cyclizing sequence that is not found in the genes of the other two genera of the Flaviviridae family (Gubler et al. 2007). So far, 73 members of the genus Flavivirus, which are divided into 12 serogroups (Poidinger et al. 1996), have been described. The West Nile virus belongs to the serogroup of the Japanese encephalitis virus (JEV). Kunjin virus endemic to Australia and Asia is now considered a subtype of the West Nile Virus (Scherret et al. 2001). Although all virus isolates belong to one serotype, they are grouped into two genetic lines (1 and 2) based on the amino acid sequences and deletions that comprise protein sequences (Berthet et al. 1997). All the isolates of the West Nile Virus that have so far caused human epidemics belonged to line 1 (Lanciotti et al. 1999). Line 2 is limited to endemic human infections, but recent trials have also been found in Hungary and Serbia.

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Results and Discussion

In Europe, the presence of the West Nile Virus was first proven in 1958 when seropositive people in Albania were identified. At the same time, for the first time, the virus has been isolated from humans and mosquitoes in the Rhone delta in France. The first discovery of the presence of the virus is seen in the outbreak of the West Nile fever epidemic in humans in 1960 in southern France, Russia, Spain, Romania, Belarus, Ukraine and Czechoslovakia. The new wave of epidemics was largely followed by significant morbidity and mortality in humans, and the phenomenon occurred in 1990 in Algeria, Romania, Tunisia, Israel and Sudan. In 2003, the disease broke out in southern Hungary and captured a flock of domestic geese, and it was the line 1 of virus. The first occurrence of the line 2 of the West Nile Virus in the territory of Europe is related to infected birds of prey in the southeastern part of Hungary in 2006. This virus line has become an enzootic for the Carpathian region, expanded to Austria in 2008, causing an unexpected epidemic in Greece in 2010. Line 1 continued to circulate in northern Italy during 2008-2009. Targeting primarily horses and people. Activity of line 1 was established on the Iberian Peninsula in 2001 and 2007, both in Spain and Portugal.

Many bird species can be the primary hosts of the West Nile Virus, although besides them, other mammals, reptiles and amphibians can be hosts (McLean et al. 2002). All infected host do not transmit viruses to vectors, but only those species in which the virus replicates so frequently that viremia is high enough to infect mosquitoes that feed on the viremic animals. The estimation is that female mosquitoes enter 0.5 to 1 μl of blood of the host during their blood meal, so that the infected host must have a minimum viremia of 1,000 to 2,000 MIU (Mosqito Infectious Units) per ml of blood in order to establish the transmission cycle is usually estimated as 105.5 to 107 PFU / ml (plaque-forming unit), it depends on the species of mosquitoes and the population. In North America, birds developed a titre of viraemia to 1012.6 PFU / ml. A high blood bird titer is necessary for the infection of a less efficient vector such as *Cx. pipiens pipiens*. The natural enzootic cycle of WNV in North America mainly involves transmission between birds (order: *Passeriformes*) and *Culex spp*. mosquitoes. For this reason, the term "host competence" is being used, it's meaning that the host develops a enough titer virus to infect mosquitoes and it is 105.5 to 107 PFU / ml (Komar et al. 2003) for mosquitoes of the genus *Culex*. Competent hosts for the West Nile Virus are almost exclusively among the bird class (Van der Meulen et al. 2005).

In North America after the virus entered the west hemisphere in 1999, there was the high level of illness and high mortality recorded in wild birds, but the virus circulation in the "Old World" territory did not result in massive disease or mortality in domestic European bird species. However, more detailed monitoring and targeted trials reveal that some species of birds are regularly subject to infection with the West Nile Virus in Europe. Clinical cases of disease and mortality associated with the West Nile Virus, both in wild and in captive birds, have been regularly recorded in Hungary since 2004. Disease was most commonly diagnosed, often in high mortality (for example, 27 WNV related deaths in Hungary have been confirmed both pathomorphologically and virologically between 2004 and 2009) among northern goshawk (*Accipiter gentilis*). The non-autochthonous species of North Falcon (*Falco rusticolus*) and Haris's Falcon (*Parabuteo unicinctus*) and an autochthonous Falcon peregrinus used in falconry, were also affected by the mortality associated with the West Nile Virus. The diagnosis for WNV infections with mortality was set up in the case of birds such as nestling females free-flowing the Gray Vetrus (*Falco vespertinus*) and sparow hawk (*Accipiter nisus*) in Hungary. The goshawk and the Gypaetus barbatus, the snow owl (*Bubo scandiacus*) and kea (*Nestor*

notabilis) are infected with WNV during the expansion of line 2 in Austria. In Spain, the disease and death associated with WNV infection was diagnosed with the Spanish Crusader (*Aquila adalberti*) between 2001 and 2005 (Höfle et al. 2008) and in the Raw eagle (*Aquila chrysaetos*) in 2007. Other cases of WNV infections without death cases were diagnosed with a golden eagle and a mountain eagle (*Hieraaetus fasciatus*) during 2007. Virus strains isolated from these birds belonged to the WNV line 1. Mortality as a result of infection with WNV in young white cubs (*Ciconia ciconia*) was recorded in Israel during the autumn migration in 1999 (Malkinson et al. 2000).

The West Nile Virus is also replicate in many mosquito species among arthropods. It has been found that in the United States, 59 different types of mosquitoes were established to support replication of the virus and 28 in Italy (Hayes et al. 2005). Although members of the genus *Culex* are the most important vectors, because they are the predominantly ornithophilic species (preferring birds for taking a blood meal), other species of mosquitoes are also significant as a "bridge" vectors. Those species feed equally on both birds and mammals and in this way transmit the bird virus as a reservoir of infection to other susceptible species. What is even more interesting is that mosquitoes of the genus *Culex*, which are said to be ornithophilic species, when most migratory birds go, start to predominantly affect mammals and humans, and this is one of the explanations why the West Nile fever epidemic in the human population occur mostly at the end of summer and early autumn. Other types of arthropods, such as ticks, are sensitive to infection by the West Nile Virus, so the virus is isolated from both Argasid and Ixodid ticks, but their role in virus transmission is still unexplored completely (Lwande et al. 2013).

Although WNV infects many of non-bird vertebrates, most mammals including humans are tangential or so-called "Dead-end" hosts because they do not develop viremia in sufficient titers to infect mosquitoes that are fed on them. However, in some rodents, rabbits and squirrels, the experimentally obtained virammic titer is capable of infecting mosquitoes. In addition to WNV, infected alligators can develop a viable blood titer sufficient to infect mosquitoes and they can play a role in the maintenance and transmission of viruses.

The West Nile virus causes a serious neuroinvasive life-threatening disease in humans and horses. However, most of these infections are asymptomatic. In humans, about 20% of the infected person develops a febrile illness called West Nile fever with similar flu-like clinical symptoms that lasts for about 6 days where elevated temperature, headaches, muscle pain, vomiting, diarrhea and other mild symptoms occur. One of the 140 infected people develops a neuroinvasive disease characterized by meningitis, encephalitis, paresis, or paralysis with a death outcome in 4 to 14% of cases. The risk of neuroinvasive disease is significantly increasing with the age of the diseased.

With the horse, most infections are also mostly asymptomatic. About 10% of clinically diseased horses develop a serious neuroinvasive disease characterized by encephalitis and neurological signs (paralysis limbs, facial tremor...) that has lethal outcome for one third of diseased horses.

The group of wild pheasants is the largest and most productive hunting species of birds in Serbia and in hunting areas around the world, all which thanks to their high adaptability to different habitats and all the changes that occur in nature. In support of these facts, there are also data showing that the increase in the number of pheasants in the hunting grounds has had a positive impact on the increase in hunters interest in hunting this type of bird, as well as increasing the demand for the market for quality of their meat.

Pheasant (*Phasianus colchicus*) is a widespread species from the genus *Galliformes*. The Pheasant originates from the region of Central Asia, but in the time of ancient Rome, he was brought to

Europe, where he quickly adapted to the existing conditions and spread in large numbers throughout Europe. It settles predominantly forest and meadow zone, often also appears in agricultural crops, as well as in coastal areas where there is plenty of food.

The aviary are semi-open type objects used for breeding, reproduction and preservation of pheasants until the moment they are released into the hunting ground. That area mainly has a rounded production cycle, with different age categories of animals, and consist of aviary for breeding flocks, egg storage facilities, incubator stations, rooms for the upbringing of young pheasants and aviary in which the pheasant is raised up to the moment of release into the hunting ground. There is a high risk of contact with mosquitoes if we have in mind that these are semi-open type objects that are mostly located nearby or in the hunting areas outside the settlement. The semi-open method of keeping pheasants implies that the individuals are in the breeders which have fences that allow contact of those birds with mosquitoes. In this way there is a high probability of infecting birds with bite of infected mosquitoes.

Having in mind the presence of West Nile virus in the mosquito population in 2012 in Belgrade, and that the virus is constantly maintained in the mosquito population over the coming years, there is a possibility that the pheasants are reservoir of the virus. In support of this, the research carried out by Đorđević et al. (Đorđević et al 2017) where the presence of the virus in the mosquito population was demonstrated in the pheasant pavilion from July to September. There is not enough research about the West Nile Virus and wild pheasants. In a study conducted by Komar with associates in 2003 (9), it has been proven that an experimental infection of the Phasianidae family which includes red-headed partridge (*Alectoris rufa*), the Japanese quail (*Coturnix japonica*), the pheasant (*Phasianus colchicus*) and the Canadian tetreb (*Centrocercus urophasianus*) can develop viraemia. Birds was infected with the NY99 strain of the West Nile Virus and, depending on the species, developed a different degree of viremia. Thus, in the partridge viremia was high, in quails and pheasants were low, and in the tetrao viremia was on middle level. This study has shown that pheasants can be reservoirs of the West Nile Virus, i.e. that they represent a target population, but recent research did not show how they can implicate the spread throughout mosquitos.

Conclusion

The presence of West Nile virus in the territory of Belgrade and Serbia general has been reported in long period of time in less or higher volume. No massive dead birds were recorded, which was the case in New York in 1999, when many birds were found death by city parks. This is probably due to the presence of virus on the field for a long time before it was first detected in the mosquito population in the territory of Belgrade and the adaptation of the reservoir animals (birds) to the virus. In accordance with the previous said and because pheasants may be West Nile reservoirs, it is necessary to determine the status of mosquitoes in the pheasant station of the presence of the West Nile Virus, as well as the status of pheasant specimens for the presence of the West Nile Virus by taking oropharyngeal swabs. Upcoming research can give a clearer picture of the risk assessment carried out by pheasant station as a farm facility that are often located in the zone of the settlement and potentially can bring health risks to animals and the population.

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RECENT AND FUTURE SPATIAL DISTRIBUTIONS IN GOLDEN JACKALS, WOLVES AND FOXES IN SLOVENIA

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Abstract: During the last decades, the golden jackal (*Canis aureus*) population in SE Europe has expanded across the Balkan and also further into Europe. The species is highly adapted to thrive in human-dominated landscapes. A similar trend is significant also for Slovenia, which has experienced a rapid increase in the jackal distribution range, which however overlaps with the range of wolves (*C. lupus*) and red foxes (*Vulpes vulpes*). Moreover, during the last decade the abundances of both jackals and wolves have markedly increased in Slovenia putting in question hypothesis on "decreased competition" with wolves as a main driver for a recent jackal expansion. The red fox is widely distributed and the most common canid in Slovenia, that lives in all of the potential jackal habitats. The current evidence for interspecific competition between these two canids is mostly circumstantial and indirect, however studies indicate that effects can be seen through strong food niche overlap between the two canids, or in some areas, strong competition has been indicated indirectly, i.e. by remarkable declines in red fox hunting bags.

To explore the hypothesis on competition and to predict potential overlapping between the three species, we developed habitat suitability models/density distributions for each species. All data on their distribution densities in Slovenia were collected from the national Hunting and Monitoring databases. Overlapping distributions and HS models of the three species indicate: (1) that jackal's current distribution in Slovenia is not overlapping with resident wolf areas as well as not with suitable habitats for wolves even where wolves are not present, (2) jackals are present primarily close to human settlements, highly forest-fragmented areas and areas with favorable snow conditions. (3) Jackals' distribution completely overlaps with high density fox areas in Slovenia, and 10-year trend in red fox harvest in these areas has not shown any decline in their abundance yet. (4) According to jackal's HS model and current red fox densities, we can expect highest competition between the species in NE Slovenia in the future.

Keywords: golden jackal, habitat suitability, distribution overlapping, grey wolf, red fox

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TRAFFIC-RELATED MORTALITY OF WILD UNGULATES AND LARGE CARNIVORES IN SLOVENIA: SITUATION AND MITIGATION MEASURES

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Abstract: Worldwide, collisions with large mammals (particularly wild ungulates) provoke a considerable risk for road safety and represent an important economic loss; moreover, they significantly contribute to wildlife mortality, represent an important risk for viability of endangered species, and hamper the outcome of the population management. In Slovenia, annual road-kill of wild-living ungulates is between 4,900 and 6,800 individuals, and railway-kill between 170 and 250 animals. In the period 2010–2018 (till the end of October), a traffic (road + railway) related mortality by species was as follows: 46,997 (45,891 + 1,106) roe deer, 1,667 (1,265 + 402) red deer, 1,090 (906 + 184) wild boar, 76 (70 + 6) fallow deer, 51 (46 + 5) chamois, and 29 (21 + 8) mouflon. Considering large carnivores, those figures were as follows: 120 (62 + 58) brown bears, 12 (10 + 2) wolfs, 1 (1 + 0) lynx, and 33 (31 + 2) golden jackals, respectively.

Since 2005, several activities for reducing problematics of wildlife-vehicle collisions (WVC) have been systematically performed in Slovenia by road managers, wildlife managers (hunters) and research institutions, and unique databases providing crucial data on spatiotemporal patterns of WVC have been developed. All these allow understanding of basic characteristics/patterns of WVC, which is a perquisite for rational implementation of adequate mitigation strategies/measures.

The following data/information will be presented in the contribution: (i) General information on problematics of WVC in Slovenia and Europe. (ii) Inter-annual variability in traffic-related mortality of roe deer, red deer and wild boar in Slovenia, and main reasons for decreasing trend in road-kill of roe deer in the period 2010–2017. (iii) Spatial patterns of WVC in Slovenia, with emphasis on the importance of collecting data in a high spatial resolution. (iv) Effectiveness of deterrents, which have been implemented and regularly monitored on >150 problematic road sections since 2006. In conclusions, recent activities for reducing the traffic-related mortality of ungulates and brown bear will be highlighted, and management implications will be discussed.

Keywords: wildlife-vehicle collisions, ungulates, brown bear, mitigation measures

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TOLERABILITY OF CARNIOLAN BEE TO RESIDUAL CONTACT TOXICITY ON FIR (ABIES ALBA) AND BLACK PINE (PINUS NIGRA) ESSENTIAL OILS

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Abstract: Honey bee *Apis mellifera carnica* is adversly affected by different harmful organisms. In order to suppress them, various synthetic medicaments with negative side effects manifested through the toxic residues which remains in bee products, are used on a large scale. The use of essential oils is an alternative way of pest elimination, however, honey bees are very susceptible to such substances. Therefore first it would be necessary to study tolerability of honey bee to essentials oils. In order to study residual toxicity of essential oils of fir (*Abies alba*) and black pine (*Pinus nigra*) a trial was conducted in laboratory conditions. Young individuals of honey bee of Carniolan breed were exposed to different doses of fir and black pine essential oils dissolved in acetone (10 - 40 µL/Petri dish). About ten adult bees were put into each Petri dish which were then placed in incubator under regulated microclimate conditions. Surviving of individual bees was monitored after 24 h and after 48 h. The lower tolerance of the honey bees is determined by the application of fir essential oil. The results of study of essential oils residual contact toxicity show the necessity to further study suitable doses and to determine the action of essential oils in combined trial with bees and varroa mite.

Keywords: Apis mellifera carnica, essential oils, residual contact toxicity

Introduction

Honey bee (*Apis mellifera* L.) is a highly adaptable species, it can be bred in different climate regions what facilitated its spreading over a vast area. Direct products obtained from honey bee which is honey, in the first place, are used in human nutrition but have also been used in apitherapeutic purposes (Basa et al., 2016; Plavša and Nedić, 2015). Besides direct benefit of bee products there is also an indirect benefit of bee which is provided through pollination, the process of vital importance for survival of land ecosystem and human species (Geslin et al., 2017; Stanisavljević and Nedić, 2008). Since 2006 bee keepers have reported significant losses in beehives throughout the whole world and this situation is still present in many different regions today. Among the factors that can be indicated as the causes of global decline in honey bee population there are intensive agricultural production, poisoning of bees due to application of pesticides, inadequate nutrition and different kind of diseases and parasites (Jemec-Kokalj and Glavan, 2017; Vanegas, 2017). Varroa mite (*Varroa destructor*) is a noxious ecoparasite which inflicts losses to bee societies worldwide. Besides direct losses that it can inflict to bees and bee brood the varroa is a virus vector which can significantly contribute to weakening of bee societies and even to their complete loss. Struggle against varroa is most often conducted by application of chemical and synthetic substances but mite can develop resistance to such substances in time. In addition, some harmful residues which get retained after

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treatment of bee societies with chemical synthetic substances can be found in bee products, first of all, in beeswax (Boecking and Genersch, 2008). In order to evade secondary negative effects of chemical and synthetic acaricides, the research of natural acaricides based on essential oils has been conducted (Ruffinengo et al., 2014; Nedić et al., 2014, Ghasemi et al., 2016).

The application of essential oils in treatment of bee societies in beehives should be preceded by determination of the appropriate doses and time of bee exposing to these natural plant products since certain natural constituents can provoke increased mortality in bees (Ebert et al., 2007).

The aim of the research was to study the residual toxicity of essential oils of fir (*Abies alba*) and black pine (*Pinus nigra* J.F.Arnold) on worker bees in laboratory conditions.

Materials and Methods

The fir essential oil (*Abies alba*) and black pine essential oil (*Pinus nigra* J.F.Arnold) used in experiment, was purchased as commercial samples from "Herba d.o.o.", Belgrade, the Republic of Serbia. Essential oils were analyzed by gas chromatography (GC-FID) on apparatus HP-5890 Series II equipped with autosampler (ALS), split-splitless injector, attached to HP-5 fused silica capillary column (25 mm × 0.32 mm, 0.52 Pm 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).

The experiment was conducted with worker bees of Carniolan breed being from 0 to 3 days old. The essential oils were diluted by acetone and in different doses (10-40 µL/Petri dish) applied to the bottom of Petri dish and left to dry at room temperature for 20 minutes. For the purposes of studying the tolerance of bees to residual toxicity of essential oils 10 bees were put in Petri dishes (11 cm in diameter) in four repetitions per studied dose. Bees were fed 3 g of candy and watered with water from a plastic micro tube (1.5 mL). Control group was only on applied acetone at the bottom of Petri dish. Such prepared variants were inserted in the incubator (T = 30° C; relative humidity = 60%). Survival of bees in each Petri dish was controlled after 24 h and 48 h. The obtained results were evaluated using the ANOVA procedure and means were separated by the Duncan test.

Results and Discussion

A chemical composition of oils used in experiment is shown in Table 1. Fir essential oil used in our experiment contained five main components, a-pinene (36.4%), camphene (14.4%), b-pinene (18.9%), b-phellandrene (18.3%) and *cis*-b-Caryophyllene (5.5%). These five components made 93.5% of fir oil content. Black pine essential oil contained three main components, a-pinene (83.6%) as dominant one, and sylvestrene (5.6%) and b-pinene (4.4%). These three components made 93.6% of total content of black pine oil.

Table 1. Chemical composition of essential oils of *Abies alba* Mill. and *Pinus nigra* J.F.Arnold

RI	Components	% *Abies alba*	*Pinus nigra*
915	Tricyclene	2.5	0.3
917	a-Pinene	36.4	83.6
930	Camphene	14.4	2.6
958	b-Pinene	18.9	4.4
993	a-Phellandrene	0.7	
1009	*para*-Cymene	0.6	0.3
1012	Sylvestrene		5.6
1013	b-Phellandrene	18.3	
1079	a-Pinene oxide		1.1
1130	*trans*-Sabinol		1.0
1266	Isobornyl acetate	2.3	
1397	*cis*-b-Caryophyllene	5.5	
	Monoterpene hydrocarbons	91.8	96.9
	Oxygenated monoterpenes	2.3	2.1
	Sesquiterpene hydrocarbons	5.5	0.0
		99.6	***99.0***

The results of toxicity of essential oils applied in different doses on worker bees are shown in tables 2 and 3.

Table 2. Tolerability of *A. mellifera* to toxic effect of the *Abies alba* essential oil

Nºof variants	Doses assayed (µL/Petri dish)	Mortality after 24h	Mortality after 48h
		$\overline{X}$ ± SE	$\overline{X}$ ± SE
1.	10	0.00±0.00^a	0.00±0.00^a
2.	15	0.00±0.00^a	0.00±0.00^a
3.	20	0.00±0.00^a	0.00±0.00^a
4.	25	1.25±0.25^a	2.00±0.71^a
5.	30	0.50±0.50^a	1.50±0.96^a
6.	36	3.75±0.75^b	4.25±1.03^b
7.	40	9.00±0.71^c	9.00±0.71^c
8. (control)	0.0	0.00±0.00^a	0.00±0.00^a
		LSD_{005}=1.58	LSD_{005}=1.92
		LSD_{001}=2.15	LSD_{001}=2.61

By applying *Abies alba* essential oil in doses of 10 to 30 µL per Petri dish there was no statistically significant difference in worker bee mortality in the first 24 h (Table 2). A significantly higher bee mortality in this period was determined with 36 µL dose, while the highest mortality rate was determined with 40 µL/Petri dish dose. After 48 h worker bee mortality rate, similarly to previous period of examination, did not differ in doses from 10 to 30 µL per Petri dish. A significantly higher bee mortality was determined for the fir oil dose of 36 µL per Petri dish (4.25±1.03), while for the dose of 40 µL/Petri dish it remained unchanged and accounted for 9.00±0.71 bees.

Table 3. Tolerability of *A. mellifera* to toxic effect of the *Pinus nigra* J.F.Arnold ess. oil

Nºof variants	Doses assayed (µL/Petri dish)	Mortality after 24h	Mortality after 48h
		$\overline{X}$ ± SE	$\overline{X}$ ± SE
1.	10	0.00±0.00a	0.00±0.00a
2.	15	0.00±0.00a	0.00±0.00a
3.	20	0.00±0.00a	0.00±0.00a
4.	25	0.00±0.00a	0.25±0.25a
5.	30	0.50±0.29a	0.50±0.29a
6.	36	1.25±0.48a	1.25±0.48a
7.	40	4.25±1.38b	4.25±1.38b
8. (control)	0.0	0.00±0.00a	0.00±0.00a
		LSD_{005}=1.50	LSD_{005}=1.56
		LSD_{001}=2.04	LSD_{001}=2.13

By application of J.F.Arnold *Pinus nigra* essential oil in doses from 10 to 36 µL per Petri dish there was no statistically significant difference in worker bee mortality in the first 24 h observed (Table 3). With the highest dose of oil of 40 µL/Petri dish the highest bee mortality was determined (4.25±1.38) and it statistically significantly differed from the other variants. After 48 h of application, there was no statistically significant difference in residual toxicity of black pine oil on the bees at given doses (10 to 36 µL/Petri dish) as was the case after 24 h. The highest toxicity was determined in the variant with the dose of 40 µL/Petri dish and it caused bee mortality which statistically significantly differed from all other doses. The effect of essential oils and some of its components on honey bee and varroa mite was studied in a previous period. Although a number of essential oils was studied the bees expressed tolerance to a small number of them (Imdorf et al., 2006).

In our research worker bees showed higher tolerance to application of black pine essential oil in relation to application of fir oil. By application of fir oil in dose of 36 µL/Petri dish the oil caused a significantly higher residual toxicity compared to other applied doses. By application of black pine oil in the same dose bee mortality was not statistically different compared to doses of 10 to 30 µL/Petri dish. Imdorf et al. (1999) reported, according to Bunsen, (1991) that application of fir needle essential oil can provoke bee brood high mortality, while application of pine essential oil causes low mortality of bee brood. Good tolerance to black pine oil by worker bees may be due to the high content of α-pinene (83.6%). Imdorf et al. 2006. reported that application of α-pinene, even at high concentrations, produced little mortality of either mites or bees. Chemical composition of fir essential oil, besides α-pinene and b-pinene marked as of little harm to bees, had also a significant quantity of b-phellandrene. According to the research of Ruffinengo et al. (2005) this chemical component is low toxic to bees. Bravoa et al. (2017) studied oral toxicity of β- phellandrene (20 µg/bee) and determined that in the course of 8 days it was not harmful to studied bees. Bee lower tolerance to fir essential oil might be due to a considerable presence of camphene in its composition. The application of pure camphene in the research by Imdorf et al. 2006. at concentration of 2000-3000 µg/l in the air, caused increased mortality in varroa (from 60 to 100 %), but at the same time also affected high mortality in bees (from 20 to 40 %). Besides main chemical constituents some minor components or synergy between certain constituents of studied essential oils might be expressed on residual toxicity of bees.

Conclusion

Residual toxicity of *Abies alba* and *Pinus nigra* essential oils on worker bees in laboratory conditions was studied.

Fir essential oil applied in doses of 36 and 40 µL/Petri dish caused a contact residual toxicity of bees at an unacceptable level.

The bees showed greater tolerance to the application of black pine essential oil and only with the highest applied dose of 40 µL/Petri dish bee mortality was at unaccepted level.

In further studies which should simultaneously be conducted both on honey bees and honey bee mite *Varroa destructor*, the residual toxic effect of fir essential oil can be studied in doses lower than 30 µL/Petri dish while black pine essential oil can be studied in doses lower than 36 µL/Petri dish.

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A SURVEY OF FISH CONSUMPTION IN BULGARIA

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Abstract: Bulgaria has one of the lowest rates of fish consumption per capita in European Union (EU). In order to study the causes of this a research was conducted with 677 volunteers. They filled a poll about their fish consumption habits. The survey was disseminated through the Internet and Social Network (*Facebook*), via emails and leaflets. The data from the survey show that the price and peculiarities of the culture of nutrition are the main reasons. The largest percentage of respondents (45.8%) consume fish 1-2 times a month. Among the most sought-after species are trout, carp, pike-perch and mackerel. Leading factors in the purchase of fish products are taste, price and appearance of the product. Most of the people buy fish mainly from neighborhood shops and supermarkets. They prefer fresh fish from Bulgarian origin. According to the respondents, the increase in the consumption of fish can be achieved in addition to lowering prices even by increasing the awareness of people about its benefits to human health. Analyzing the data obtained and identifying the profile of the fish consumers in our country will help aquaculture producers to develop marketing strategies, optimize their farms and orient their production to the desired products.

Keywords: consumption, fish, marketing, questionnaire, survey

Introduction

Consumption of fish meat has a proven positive impact on human health. It has a rich mineral composition consisting of over 60 chemical elements (I, F, Ca, Cu, Zn, Fe) and a high content of vitamins A, D, K, E, B_2, B_{12}. Proteins in fish contain all the amino acids needed, especially lysine, methionine and tryptophan (Sándor et al., 2011). Fish oils are rich in highly soluble fatty acids, especially omega-3 polyunsaturated fatty acids (PUFAs). They help reduce cholesterol levels, have a positive effect in people prone to atherosclerosis, lowering blood pressure hypertension, strengthening the body against infectious diseases and reduce the risk of heart attack and heart disease (Pieniak et al., 2008). According to data from the European Observatory on Health Systems and Policies (OECD, 2017), the mortality rate in Bulgaria from these diseases is 4 folds higher than average for the EU. That is why it is extremely important that fish should be present more frequently in menu of Bulgarian consumers.

The production of fish and aquatic organisms in the country has grown almost double in the period of four years, from 7557 tons in 2012 to 15 432 tons in 2016. Imports of fish, crustaceans and mollusks were also 8% higher in the last year only. According to the information from annual Agrarian report of Ministry of Agriculture, Food and Forestry (MAFF, 2017) the number of fish farms in the country has also increased. Nevertheless, according to the data from European Commission (EC, 2018), Bulgaria holds penultimate place in the EU for consumption of fish and fish products per capita. Compared to EU average of 25.1 kg per person, Bulgarian fish consumption is only 6.2 kg per capita.

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The purpose of this study is to gather information on the causes of low consumption of fish in Bulgaria, what are the preferences of consumers of fish products and what would lead to increased consumption of fish in the country. Obtained results could be used by aquaculture sector to analyze the market situation in the future. According to Food and Agriculture Organization of the United Nations (FAO, 2007, 2018) aquaculture is expected to satisfy the world ever-increasing demand for fish products, which is projected to reach 24 kg per capita in 2030. By analyzing the results of various socio-economic groups of consumers, producers can find solutions for future marketing strategies, optimizing farm processes, improving product quality, and orientating aquaculture production to the desired fish products.

Material and Methods

Nature of research and data collecting

The research was conducted by distributing and filling in an anonymous questionnaire. It was compiled using *Google forms.* The poll consisted of 16 questions that are grouped according to the information they are expected to collect: first group - factual information on fish consumption in Bulgaria, second group – consumer preferences for fish products, third group of questions - socio-economic data for the profile of the respondents. Some of the questions were multi-responsive and, before being disseminated, the poll was tested by a group of people, lecturers in the relevant scientific field. It was then distributed via social network *Facebook*, e-mails and leaflets. Research has shown that the Internet has already proven itself to be a reliable data collection tool (Maciel et al., 2013 and 2014). The survey was conducted in period July-October 2018.

Data analysis

For data processing we performed descriptive analysis. Methodology was adapted from Maciel et al., 2016. The focus of the study was on characterizing main fish consumer profile. That is why there is no significant interest in statistical comparisons in this study.

Results and Discussion

The research involved 677 volunteers (55.8% men, 44.2% women). Of these, the most active were those aged 19-35 (56.6%) and those living in district towns (80.5%). Regarding employment, 83.7% of the respondents are working, and 67.5% of them have higher education. All the characteristics of the survey sample are shown in Table 1.

Table 1. Sample characteristics (%)

Gender	n	%	Age	n	%	Employment	n	%	Education	n	%	Live in	n	%
Male	378	55.8	< 18 years	9	1.3	Student	65	9.6	Primary	14	2.1	District town	545	80.5
Female	299	44.2	19-35 years	383	56.6	Employee	568	83.7	Secondary	206	30.4	Municipal center	89	13.1
Total	677	100	36-65 years	267	39.4	Retired	31	4.6	Higher	457	67.5	Village	43	6.4
			> 65 years	18	2.7	Unemployed	13	2.1	Total	677	100	Total	677	100
			Total	677	100	Total	677	100						

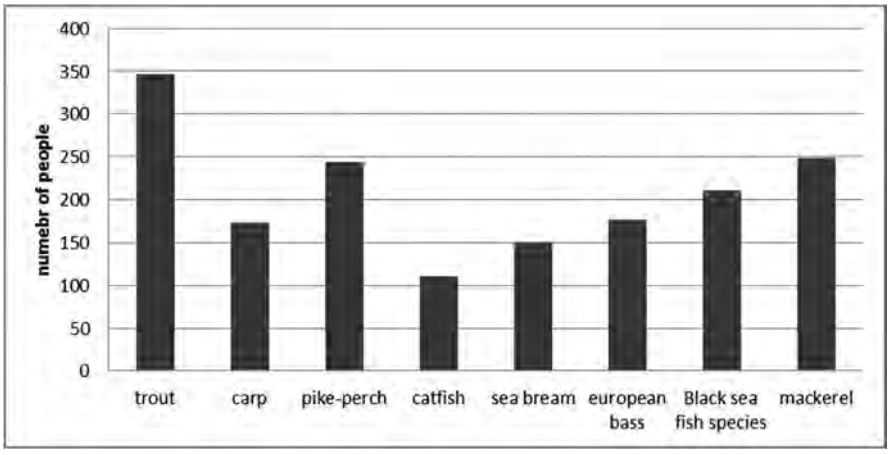
The study showed that as the main reasons for the low consumption of fish products, the respondents pointed to the high price and peculiarities in the food culture and the lack of traditions in the consumption of fish.

Concerning the frequency of consumption of fish products, the largest percentage of respondents has fish in their menu 1-2 times per month (Table 2).

Table 2. Frequency of fish consumption and reasons for the low consumption in Bulgaria

Frequency	n	%
1 to 2 times per week	175	25.8
More than 2 times per week	36	5.3
1 to 2 times per month	310	45.8
Rarely	146	21.6
Never	10	1.5
Total	677	100
Reasons for the low consumption		
High price of fish products	336	36.7
Peculiarities in the culture of nutrition and lack of traditions in the consumption of fish products	323	35.3
Low quality of the offered fish products	180	19.7
A small variety of products	61	6.7
Other	15	1.6
Total	677	100

Some of the most commonly consumed species are listed in Graph 1. The top three places occupy respectively trout, mackerel and pike-perch, which are most often present in the sample answers. But there are some differences between preferred species in district centers comparing to municipal centers and villages. The most consumed species of the respondents in district centers are trout, pike-perch and mackerel and in municipal centers and villages are trout, carp and mackerel.



Graph 1. The most commonly eaten fish according to the questionnaire

The results indicate that the most favored fish products are fresh, and processed fish products (tinned, fillets).

More than half (56.2%) of the participants responded they prefer fish products originating from Bulgaria. Asked whether they would pay a higher price for fish produced in certified farms, 58.5% responded positively and women were more likely to pay for higher prices (H chi squ. = 5.2, p <0.01).

The answers of 36.8% of respondents are disturbing, since they do not know the origin of the fish products they consume. Responders buy fish mostly from neighborhood stores and retail chains, and most rarely buy fish products online. It is alarming that there are people who say they have no access to fish products.

With the potential to increase fish and fish products consumption in the country, respondents pointed to price drop (29.4%), followed by increased awareness of the health benefits of fish consumption (25%), and product quality (24%). On the question of what is decisive in the purchase of fish products, the highest number of respondents (30.8%) singled out taste as the most important, followed by the appearance of the commodity (29%) with the price of fish ranked only in the third place (17%).

For prevention of cardiovascular diseases and good health, the World Health Organization (WHO) recommends eating fish 1-2 times a week. However, the results of the poll show that in Bulgaria only 27% of the people fulfill this recommendation. The highest is the percentage (45.8%) of people who consume fish products 1-2 times a month. As with other similar studies conducted in different countries, the main reasons for low consumption are high price (Maciel et al., 2015, Haq et al., 2014, Myrland et al., 2000,), the peculiarities of the culture of nutrition and the lack of traditions in fish consumption (Boyadzhiev, 2011). Mentioned factors, combined with the low income level of citizens of Bulgaria, determine bottom place for fish consumption in EU. On the other hand, in Spain, the EU country with the highest consumption of fish per capita (55.9 kg) and ranked on third place in the world (Almeida, 2015), 77% of the population consume fish 2 or more times per week (Maciel et al., 2016).

A number of artificial lakes on the territory of Bulgaria have unused capacity for aquaculture development (Boyadzhiev, 2011). The results of the survey show that trout, which is one of the most frequently farmed species in Bulgarian farms (MAFF, 2017), will continue to be one of the most popular species in the market. Producers of fish and aquatic organisms can increase the added value of their products by certifying the farms. Over half of polled people (58.5%) would pay a higher price for fish products reared in line with best aquaculture practices.

A total of 36.9% of respondents responded that in order to increase the consumption of fishery products in Bulgaria, with the participation of the Ministry of Health and MAFF, a large-scale campaign could be made to raise awareness of the health benefits from the consumption of fish products. Advertising (television, internet, radio and newspapers) of fish and fish products would also encourage consumers to buy them more often.

Conclusion

This research presented some information as main reasons for low fish consumption in Bulgaria. In the survey, there are no significant differences in the responses to socio-economic groups surveyed. As a limiting factor for consumption of fish and fish products in Bulgaria, the respondents determine its high price and the lack of traditions in fish eating. The highest consumption of traditional species such as trout, white fish, mackerel and carp is maintained, but market demand for product diversity

is increasing. Participants in the survey pay close attention to the condition of the products they buy and are willing to pay a higher price for better quality products. The analysis of the results shows that if a large-scale campaign to raise awareness of the benefits of chewing meat consumption and advertising (TV, radio, print media) of fish products; this would lead to an increase in consumption. Identifying the profile of the fish consumer is very important for the aquaculture producers because the market demand is decisive for the development and the planning of their activity.

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AQUACULTURE INSURANCE APPLICATIONS IN TURKEY

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Abstract: Today, the aquaculture sector faces many risks and uncertainties, especially natural risks such as floods, storms, landslides and diseases. As a result of these risks and uncertainties, the quantity and quality of the fisheries products obtained from the aquaculture sector can be reduced. These declines caused by unexpected risks affect adversely the amount of fish production in Turkish aquaculture sector. The most important tool to ensure the losses caused by these risks and uncertainties is the aquaculture insurance. Risks that are affecting fish production are transferred by the aquaculture insurance from the fish farmers through the insurance sector. The first aquaculture life insurance application was started in 1990s in the World. However, the first Turkish aquaculture life insurance was applied in 2007 after publication of Agricultural Insurance Act in Turkish Official Gazette and establishment of Turkish Agricultural Insurance Pool (TARSIM) in the year 2005. In this study, aquaculture insurance, its coverage and encountered risks have been evaluated with a comprehensive review of insurance practices in Turkey.

Keywords: Aquaculture, risk, insurance, underwriting, premium, claim

Introduction

The aquaculture sector is a very important and strategic sector with agriculture and livestock sector for basic needs of all countries such as nutrition, employment and development. However, production is very vulnerable and sensitive because it depends on natural conditions. Aquaculture is one of the most affected sectors by natural, economic and social risks. The agricultural, livestock and fisheries sector is a very important sector that needs to be supported both economically and strategically throughout the world which is necessary to take measures against all possible risks (Keskinkılıc, 2013; Sumer and Polat, 2016).

There is a need for a kind of player such as insurance that can take all the risks in agriculture, livestock and aquaculture and eliminate the fears caused by uncertainties. Insurance is a risk transfer system that is used to cover the losses of insured person who is faced with the same or similar hazards caused risks. Risks that are affecting aquaculture are transferred by the aquaculture insurance from the fish farmers through the insurance sector (Dogan, 2012). The most general definition of insurance is a risk management system designed to reduce or eliminate the negative consequences of possible risks that people and companies may face in the future (Keskinkılıc, 2013)

Today, the most important and effective risk management tool for the agricultural sector is seen as agricultural insurance. Many countries understand the importance of agricultural insurance in terms of sustainable agricultural production and continue to develop agricultural insurance systems (Sumer and Polat, 2016). The first agricultural insurance practices started in the 18th century in the world and the first agricultural insurance was carried out in 1797 against the hail risk of crops in Europe. In Turkey, insurance companies have started it by making insurance against the hail risk

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of crop production since 1957. Then, in 1960 livestock life insurance, in 1984 poultry life insurance, and in 1990 aquaculture life insurance were started in Turkey (Keskinkılıc, 2013).

The aquaculture sector is one of the most important sectors within the agricultural sector both economically and strategically. Production is generally carried out in open areas, so the sector is directly affected by many risks and uncertainties, especially natural risks. In order to make a healthy, productive and sustainable production, it is important to minimize the effects of these risks and uncertainties. The most important risk management tool that provides protection against these risks and uncertainties is Aquaculture Life Insurance (Sevim, 2010).

In this study, the overall structure of the aquaculture insurance, risk management issues, aquaculture insurance applications in Turkey are reviewed. Some information and recommendations related to the Agricultural Insurance Pool (TARSIM), which was newly established in the year 2007 by the Agricultural Insurance Act in Turkey were evaluated.

History of Agriculture Insurance Applications in Turkey

The establishment of agricultural insurance system and its implementation in Turkey have been consistently thought for a long time by Turkish Government. However, this idea was not put into operation until the Agricultural Insurances Act, which came into force in 2005. Studies on the agricultural insurance in Turkey began in 1920s, had dealt with various institutions and organizations and had conducted various studies on the subject. In 1926, the first agricultural insurance act was stated in the Turkish Commercial Code as articles 1316, 1317, 1318, 1319. The first agricultural insurance policy was made by Seker Insurance Company in 1957 for sugar beet producers as herbal crop insurance policy. In 1960, Basak Insurance Company began its crop insurance against hail damage and animal life insurance activities (Çetin 2007; Çetin & Turhan, 2013).

In 1995, the Agricultural Insurance Foundation (AIF) was established with the insurance companies operating in the field of agricultural insurance. Since 1995, AIF has provided risk assessment, damage expertise and claim payment procedures until 2006, with the establishment of the Agricultural Insurance Pool in 2006, and all of AIF data and systems were transferred to the Agricultural Insurance Pool (TARSIM) (Cetin 2007).

Aquaculture Insurance Applications in Turkey

Although aquaculture insurance has a 1% share in global agricultural insurance market, it is foreseen to increase its market share due to increasing fish farms due to the rapid decrease in natural fish stocks. Flatfish, shellfish and mollusks and commercially important algae production are also covered by this type of insurance. Aquaculture insurance covers meteorological events, natural disasters, pollution, predators, oxygen depletion, collision, and changes in pH and salinity rates of water, protection against theft and escaping risks (Iturrioz, 2009).

Aquaculture insurance requires more expertise than other agricultural insurances due to the complex production stages of aquaculture. In this context, underwater assessment is carried out during insurance and damage assessment (Iturrioz, 2009). In Turkey, aquaculture life insurance has been provided by TARSIM to fish farmers since 2007 after the risk analysis of fish farm in marine and inland waters (TARSIM, 2018). Aquaculture insurance policy covers the following risks. (1) For farmed aquatic organisms: All kinds of diseases except written in general conditions, pollution

and poisoning except caused by farmers, all kinds of natural disasters, accidents, predators, algal bloom due to the mass mortality and financial losses in the farmed stocks. (2) For cages and nets: The financial losses caused by all kinds of natural disasters, accidents, predators are included in the coverage (TARSIM 2018).

According to the data obtained from TARSIM (2018), there are 63 trained expert and 48 of them actively serve in aquaculture insurance field (Table 1). Although the number of experts actively working in the field seems to be less, this is sufficient for now when the available farms number and insurance companies are considered. Year by year, there is an increase in policy number, insurance amount and collected premium rates (tables 2 and 3)

Table 1**.** Number of aquaculture and other agriculture insurance experts in Turkey (TARSIM, 2018)

Type of Insurance	Trained and certified expert number	Active employee expert number
Aquaculture Life	63	48
Other Agricultural	3025	1869
Total	3088	1917

Table 2. Changes in aquaculture insurance policy number and premium amount rates (%) (TARSIM, 2018)

Years	2013	2014	2015	2016
Policy number	-57.5	29.4	11.4	24.5
Insurance amount	-89.3	93.5	-14.5	46.6
Collected premium	-89	109.5	-14.6	51.3

Table 3. Changes on aquaculture insurance policy, premium, government subversion and claim payments (TARSIM, 2018)

Years	Policy number	Insurance amount (€)	Collected premiums (€)	Government subversion for premiums (€)	Claim Payment (€)
2013	34	5,137,185	223,637	111,818	2,785
2014	44	9,938,840	468,595	234,297	27,746
2015	49	8,495,100	400,017	200,008	172,541
2016	61	12,453,283	605,178	302,589	224,803

Policy Request, Underwriting, Premium Payment

The fish farmer applies to insurance companies or agencies that are member of Agricultural Insurance Pool (TARSIM) for an aquaculture insurance policy. TARSIM begins to examine the risks after the registration of the preliminary information form which is regulated by the policyholder's declaration. According to the results of the risk assessment process conducted by TARSIM, the

government, which subsidized aquaculture insurance policy, is issued by the agency in TARSIM system according to general conditions and tariffs and instructions (Figure 1). Firstly, information form was printed from the system as two copies and one copy was given to the farmer and a form was signed by farmer. Then insurance agency arranges the policy as two copies and gives a copy to the insured farmer (TARSIM, 2018).

Figure 1. Flowchart for insurance request, request handling process and underwriting. ARS: aquaculture record system (TARSIM, 2018)

The 50% of the premium stated in the policy shall be paid by the Turkish Government. The 25% of the temporary premium amount calculated according to the monthly average stock value specified in the breeding plan is charged from the insurance. The remaining amount of premium is charged in 5 installments. When the insurance policy expires, premium arrangement are done according to the monthly average stock values calculated during the production period (TARSIM, 2018)

Insurance Claim Request, Inspection and Claim Payment

If there is any risk within the scope of the guarantee for the aquaculture life insurance, the insured farmer shall be notified to the phone numbers specified in the policy within 24 hours at the latest. After a claim request, an insurance expert is appointed by TARSIM for assessment of the damages in insured aquaculture stocks as soon as possible and makes the necessary inspections. Afterwards, aquaculture insurance expert prepares damage assessment report. When the damage inspection is completed, the finalized compensation amounts are remitted payment to the insured farmer's bank account by TARSIM within 30 days at the latest (TARSIM, 2018) (Figure 2).

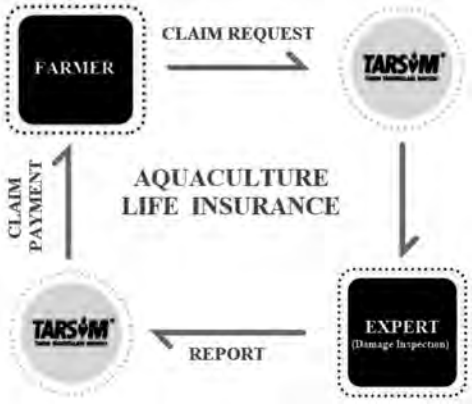

Figure 2. Flowchart for claim request and claim payment process (TARSIM, 2018)

Conclusion

Agricultural insurance guarantees the livestock and crops of farmers against various risks, and tries to prevent the producers from economic losses. In addition, this system contributes to the sustainable agricultural production by encouraging the farmer for production (Sumer and Polat, 2016). In Turkey, many agriculture insurance applications from other countries have been examined and evaluated. Turkish Agriculture Insurance System is based on the Spanish Agriculture Insurance System that made significant advances in the field of agricultural insurance. In accordance with this model, the Agricultural Insurance Act was enacted in 2005 and the Agricultural Insurance Pool (TARSIM) was established in 2006 (Sumer and Polat, 2016).

As a result of enacting the Agricultural Insurances Act, a remarkable success was achieved with the cooperation of public and private sector in the aquaculture sector. After enacting the agriculture insurance act, within a short period such as 12 years, it has been made significant progress in all areas of agricultural insurance with TARSIM in Turkey.

In the light of the data presented in tables (1, 2, 3), it is seen that farmers have an understanding of the importance of insurance at positive levels and tend to increase underwriting policy, but could not reach the desired levels. However, in order to operate the system more effectively and properly, efforts should be ensured to join more producers to the system and the amount of premiums should be minimized. Lower premiums will also lead to an increase in the number of insurance policy. The costs will be reduced and sustainability of the system will be ensured by this increase. However, for more effective system, rapid and realistic damage assessment is very important during claim process. The government support is also important for the system sustainability.

In order to eliminate such deficiencies, TARSIM, the Ministry of Agriculture and Forestry and the Fish farmer's associations should work together and organize information meetings and should reach to all farmers individually. Efforts should be made to expand the risk coverage of insurance to ensure sustainability in the aquaculture sector in the future.

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THE EFFECTS OF TAX BURDEN IN THE CATTLE PRODUCTION

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Abstract- Fiscal policy represents a complex area through which the state sets out the operating conditions through various instruments. The aim of this study was to demonstrate the importance of optimizing the rate of value added tax (VAT) in the case of beef cattle production. It is analysed how much tax burden affects the production volume and the budget revenues. The analysis showed that at different VAT, tax revenues vary. The difference between the largest and smallest analysed the VAT rate was 8% (10 % vs 18%), while the monetary amount of taxes was multiplied by 8.56 times. Furthermore, the state can also have negative consequences if it wants to raise the VAT rate above the realistic framework. By imposing large taxes, taxpayers feel less incentive to make money because everything they earns goes to the state. Therefore, in determining the VAT, its creators must be very vigilant and the objectives must be well defined in the short and long term, in order for the fiscal measures to be stimulating and in function of the growth of the economy.

Keywords: tax rate, tax revenue, cattle production, value added tax (VAT)

Introduction

Cattle production in the Republic of Croatia represents a very significant part of the economy. Various theoretical studies show that at a tax rate of zero percent and at a tax rate of one hundred percent the tax revenue is equal to zero. With the increase in tax rates, the tax revenues also increase, but after some point, the tax rate increase does not fill the state budget (Baumol and Blinder, 1991), but vice versa. The high tax rates discourage people from working and saving. In that case they pursue their economic activity to an unofficial economy, or decide on more leisure and less work. By increasing the tax rate, the state has failed to reach an acceptable measure of fiscal policy: state revenues are smaller, less labour, lower investment and lower growth. When the factors of production increase, domestic products are not competitive and the export of agricultural products is reduced (Messere, 2009). Therefore, the aim of this study was to demonstrate the importance of optimizing the rate of value added tax in the case of cattle production.

Materials and methods

Calculation of beef sales prices was made on the base of 10,000 cattle on an average of 500 kg, and fixed costs, variable costs, mixed costs and earnings were calculated. Furthermore, the offer of company and offer of branches was analysed. This analysis was made based on the microeconomic model of Hal Variana. Concluding remarks was adopted on the basis of comparison of tax rates with the amount of tax revenue. This method showed the consequences for the revenue side of the budget if the tax rate varies, and how this reflects on the total volume of production. The data analysed

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were obtained from producers Belje d.d., Gavrilović Ltd., Pivac Ltd. and PIK Vrbovec and refer to average wages. The following methods were used in this study: the analytical method and case study method. Prices are expressed in Euro.

Table 1 shows the average costs for the production of cattle weighing 500 kg, and the calculated price per kilogram of meat in Euro.

Table 1 Elements of the formation of selling prices in the Republic of Croatia

Elements of calculations	Price of fattening (500 kg)	Price of meat (kg)
Fixed costs	106.70	0.21
Variable costs	696.20	1.39
Mixed costs	104.30	0.21
Cost price	**907.20**	**1.81**

Results

In the analysis of operating costs form the pricing of beef at different rates of value added tax (hereinafter VAT). VAT represents a multistage sales tax that is levied at each stage of production and sales cycle, but only on the amount of added value that is created at this stage, rather than on the overall value of production. It should be noted here that the EU legislation allows each member to apply a reduced VAT rate to certain goods. By analysing the production of 10,000 cattle with an average weight of 500 kg, a calculation of the selling price was made at different rates of VAT. Calculation of selling price of cattle regarding the different rates of VAT is shown in Table 2.

Table 2 Calculation of selling price of cattle regarding the different rates of VAT

Elements of calculations	Calculation of selling price of cattle regarding the different rates of VAT			
	10%	13%	15%	18%
Fixed costs	1,067,000.00	1,067,000.00	1,067,000.00	1,067,000.00
Variable costs	6,962,000.00	6,962,000.00	6,962,000.00	6,962,000.00
Mixed costs	1,043,000.00	1,043,000.00	1,043,000.00	1,043,000.00
Cost price	9,072,000.00	9,072,000.00	9,072,000.00	9,072,000.00
VAT	907,200.00	1,179,360.00	1,360,800.00	1,632,960.00
Selling price	9,979,200.00	10,251,360.00	10,432,800.00	10,704,960.00

From this analysis it can be seen that at various VAT rates (10%, 13%, 15% and 18%) the amount of VAT varies. The lowest amount of VAT was EUR 907,200.00, while the highest VAT was 1,632,960.00 EUR. The difference between the largest and the lowest VAT was 725,760.00 or 44,44%. The difference between the largest and smallest analysed the VAT rate was 8%, while the monetary amount of taxes was multiplied by 8.56 times. The offer of the branches is shown on Figure 1.

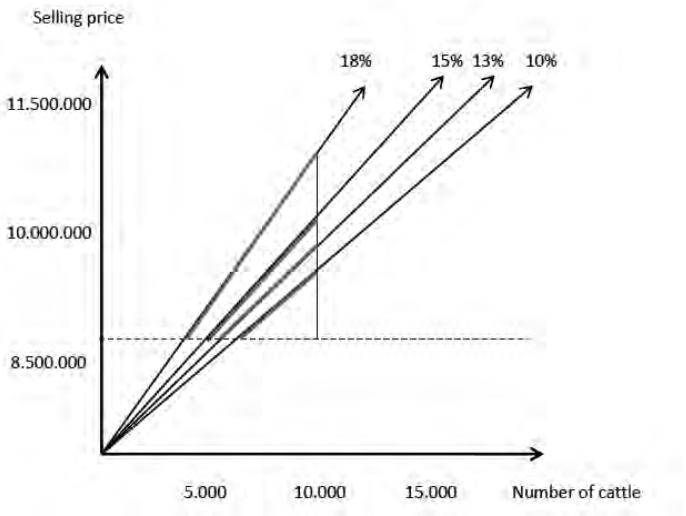

Figure 1 Offer of the branches

Offer of the branches was created based on costs and different VAT rates. The parts of the offer curve marked with red are a taxable part. It can be seen that the sections in which the tax rate is lower are flatter comparing to those where the VAT rate is higher. As the VAT rate increases, the supply curve becomes more upright and vice versa, when the VAT rate decreases, the supply curve becomes more and more straightforward.

In the branch with free entry and expulsion of goods to the market a long-term average cost curve would have to be a straight line at a price level equal to the minimum average cost. This is the long-term supply curve of a company that has constant yields on the scale (Varian, 2008). Since our market is free, and that the entry and exit from the market free, short-term supply curve branches will have a positive slope (ascending), while in the long run it becomes flatter at the level of prices with the same average cost (Barro, 1987). Samuelson (1992) stated that the introduction of higher taxes facing end consumers with higher prices.

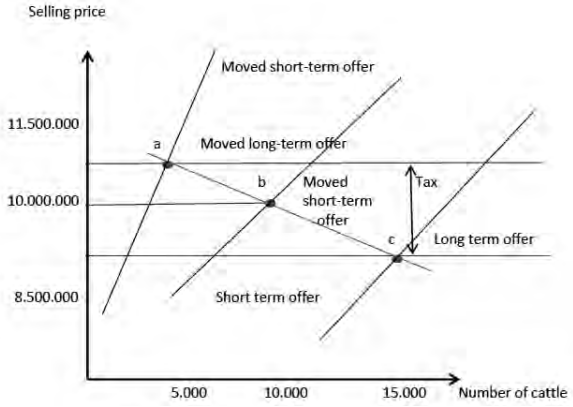

Figure 2. Changes in tax rates and the impact on the supply and demand of fattening

The variations in supply and demand curves due to changes in the VAT rates that reflect on the change in price. In the Figure 2 blue horizontal lines represent a long-term supply curve, black vertical lines represent a short-term supply curve, while red line represents the demand curve. The introduction of tax represents point c. At this point, a demand of 15,000 cattle is projected, and the short-term supply curve is under 45 degrees slope. Point b represents the limit of profitability of cattle production. This shows that in the case of introduction of VAT rate of 13%, the price amounted 10,251,360.00 EUR, the demand is reduced to 10,000 cattle and short offer becomes steeper. What is more price increases due to the introduction of higher VAT rates, the market suffers from the pressure, and the demand is reduced to 5,000 cattle, short-term supply curve becomes completely upright, moving the long-run upward and intersection with the curve of demand in point a.

Conclusion

The tax policy of the state is very important instrument which determines the market conditions. As it is presented in this analysis, at different VAT, tax revenues vary. Furthermore, the state can also have negative consequences if it wants to raise the VAT rate above the realistic framework. Based on performed analysis it could be concluded that in determining the VAT, its creators must be very vigilant and the objectives must be well defined in the short and long term, in order for the fiscal measures to be stimulating and in function of the growth of the economy.

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GENETIC AND PHENOTYPIC TRENDS FOR MILK YIELD HOLSTEIN FRISIAN COW IN THE R. OF MACEDONIA

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Abstract: In dairy cattle production systems milk production has high economic significance therefore it is included in breeding programs. The aim of this research was to evaluate genetic and phenotypic trends for milk yield trait of Holstein Frisian cow in the R. of Macedonia.

As a material for research were used data of animals calved in the period from 2011 to 2017. The data set consisted of 2520 measurements of milk yield during 305 days lactation. A phenotypic trend was partitioned into genetic and environmental components through fitting of mixed linear models that include usually fixed environmental and random genetic effects. The genetic trend was expressed as changes in averages of breeding values across calving years and the environmental trend was calculated as changes in GLM solution for fixed effect of calving years. Estimation of variance components and heritability was performed using the restricted maximum likelihood (REML) and mixed model methodology (BLUP) was used to predict the breeding value of sire. The model contained fixed effect of farm, lactation number, calving year and random effect of sire. Statistical data processing was performed using the WOMBAT software (Meyer, 2006).

Heritability estimate amounts 0,294 and indicate that effective genetic improvement should be accompanied by a corresponding improvement of the production environment. Based on regression analysis we calculated the positive phenotypic and environmental trends (on average 63.88 and 153.23 kg, respectively) and negative genetic trends (on average 89.35 kg). Regardless of the achieved increase in milk production at the phenotype level, a genetic trend represents negative genetic response across a period of time. This may indicate that environmental factors such as management, feeding, housing not supporting and limit the expression of genetic improvement.

Keywords: cattle, Holstein cows, milk yield, trends

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THE IMPACT OF THE RELATIVE LEVEL OF MILK PRODUCTION, THE SHARE OF HOLSTEIN FRIESIAN GENES AND LACTATION ON METABOLIC DISEASES OF DAIRY COWS

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Abstract: High incidence of metabolic diseases in dairy cattle populations together with disturbed milk production is an increasing problem in modern cattle production. A major objective of this paper was to study the impact of relative level of milk production, lactation and share of HF genes on the occurrence of incidence of metabolic disorders as the reasons for culling the dairy cows. The trial was conducted on population of Black and White cows which were raised on 7 PKB farms and realised their production in the period from 2000 to 2012. The analysis included 897 cows, which realised 5 lactations maximally. The incidence of four clinically diagnosed bovine metabolism diseases of great economic importance to milk production such as milk fever, ketosis, fatty liver infiltration and abomasum displacement were studied. The incidence of milk fever and ketosis was highest in IV, and lowest in I lactation, while the incidence of fatty liver infiltration and abomasum displacement was slightly higher in first two and first three lactations compared to the last ones (IV and V lactation). The highest ratio of cows culled due to metabolic diseases was observed in the cows that were located in the part of the herd which realised production in the range of one standard deviation below and one standard deviation above the average of given herd, i.e. farm. The cows with the lowest share of HF genes (up to 50%) manifested highest occurrence of incidence of milk fever and ketosis, cows with share of HF genes ranging from 75 to 87.5% manifested highest incidence of fatty liver infiltration while abomasum displacement had highest incidence in cows with maximum share of HF genes (over 93.75%).

Keywords: culling reason, dairy cattle, incidence, metabolic diseases

Introduction

The incidence of organism dysfunction manifested through different metabolic diseases is not so rare. The most common metabolic diseases in dairy cattle are: ketosis, tetany, milk fever, fatty liver infiltration, abomasum displacement and other. These diseases can to a great degree disturb bovine health state and are reflected in reduced milk production, decline in fertility and working capability, decreased immune response, degeneration of muscles and other diseases which can result in an overall weakness of organism besides its other dysfunctions (Webster, 2000; Oltenacu and Algers, 2005; McLaren et al., 2006; Mulligan and Doherty, 2008).

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In addition, all these body changes can make the organism susceptible towards secondary diseases. There is an opinion that in order to include certain disease into a programme of systemic monitoring that would prove justified from an economic and health state aspect it should fulfil certain conditions, first of all, high frequency (up to 5% and more) and disturbed milk production.

Standards for defining metabolic diseases are prerequisites for any breeding programme formulated for monitoring and analysis of these disorders (Kelton et al., 1998). In order that bovine metabolic diseases should be treated properly it is necessary first to define the disease on the basis of clinical and subclinical diagnosis, and second, given that disease is measured consistently, to begin assessing the frequency of each individual disease. Disease frequency can be determined as the incidence, i.e. the rate of new cases per time unit, or prevalence, i.e. the percentage of affected animals at one moment (ICAR, 2014). Programmes of monitoring can be different between the countries. Health data can be collected either by breeder or veterinarian. In Nordic countries certain health traits have been included in routine genetic estimations. In Austria a system based on veterinary diagnoses of health events (Egger-Danner et al., 2012) was started in 2006. A similar, obligatory farm register was started in Belgium in 2016 while Great Britain in 2011 began to develop system both for monitoring health and welfare of domestic animals. For the countries that have no health record established system, recommendations for collecting data and defining the traits, as well as use of health data for genetic estimations are provided by ICAR (2014). ICAR guidelines for disease scanning give description of complete register of diagnoses with approximately thousand entry options. In addition, diseases can be classified regarding whether they occur once in a lifetime of individual, once per lactation or more than once during lifetime.

In this paper, frequency of incidence of metabolic disorders has been founded on database which incorporated the reasons for culling of the Black and White cows. Cows can be culled in different life and productive age and reasons for culling can be quite various. Beaudeau et al. (1993) analysing the reasons for culling, determined that more than half culled dairy cows had some form of health disorder. In USA the share of culled animals due to health problems can reach even 79%, prevalent consequences thereof being reproductive disorders, injuries and metabolic diseases (Hadley et al., 2006).

Material and Method

The trial was conducted on a population of Black and White cows which were raised on 7 PKB farms and realised their production there in the period from 2000 to 2012. The analysis included 897 cows which realised maximally 5 lactations and whose origin and production results were known and recorded. These animals also had some of monitored metabolic diseases observed registered as the reasons for culling. Following metabolic diseases, as reasons for culling, were monitored: milk fever, ketosis, fatty liver infiltration and displacement of abomasum. The share of animals culled according to each of monitored metabolic disease depending on lactation in which animal was culled, relative milk production in first lactation and share of genes of Holstein Friesian breed were analysed. Relative milk production in the first lactation is obtained when total milk quantity in first lactation is regarded as relative yield of milk in relation to average production of the farm which given animal originates from. Differences that can occur in this way are expressed as the number of standard deviations below or over the farm average on which the animal was producing. According to this procedure all monitored animals were divided into 9 classes.

Table 1: Classes according to relative level of production

Class	Range expressed in standard deviations in relation to farm average
I	x < -1.5 SD
II	-1.5 SD < x < -1 SD
III	-1 SD < x < -0.5 SD
IV	-0.5 SD < x < -0.2 SD
V	-0.2 SD < x < 0.2 SD
VI	0.2 SD < x < 0.5 SD
VII	0.5 SD < x < 1 SD
VIII	1 SD < x < 1.5 SD
IX	x > 1.5 SD

An analysed population of dairy cows was created by introducing the genes obtained by crossing the animals which belonged to the group of European Black and White cattle and the animals of Red Danish breed into a Holstein Friesian breed because the analysed animals were in different phases of crossing and with different share of genes of Holstein Friesian breed. Based on the share of Holstein Friesian breed genes the trial animals were divided into 5 groups:

- Group 1- animals with share of Holstein Friesian genes from 0 to 50%,
- Group 2- animals with share of Holstein Friesian genes from 50 to 75%,
- Group 3- animals with share of Holstein Friesian genes from 75 to 87.5%,
- Group 4- animals with share of Holstein Friesian genes from 87.5 to 93.75%,
- Group 5- animals with share of Holstein Friesian genes over 93.75%.

The share of animals culled due to some metabolic diseases was calculated by means of PROC FREQ procedure within SAS programme package (SAS; SAS Institute, Cary, North Carolina, USA).

Results and Discussion

The results in Table 2 show that incidence of milk fever and ketosis as reason for culling was highest in IV and lowest in I lactation, while the incidence of fatty liver infiltration and displacement of abomasum was slightly higher in the first two and three lactations in relation to the last two lactations (IV and V lactation). Thus, for instance, in first lactating cows and cows in succeeding lactations a similar incidence of fatty liver infiltration was observed as the reason for culling (41% in I and 44% in V lactation). The herds with high prevalence of ketosis in the first lactations usually display increased incidence of abomasum displacement (Oetzel, 2004), what has been confirmed by our research as well.

According to Kelton et al., 1998, reported incidence of milk fever in cows expressed through median line on the basis of records of 33 cited papers from 1979 to 1995 was 6.5% calculated as incidence of risk in the course of lactation, while the incidence of ketosis calculated in the same way was 4.8%. Fatty liver infiltration can be associated with postpartum disorders such as hypocalcaemia, ketosis and displacement of abomasum (Allen and Piantoni, 2013). Fiorentin et al. (2018) determined that 9% cows with fatty liver infiltration also had abomasum displacement,

compared to only 1% of healthy cows. Bobe et al. (2004) on the basis of records of great number of European dairy cows in the period between 1980 and 2001 determined that incidence of moderate symptoms of fatty liver infiltration ranged from 20 to 65%, while the incidence of serious lipidosis ranged from 5 to 24%.

Table 2: Share of animals (%) culled due to metabolic diseases depending on lactation

Metabolic disease	Lactation				
	I	II	III	IV	V
Milk fever	15	17	23	34	21
Ketosis	7	10	12	15	13
Liver lipidosis	41	46	46	36	44
Abomasum displacement	37	27	19	15	22

Asl et al. (2011) reported that occurrence of subclinical ketosis was highest in high-yielding dairy cows and cows that realised two or more lactations. Increased risk of ketosis as one of the most common metabolic disorders in cows that realised more lactations compared to first lactating cows can occur because gravidity and lactation happen almost simultaneously and in that way can exhaust the energy reserves in older cows. Coffei et al. (2004) described the ways of how cow's body and energy profiles can vary during lactation and within first three lactations. Rajala-Schultz et al. (1999) reported that milk production loss due to ketosis varied from 3.0 to 5.3 kg/day and that the cows in 4th lactation were the most affected by ketosis in total loss per cow being over 300 kg/ lactation on average. The highest incidence of ketosis in IV lactation was also determined in our research although we did not analyse losses in milk production caused by this metabolic disease. The same authors studied the loss in milk production caused by milk fever concluding that milk fever caused greater economic losses compared to the occurrence of ketosis.

Economic loss caused by milk fever regarding prophylaxis and clinical treatment, as well as decrease in milk yield and increase in service period was estimated to amount to about 335 $ (USA) per head in total, while the same loss caused by ketosis amounted to 145 $ (USA) per head (Guard, 1994).

The results of occurrence of monitored metabolic diseases in cows on different levels of production depending on relative milk production in first lactation are presented in Table 3. Group I represents the animals with milk production of -1.5 SD and group IX the animals with yield of +1.5 SD above average of farm on which the animal produced.

Table 3: Share of animals (%) culled due to metabolic diseases depending on relative milk production in the first lactation

Metabolic disease	Groups according to relative milk production in first lactation								
	I	II	III	IV	V	VI	VII	VIII	IX
Milk fever	25	17	15	23	19	28	21	27	23
Ketosis	19	19	19	10	11	12	6	11	7
Liver lipidosis	31	46	48	39	48	44	44	37	40
Abomasum displacement	25	18	18	28	22	16	59	25	30

If we take a relative production of dairy cows in first lactation, within the herd in which the animal realised its production, as a criterion for analysis of reasons of culling, it can be seen that highest share of animals culled due to metabolic diseases had animals which were placed in the part of the herd which produced in range of one standard deviation below and one standard deviation above the average of given herd. Physiologically regarded a higher share of animals culled due to metabolic disorders should be expected among the most productive groups in the herd, what was not the case in this research. The obtained results could be a consequence of preferred attention to and proper nutrition of high-yielding dairy cows on monitored farms.

The research papers on phenotypic and genetic correlations between metabolic diseases and yield of milk are quite rare. There are few studies which indicate the existence of genetic correlations between the traits of milk yield and ketosis, and of milk fever and abomasum displacement. Two studies reported very high unfavourable genetic correlations between the yield of milk and ketosis, from 0.65 for Norwegian Red Cattle (Simianer et al., 1991) to 0.77 for Holstein Friesian cattle (Uribe et al., 1995). In the same study a strong negative genetic correlation between milk fever and milk yield (-0.67) was determined. Limited number of studies, substantial standard errors and large range of estimated correlations make it difficult for drawing some general conclusions regarding the correlation between milk yield and metabolic diseases.

Since heritability of metabolic diseases is low (Pryce et.al., 1997, 1998) the application of selection from the aspect of improving the health in dairy cows cannot bring desirable improvements in the near future. In the meantime, cows will continue to contract metabolic diseases while the yield of milk per cow and lactation will be more increased still. Necessary prerequisite for preventing incidence of metabolic disorders seems to be the readiness for high- yielding dairy cows to reduce negative energy balance in early lactation. It is probable that feedback in high-yielding dairy cows is weak, for example, reduced yield of milk would decrease the exhaustion of body reserves to a degree which would prevent metabolism disorders and incidence of disease. Therefore, it is the responsibility of farm management, to provide, by their organisational capabilities and resources, quality nutrition with special attention being paid to intake of dry matter.

The results of Dohoo et al. (1984) who studied the impact of the levels of milk production in Holstein Friesian cows on the risks of occurrence of metabolic disorders for 2875 lactation in 32 commercial dairy herds in Guelph, Ontario, have shown that metabolic diseases tend to occur in second lactation, while level of milk production was not significantly associated with the risk of incidence of monitored metabolic diseases. The incidence of milk fever was the only disorder which seemed to be significantly associated with the level of milk production.

Kelton et al., 1998, state that significant differences in frequency of incidence of metabolic diseases in dairy cattle can originate from and due to geographical and management differences and/or differences in genotype (breed).

In order to study the impact of genotype on frequency of cows culled due to metabolic disorders all the animals in this paper were divided into 5 groups according to the level of HF genes (Table 4).

Table 4: Share of animals (%) culled due to metabolic diseases depending on the share of HF genes

Metabolic disease	Groups according to share of HF genes				
	I	II	III	IV	V
Milk fever	38	21	16	24	23
Ketosis	19	14	14	9	6
Liver lipidosis	29	51	50	40	30
Abomasum displacement	14	14	20	27	41

In our trial the cows with the lowest share of HF genes (up to 50%), displayed the highest incidence of milk fever and ketosis, fatty liver infiltration had highest frequency in cows with the HF genes share ranging between 75 and 87.5%, while abomasum displacement had highest incidence in cows with maximum share of HF genes (more than 93.75%). Since greater share of HF genes has a positive effect on increasing the milk yield the results obtained regarding the incidence of ketosis and milk fever as reasons for culling were not the expected ones. Contrary to our results McLaren et al., 2006, determined that in the herds with higher milk yield there is an increased risk of clinical ketosis ($p=0.03$). It is interesting that in this same research, McLaren et al., 2006, a significant correlation between the milk yield and frequency of incidence of abomasum displacement was not determined ($p = 0.44$). The incidence of occurrence of clinical ketosis is positively connected with increased milk production in a herd (Dohoo and Martin, 1984).

Due to a lack of greater number of research papers on the effect of genotype/breed on incidence of metabolic diseases it is not easy to make general conclusions about this issue. However, it is interesting to mention the paper by Aikman et al. (2008) who determined that due to differences in nutritional requirements and digestive capacity of Holstein and Jersey breeds, particularly during periparturient period (3 weeks before parturition and 1 week after parturition), Jersey breed showed to be more susceptible to incidence of metabolic diseases.

Conclusion

There is a lack of documentation and programmes of monitoring the diseases in dairy cows in our country. In order that a disease should be included into a programme of systemic monitoring it should fulfil certain conditions. In the first place, it should display high frequency and it should impact adversely an economically important trait of interest to producers. It is necessary to permanently exert an influence on breeders and farm managers' conscience to pay greater attention to animals' welfare and health. Monitoring the incidence of diseases represents introduction into genetic estimations for metabolism diseases which have already been calculated in some developed countries of Europe, as well as in the USA and Canada. In these countries they have already been making estimates of breeding values for the traits of metabolic diseases at the national level.

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HIGH MOLECULAR WEIGHT PROTEINS IN CANINE SEMINAL PLASMA AND THEIR INFLUENCE ON HYPERACTIVARION AND CAPACITATION OF SPERMATOZOA

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Abstract: The recent researches confirm that certain proteins contained in the seminal plasma (SP) have essential role in various processes concerning the fertilization ability of spermatozoa. The latest studies provide significant data that the SP and especially specific seminal plasma proteins (SPPs) are responsible for the changes in the motility pattern of the sperm cells occurring after ejaculation and lead to capacitation and dynamic non progressive movement in the process of hyperactivation . However, it is proven that some SPPs are involved in the process of acrosome reaction during fertilization and have significant protective effect on the cell membrane of the spermatozoa during cryopreservation. The aim of our research is to detect and characterize canine high molecular weight SPPs that lead to kinetic changes related with hyperactivation and detected by Computer Assisted Sperm Analysis (CASA) during *in vitro* capacitation. Ejaculates were collected from 10 healthy dogs with normozoospermia. SP was isolated and separated by HPLC. SPPs fractions from each sample were collected and analyzed by 15% SDS-PAGE. The influence of the SPPs fractions on sperm total motility, velocity and motion kinetics during in vitro capacitation was evaluated by CASA. 2-D electrophoresis was used to identify and characterize selected SPPs with proven impact on CASA parameters after in vitro capacitation. Further identification and following mapping of the proteins was performed using Western blot. The identification and effect of high molecular weight SPPs on sperm velocity and motion kinetics after incubation was confirmed. The analysis on two specific CASA parameters shows accurately the path of hyperactivation during incubation with these SPPs which is a sign of *in vitro* induced capacitation. Canine SSPs were characterized and analyzed. The research shows that HMW proteins lead to changes in velocity and CASA motion parameters. In conclusion, our research found that these SPPs could be a factor in the process of hyperactivation of canine spermatozoa and lead to capacitated sperm cells in *in vitro* conditions

Keywords: proteins, hyperactivation, 2-D electrophoresis, Western blot

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HERITABILITY AND REPEATABILITY ESTIMATES FOR MILK PRODUCTION TRAITS IN ORGANIC AND CONVENTIONAL CATTLE PRODUCTION

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Abstract: Estimates of genetic parameters for milk production traits in organic dairy cattle production have not yet been published in our country. However, this production is growing worldwide and basic information on genetic and phenotypic variability is needed primarily to define the breeding strategy. The aim of this study was to estimate the heritability and repeatability for milk production traits in the population of dairy cows from organic and conventional farming. As a material for research were used data of animals calved in the period from 2012 to 2016. The data set consisted of 1.445 measurements of milk yield, fat yield, fat percentage, protein yield and protein percentage during 305 days lactation from organic and 2.073 measurements from conventional milk production. Estimation of variance components, heritability and repeatability were performed using the restricted maximum likelihood method (REML). The model contained the fixed effect of the contemporary group (herd × birth year), calving year, calving season, lactation number and the random effect of animal and permanent environmental effect. Statistical data processing was performed using the WOMBAT software (Meyer, 2006). In population from organic dairy farming heritability estimates ranged from 0.342 for milk yield to 0.375 for fat yield. Repeatability estimates ranged from 0.684 for protein percentage to 0.750 for milk yield. In population from conventional dairy farming heritability estimates ranged from 0.340 for protein percentage to 0.378 for protein yield. Repeatability estimates ranged from 0.680 for protein yield to 0.756 for fat yield. On the basis of the obtained results, we can conclude that there are no large differences in heritability and repeatability estimates between populations from organic and conventional system of production. All estimates of parameters were middle to high, which creates opportunities for selection and genetic progress under both production circumstances.

Keywords: heritability, repeatability, milk production traits, cattle

Introduction

Organic agricultural production in Europe, including the production of food of animal origin, is growing steadily (Anon, 2001; In: Wytze, 2009). The reason for this can be the obviously disrupted environment as well as the needs of consumers for quality and safe food. In the last decades, the volume of organic production in the world has increased significantly, so that in the world scale, organic production has covered over 43 million hectares. In Serbia organic farming is carried out on about 8.228 ha, which is only 0.2% of the total agricultural area. In conventional agriculture, there is in general a tendency to control production conditions in order to maximize animal yield.

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By contrast, organic agriculture is based on natural processes and closed cycles, which implies equilibrium rather than control and a less intensive land use.

Animal breeding in organic dairy farming is a complex issue. When organic dairy production first took off, little attention was paid to breeding livestock that specifically met the requirements of organic farming (Baars and Nauta, 2001). According to Wytze (2009) two broad areas raise concern: (1) the adaptation of animals to the organic environment, i.e. the selection of animals that can produce efficiently in the organic environment, and (2) the naturalness of breeding, i.e. the technologies used for the selection and reproduction of such animals. In cattle organic production in Europe, so far in the process of organic milk production, good experiences have been achieved primarily with dual purpose breeds (Simmental and brown and black alpine cattle) and dairy type (Holstein-Friesian black and red white, Jersey). Based on the results Wytze (2009) three distinct options are formulated for breeding in organic dairy farming: (1) use of adapted conventional breeding, (2) a separate breeding programme and (3) a breeding system based on natural mating.

Defining and implementing of breeding programs is an investment in increasing of dairy production profitability, primarily through genetic improvement of dairy cattle for economically important traits, along with the optimization of management. Selective breeding of dairy cattle combined with artificial insemination (AI), has led to a significant improvement of dairy cattle, especially through enormous improvement of production traits of dairy cows. There are different views on animal breeding within the organic sector. While pragmatic farmers simply keep using AI bulls from conventional breeding programs, others want to obtain animals that fit right for their farm and explore breeding systems based on the integrity of animals, often keeping bulls at the farm for natural mating (Baars, 2005; In: Wytze, 2009).

Estimates of genetic parameters for milk production traits in organic dairy cattle production have not yet been published in our country. However, this production is growing worldwide and basic information on genetic and phenotypic variability is needed primarily to define the breeding strategy. Variance components and genetic parameters are needed for genetic improvement programs to predict the breeding values of candidates for genetic selection, to choose among mating plans and to predict selection response (Montaldo et al., 2012). Thus, the evaluation of genetic parameters, heritability in particular, is crucial for better understanding of genetic mechanism and designing of effective programs for genetic improvement of dairy cattle. The aim of this study was to estimate the heritability and repeatability for milk production traits in the population of Holstein Frisian cows from organic and conventional farming.

Material and Method

As a material for research were used data of animals calved in the period from 2012 to 2016. The data set consisted of 1.445 measurements of milk yield, fat yield, fat percentage, protein yield and protein percentage during 305 days lactation from organic and 2.073 measurements from conventional milk production. Total numbers of cow was 961 in organic and 1.235 in conventional farming which originated from 276 and 67 sire, respectively. Average number of lactation per cow was 1.5 in organic and 1.65 conventional farming. Records were obtained from the Main breeding organization (Faculty of Agriculture in Novi Sad, Department Animal Science). In order to analyze and determine the fixed effect and their significant levels we applied the general linear model (GLM) in Statistica 13 (StatSoft, 2015) with the following model:

$$Y_{ijklm} = \mu + CG_i + Yc_j + Sc_k + L_l + e_{ijklm}$$

Where:

Y is milk (MY), fat (MF) and protein (MP) yield (kg), fat and protein percentage; μ is general average; *CG* is fixed effect of contemporary group (herd×birth year); *Yc* is fixed effect of calving year; *Sc* is fixed effect of calving season; *L* is fixed effect of lactation number; *e* is random residual with assumed normal distribution $N(0,\boldsymbol{\sigma}^2)$

Genetic analyses (estimates component of variance, heritability and repeatability) were conducted by restricted maximum likelihood (REML) procedures using the software WOMBAT (Meyer, 2007). The basic model was fitted as follows:

$y = Xb + Za + Wpe + e$

Where is:

y = vector of observations on lactation milk, fat and protein yield, fat and protein content;

X, Z, W = the incidence matrices relating observations to effects;

b = a vector of fixed effects;

a = a vector of additive genetic effect;

pe = a vector of permanent environmental effect;

e = a vector of unknown residual effects.

Results and Discussion

Phenotypic parameters of analyzed milk traits are presented in Table 1 (from conventional farming) and Table 2 (from organic farming).

Table 1: Phenotypic variability for milk traits from conventional farming

Traits	n	Mean	Standard error of mean	Minimum	Maximum	Standard deviation	Coefficient of variation
MY, kg	2073	8149.515	35.637	2444.000	17096.000	1622.548	19.910
MF, kg	2073	284.804	1.351	77.000	739.000	61.523	21.602
MF, %	2073	3.514	0.009	2.240	6.060	0.415	11.797
MP, kg	2073	261.144	1.127	80.000	596.000	51.308	19.647
MP, %	2073	3.217	0.004	2.500	6.270	0.166	5.161

Table 2: Phenotypic variability for milk traits from organic farming

Traits	n	Mean	Standard error of mean	Minimum	Maximum	Standard deviation	Coefficient of variation
MY, kg	1445	6996.961	50.125	2104.000	13725.000	1905.403	27.232
MF, kg	1445	265.902	2.067	74.400	558.000	78.592	29.557
MF, %	1445	3.816	0.014	2.070	6.580	0.542	14.200
MP, kg	1445	225.845	1.631	66.200	504.000	61.996	27.451
MP, %	1445	3.239	0.007	1.150	5.240	0.289	8.917

Milk yield is economically the most important trait in our breeding programs. According to data reported by Main breeding organization (2018) the average milk yield for Holstein Friesian population in 2017 amounted to 7722 kg which is higher in relation to farm with organic production

and lower in relation to farm with conventional milk production. In relation to results presented in the research performed by Trivunović et al. (2015), for Holstein-Friesian population (milk 6901 kg, fat 260,8 kg and 3.81%, protein 222,7 kg and 3.22%), results are higher for the most traits. Much lower results obtained Popesku (2014) for 305 days first lactation - 3034,89 kg with 3.79% fat and 115,72 kg milk fat content for Romanian Friesian cows. Wytze (2009) reported mean value for milk production traits in four environmental group: conventional (milk 7156 kg, fat 310 kg and 4.36%, protein 246 kg and 3.45%), pre-organic (milk 6991 kg, fat 299 kg and 4.32%, protein 240 kg and 3.44%), converting to organic (milk 6622 kg, fat 284 kg and 4.31%, protein 223 kg and 3.37%) and organic group (milk 6440 kg, fat 274 kg and 4.29%, protein 214 kg and 3.34%). By comparing the results in tables 1 and 2 we see a higher value for milk and fat yield on the conventional farm while other traits are higher on the organic farm.

Effects of fixed factors (herd × birth year, calving year, calving season, lactation number) on milk traits are presented in Table 3.

Table 3: Effects of fixed factors on milk traits

Source of variability	d.f.	F-values with significance level				
		MY, kg	MF, kg	MF, %	MP, kg	MP, %
Herd×birth year	2214	3.914*	0.090^{NS}	10.920**	3.429*	0.046^{NS}
Calving year	6	64.263**	30.739**	15.970**	56.562*	0.181^{NS}
Calving season	2	11.508**	0.004^{NS}	27.685**	2.938**	23.216**
Lactation number	7	34.078**	36.053**	0.551^{NS}	40.875**	4.105*

d.f. degree of freedom; ** $p<0.01$; * $p<0.05$; ^{NS} $p>0.05$

Trivunović et al. (2015) found that calving year have a significant effect only on fat content; birth year and calving season did not have a significant effect only on fat content, while lactation number and age at calving did not have significant effect on protein content. Age at calving did not have significant effect on fat content as well. Wytze (2009) defined the following model: fixed effect of herd-year-season of calving, covariable of age at first calving and covariable of days open. Radinović et al. 2013., also reported that the farm had a highly significant effect on all observed features for Holstein-Friesian, like calving year too, while season did not had effect only on milk fat content.

Heritability and repeatability estimates for milk yield, fat and protein yield and content are shown in Table 4.

Table 4: Heritability and repeatability estimates

Traits	Conventional		Organic	
	h^2	R	h^2	R
MY, kg	0.351	0.743	0.342	0.750
MF, kg	0.372	0.756	0.375	0.744
MF, %	0.367	0.734	0.371	0.741
MP, kg	0.378	0.680	0.372	0.686
MP, %	0.340	0.701	0.343	0.684

In population from conventional dairy farming heritability estimates ranged from 0.340 for protein percentage to 0.378 for protein yield. Repeatability estimates ranged from 0.680 for protein yield to 0.756 for fat yield. In population from organic dairy farming heritability estimates ranged

from 0.342 for milk yield to 0.375 for fat yield. Repeatability estimates ranged from 0.684 for protein percentage to 0.750 for milk yield. Wytze (2009) report higher results of heritability estimates. In his research the heritability of milk yield was 0.48 for conventional farms and 0.70 for organic farms. The heritabilities of fat and protein yield in conventional farming were 0.39 for both traits, whereas these were 0.58 for fat yield and 0.59 for protein yield in the organic environment. In conventional farming, the heritabilities were 0.79 for fat percentage and 0.72 for protein percentage, whereas for organic farming these heritabilities were 0.79 and 0.70, respectively. Mosharraf et al. (2014) estimated the heritability for milk, fat and protein yields in the first lactation for Iranian Holstein dairy cattle, as follows: 0,36, 0,23 and 0,29, respectively.

Conclusion

On the basis of the obtained results, we can conclude that there are not large differences in heritability and repeatability estimates between populations from organic and conventional system of production. All estimates of parameters were middle to high, which creates opportunities for selection and genetic progress under both production circumstances. This can be explained by the fact that both farm implement the same breeding program and use conventional breeding methods and production stock. Further research is required for developing information and tools to support organic farmers in realizing organic breeding. The challenge is to develop breeding strategies for organic farming that fit to organic principles in general and at the same time acknowledge the actual differences and dynamics of organic farming and animal breeding.

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METABOLIC PROFILE OF THE BLOOD OF SIMENTAL CATTLE COWS DURING A PRODUCTION CYCLE IN AN EXTENSIVE BREEDING SYSTEM

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Abstract: The aim of this paper is to look at the metabolic profile of the blood of cows of the Simmental breed during a production cycle in the extensive breeding system, from two aspects: by season (spring, summer, autumn and winter) and by stages of production (1/3, 2/3, 3/3 lactation and dry period). The cows were kept in an extensive breeding system in tie-stall housing in winter, while in the spring, summer and autumn were on the pasture. Blood samples of 10 cows were taken four times during a production cycle (winter, spring, summer, and autumn) in the corresponding production stages (1/3, 2/3, 3/3 of lactation and dry period). It was taken by puncturing of the tail vein. The content of Ca, P, Mg, glucose, urea, total proteins, total cholesterol, AST, bilirubin, and hormones: thyroxine (T4), triiodothyronine (T3) and cortisol were determined in blood serum of cows. The analyzed parameters of the metabolic blood profile, tested with the F - test, indicate that there is a high statistically significance ($p < 0.01$) in Ca, P, glucose, thyroxine and cortisol content depending on the season. The obtained results of the F - test indicate the high level of statistically significance ($p < 0.01$) only in the contents of cortisol, depending on production stages.

Keywords: Simmental breed, extensive breeding system, metabolic profile, season, production stage

Introduction

The Simmental breed of cattle is the most common breed in the Republic of Srpska, also in Bosnia and Herzegovina. According to the Vaško et al. (2016) total number of cattle in the Republic of Srpska are 223 000, and about 60-65% of the total number are Simmental breed of cattle, according to estimation. In the northern part of the Republic of Srpska, characterized by intensive agricultural production, the Simmental breed of cattle is in an intense, but in a mountainous area is in a half-intense and extensive system of keeping. These three ways of keeping the Simmental breed are different among themselves in the organization of production that includes the nutrition, care and accommodation. The Simmental breed belongs to combined type of breed for the production of meat and milk, and can tolerate all three, an extensive, half-intense and intense system of keeping.

In intensive keeping conditions, the average lactation production of milk ranges from 5 000 to 5 500 kg, and in extensive from 3 500 to 4 000 kg (Važić et al., 2005). The limiting factor for achieving milk production is the nutrition, and its quality is now valued over the metabolic profile. The assessment of the metabolic profile of cows is a laboratory blood analysis of the following metabolites: Ca, P, Mg, K, Na, Cu, Fe which are indicators of mineral status, albumin, globulin and urea which are indicators of protein status, and glucose which are indicators for energy status. The obtained values can give a "first warning" about the imbalance of some nutrients before this leads to visible

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disorders. This is very important in order to take appropriate measures to correct nutrition, so as to avoid a decline in production or clinical signs of the disease (Jovanović et al., 2001). Productive diseases of dairy cows are caused by irregular nutrition or poor farm management (Đoković, et al., 2013). The most common diseases of dairy cows are: fat liver syndrome, ketosis, oxidative stress, laminitis, mastitis, milk fever, retained placenta, metritis and infertility (Oetzel, 2004; Jozwik et al., 2012). The metabolic profile is routinely used to detect metabolic disorders in dairy cows. Due to the fact that cows are fed in different stages during the year, in order to more fully understand the metabolic profile, it is necessary to determine the blood content of the parameters monitored in order to prevent certain metabolic disorders. Based on the metabolic blood profile, it is necessary to balance the meals by the seasons. In addition to metabolic profiles in seasons, it is likewise necessary to define the metabolic profile according to production stages, because cattle are set up under different physiological conditions. The metabolic profile involves collecting of blood samples from 8 to 12 cows at 4 time periods relative to calving (dry, early lactation, peak lactation and mid lactation) and measuring selected blood metabolites (Oetzel 2004; Stengarde et al., 2008; Gross et al., 2011).

The aim of this research is to examine the blood metabolic profile of cows of the Simmental breed during a production cycle, from two aspects: by season (spring, summer, autumn and winter) and by stages of production (1/3, 2/3, 3 /3 of lactation and dry period).

Material and Methods

The experiment was done on a dairy farm of Simmental breed of cattle during one production cycle. Cows were kept in an extensive production system in tie-stall housing method, and were located in the hilly and mountainous region of Republika Srpska. The animal is fed with different composition of the meal. The winter meal was based on hay with minimum addition of corn currant. Spring meal was based on hay and grazing, depends on weather conditions. By the end of the spring and the whole summer season, the basic fed was pasture from the natural meadows. During the larger part of the autumn, cows was on pasture, just before the end, and during periods of weather, cows are fed with meadow hay with minimal addition of corn currant. Cows did not have clinical signs of any disease.

Blood samples of 10 cows were taken four times during one production cycle, ie in the winter, spring, summer and autumn, and- in the corresponding production stages (1/3, 2/3, 3/3 of lactation and dry period). It was taken by punction of the tail vein in sterile vacutainres that did not contain anticoagulants.

After spontaneous coagulation (30 minutes), the samples were centrifuged at 3000 rotation/ minute for 10 minutes. The serum was stored at -20 ° C until the analysis.

Content of the following: Ca, P, Mg, glucose, urea, total proteins, total cholesterol, AST, bilirubin, and hormones: thyroxine (T4), triiodothyronine (T3) and cortisol were determined in the blood serum of cows. The concentration of biochemical components in the blood serum was determined on the automatic veterinary biochemical analyzer (VetEvolution, Italy), while the hormone concentration was determined by the radioimmune method (RIA) using commercial kits.

The obtained results were processed by simple analysis of variance using the statistical program SPSS17. The differences between the arithmetic mean of these parameters were tested using the Duncan test. A significance of 0.05 and 0.01 was used.

Results and Discussion

According to Scamell 2006., biochemical tests evaluate the body's internal condition, the function of different organs (including the kidneys and the liver) and the course of metabolic changes in the body. It is very important to choose the right biochemical parameters that will determine the function of different organs. In cattle the concentration of glucose, free fatty acids and beta-hydroxy buteric acid are considered as indicators of energy metabolism. Protein metabolism indicators are urea, total proteins and albumin. Liver status is seen in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamaglutamyltransferase concentrations, and total bilirubin concentration, while creatinine is the basic parameter that reflects the function of the kidney (Stojević et al., 2005).

Table 1. The metabolic profile of the blood of the Simmental cattle for annual seasons

Annual seasons	Winter	Spring	Summer	Autumn	P
of cows (n)	10	10	10	10	
Ca (mmol/l)	2.78A	2.30AB	2.37AB	2.19A	<0.01
P (mmol/l)	1.89AB	1.59B	2.26A	2.13AB	<0.01
Mg (mmol/l)	1.14	1.36	1.09	1.28	NS
Glucose (mmol/l)	2.80A	2.55B	1.84B	3.41A	<0.01
Urea (mmol/l)	4.00	3.78	5.63	4.94	NS
Total proteins (g/l)	83.86	78.94	76.81	87.57	NS
Cholesterol (mmol/l)	4.06	3.63	4.06	2.73	NS
AST (IU/l)	72.50	77.34	70.61	80.88	NS
Bilirubin (µmol/l)	5.77	7.10	7.02	6.29	NS
T4 (mmol/l)	66.20B	76.10AB	102.40B	93.00AB	<0.01
T3 (mmol/l)	2.11	2.07	1.68	2.43	NS
Cortisol (nmol/l)	3.80B	6.08B	8.00A	4.01A	<0.,01

NS: non-significant; values marked by a capital letter differ high-significantly ($p < 0.01$);

The analyzed parameters of the metabolic blood profile, tested with the F - test, indicate that there is a high statistically significance ($p < 0.01$) in Ca, P, glucose, thyroxine and cortisol content depending on the season. The content of Mg, urea, total proteins, cholesterol, AST, bilirubin and tirotionin were no significantly (Table 1).

The metabolic profile of the blood of the Simmental cattle for annual seasons showed that content of Ca and P were high significantly. Extensive feeding conditions are based on cabbage fed, especially a hay, which has satisfactory content of Ca in its composition, but low content of P. This is the reason that the highest content of Ca in the blood was determined during the winter. A high significance ($p<0.01$) in Ca content in blood was determined between the winter and spring, and between the winter and summer seasons. The highest content of P in the blood of Simmental cows was determined during the summer and the smallest during the spring. A high significance ($p <0.01$) in P content in blood was determined between the spring and summer seasons.

Glucose, as an indicator of energy status, according to the average values has shown a certain variability in different seasons. The concentration of glucose in dairy cows is low, since it is

produced mostly in the process of gluconeogenesis, and great quantities are secreted in milk in the form of lactose. The value of glycaemia depends both on gluconeogenesis and consumption of glucose by mammary gland (Joksimović-Todorivić and Davidović, 2012). Glucose delivery and uptake by the mammary gland are a rate-limiting step in milk synthesis (Komatsu et al., 2005). The highest content of glucose in the blood of Simmental cows was determined during the autumn and the smallest during the summer. A high significance ($p < 0.01$) in glucose content in blood was determined between the spring and autumn and spring and winter seasons on one side, and also between summer and autumn and summer and winter on the other side.

The hormonal activity of the thyroid gland has an important role in the transitional period for determining cell metabolism intensity, metabolism of lipids and carbohydrates and the lactation course itself by its thyroid hormones (Nikolić et al., 1997). Đoković et al. (2007) suggested that a hypothyroidal status was established in ketotic cows and that the blood concentrations of free fatty acids, triacylglycerols, total cholesterol and glucose served as major biochemical indicators in determining liver steatosis in the dairy cows in the transitional period. Diagnosing liver lipidosis and susceptibility to ketosis in dairy cows may include liver biopsy or echography, but less invasive and more economical analytical method may be the measurement of blood biochemical indicators (Baird, 1982; Bobe et al., 2004). Based on blood biochemical indicators, ketosis in cow may be diagnosed when the following values match both the clinical signs (BHB>1.2 mmol/l, glucose<2.5 mmol/l, and TG<0.12 mmol/l) and blood values of NEFA>0.7 mmol/l and AST activity above 100 IU/l, which is indicative of hepatic lipidosis (Sevinc et al., 1998; Oetzel, 2004; Xu et al., 2008; Gonzalez et al., 2011). According the obtained value of glucose content the appearance of ketosis in the summer meal was diagnosed. The characteristic of this study, cows in the autumn season, were at some stage of hyperglycaemia.

The diffuse lipid infiltration of hepatocytes, impairing most of them, occurred due to reduced mitochondria capacity to oxidize fatty acids at decreased levels of thyroid hormones in the blood (Kapp et al., 1979). The smallest content of thyroxine Simmental cows was determined during the winter, and the lowest content of triiodothyronine was determined during the summer season. High milk yield and the concomitant large energy deficit were associated with: increased pituitary (re) activity, i.e. increased ACTH baseline concentrations after corticotropin-releasing hormone (CHR) (Beerda et al., 2004). ACTH stimulates the adrenal gland to the glucocorticoid secretion, leading to a process of glucagonegesis in the liver, which occurred in our research during the summer season. The lowest blood glucose concentration in cow blood was recorded during the summer season, and in the same period, the highest concentration of cortisol in the blood of Simmental cows was recorded.

Radkowska and Herbut (2014) indicated a positive effect of pasturing dairy cows on their hematological parameters. In cows that stayed on pasture, the most important parameters of hematology increased in relation to the groups of cows that were staying in the barn. Blood samples to determine hematological and biochemical parameters were collected from animals on an empty stomach before the onset of the experiment and towards the end of the pasture period. Pasturing dairy cows have a lower content of cholesterol. Obtained results of the urea, AST and cholesterol was similarly with values obtained with are results.

Based on the calculated F-test, it can be concluded that there is no statistically significant difference between the determined blood parameters by the production stages of Simmental cows, with the exception of the cortisol content (Table 2).

Table 2. The metabolic profile of the blood of the Simmental Cattle for stage of production

Stage of production	1/3	2/3	3/3	Dry period	P
No of cows	10	10	10	10	
Ca (mmol/l)	2.39	2.37	2.36	2.56	NS
P (mmol/l)	1.86	2.05	2.06	1.87	NS
Mg (mmol/l)	1.29	1.34	1.17	1.12	NS
Glucose (mmol/l)	2.46	2.56	2.89	2.65	NS
Urea (mmol/l)	4.81	4.87	4.68	3.91	NS
Total protein (g/l)	80.35	81.48	81.70	83.11	NS
Cholesterol (mmol/l)	3.87	4.08	3.23	3.90	NS
AST (IU/l)	69.81	73.62	67.73	89.87	NS
Bilirubin (µmol/l)	5.77	7.10	7.02	6.33	NS
T_4 (mmol/l)	82.70	99.30	84.50	78.80	NS
T_3 (mmol/l)	2.03	1.76	2.55	1.90	NS
Cortisol (nmol/l)	3.80^{B}	6.08^{AB}	8.00^{A}	4.01^{B}	<0.01

NS: non-significant; values marked by a capital letter differ high-significantly ($p < 0.01$);

The highest concentration of cortisol in cow blood was recorded in 3/3 lactation. A high significance ($p < 0.01$) in the cortisol content in blood was determined in 3/3 lactation in relation to 1/3 lactation and dry period. The metabolic system is closely connected with the hypothalamo-pituitary-andrenocortical (HPA) axis (Chrousos, 2000), a neuroendocrine system that orchestrates responses of the body to many different types of challenges. The HPA axis effectuates allostasis, i.e. stability through change and its normal functioning is important for successful adaptation. According to our results and cortisol content, cows in 3/3 lactation had the most stressful external influences.

Conclusion

The examined parameters of the metabolic blood profile of the cows indicate that there are statistical differences depending on the season (different diet, temperature and other paragenetic factors). The obtained results of the F - test indicate the high level of statistically significance ($p < 0.01$) in the contents of Ca, P, glucose, thyroxine and cortisol, depending on the season. The analyzed parameters of the metabolic blood profile, tested with the F - test, indicate that there is a statistically significance ($p < 0.01$) in cortisol content depending on production stages.

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THE EFFECT OF THE RAISING REGION ON PRODUCTION RESULTS IN SIMMENTAL BREED COWS

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Abstract: The research was conducted on a cattle farm in Central Serbia on which 1000 heads of Simmental breed of all categories are raised. Data on milk yield were analysed for 205 lactating cows whose lactation ended in 2013/2014. Following data on cow's milk yield were analysed: length of lactation (days), production of milk in standard lactation (PMSL - kg), production of milk fat in standard lactation (PMMSL - kg), content of milk fat in standard lactation (SMMSL - %), production of protein in standard lactation (PPSL - kg), content of milk protein in standard lactation (SPSL - %), production of 4% fat corrected milk in standard lactation (4% MKMSL - kg). Data on cow's milk yield were determined during a regular control of milk yield (AT4 control). In the group of 78 first calf heifers imported from Austria as pregnant heifers and the group of the heifers born on the farm in Central Serbia, the production traits in the first lactation and the age at first insemination and age at first calving were compared. During standard lactation the cows produced 6659.9 kg milk with 4.09% milk fat and 3.40% protein on average. The effect of the origin of animal had a statistically high significant effect on the age at first insemination and the age at first calving. In these two groups of animals a statistically significant difference was determined in milk production in standard lactation in which it can be seen that higher yield was obtained in the animals born on the farm, i.e. the group of animals inseminated at a later date.

Keywords: Simmental breed, milk production, first calf heifers

Introduction

Cattle breeding represents the most important branch of livestock production. Owing to meat and milk production a cattle breeding participates with 40% to 60% in total agricultural production of some European countries such as Finland, Denmark and the Netherlands. In Serbia cattle breeding participates with about 45% in total livestock production.

Simmental breed is one of the most distributed cattle breeds in the world and the second important breed in Europe, coming immediately after Holstein-Friesian cattle. Total number of Simmental cattle raised in the world is estimated at 40 to 60 million out of which more than half is raised in Europe (Huyghe et al., 2014). As regards the cattle in Serbia the most represented cattle breed is Simmental breed which together with Domestic Spotted cattle in type of Simmental breed make about 80% in total fund of cattle. However, a negative trend in the number of animals as well as low production of milk per cow has been perceived (Perišić et al., 2009). In Serbia, Simmental breed is raised mostly in Central Serbia as a breed of combined production capacities.

By thorough selection work Austria and Germany won the leading place among the exporting countries of breeding bulls of Simmental breed both in Europe and beyond. One of the most important export categories for European countries was a category of pregnant heifers.

Simmental breed, in majority of European countries, has kept its dual direction of production

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(Kuczma, 2009), what was also reflected on a breeding programme for this breed in our country (Main breeding programme, 2014 – Glavni odgajivački program u Govedarstvu za simentalsku rasu). However, in some countries, such as Switzerland, which is a native land of Simmental breed, the crossbreeding of Simmental with dairy breeds (Red Holstein) was performed due to an increased demand of dairy processing industry for raw milk.

In 2016 an average production of bovine milk in standard lactation of 4713 kg with 187.27 kg or 3.97% milk fat and 151.16 kg or 3.21% milk protein was realised. The average milk yield in first calf heifers was 4543 kg with 179.85 kg or 3.96% milk fat and 145.84 kg or 3.21% milk protein (Expert assay, 2016 – Stručni izveštaj i rezultati obavljenih poslova kontrole sprovodjenja odgajivačkog programa). In other countries milk yield in Simmental cows in standard lactation ranged from 2312 to 5773 kg (Konstandoglo et al., 2009; Czerniawska-Piątkowska et al., 2012).

Milk production is affected by both genetic and non-genetic factors. In non-genetic factors a statistically significant effect on milk yield traits have farm, lactation, calving season and the age at first insemination (Kučević et al., 2005; Nikšić et al. 2011; Pantelić et al., 2014; Petrović et al., 2015).

Material and Methods

The trial was conducted on a cattle farm situated in Central Serbia on which 1000 heads of Simmental breed of all categories are raised. The trail included 205 cows of Simmental breed which were first calved in the period from 2011 to 2014. The animals were kept in free range system and fed complete mixtures adapted to the stage and level of production. The cows were milked two times a day in milking parlour equipped with a "fish bone" system which can place 24 cows.

During collecting data for this paper the greatest attention was directed towards milk production. For that purpose, the data on milk yield of cows which were determined during a regular control of milk yield (AT4 controls) were analysed although the data on cow milk yield are registered daily since there is a software serving to that purpose within the milk parlour. With the help of this software the data regarding certain animal can be obtained at any moment. By inserting the number of an animal all data are being available, starting from the quantity of milk milked during a given day, quantity of milk produced by a cow from the start of lactation all through to a given day, as well as data for all previous lactations. Besides these there are also records regarding all veterinary interventions such as: date of insemination, date of calving, therapy treatments, etc.

For the purpose of this paper, the records on milk yield for total of 205 lactating cows whose lactations finished in 2013/2014 were analysed. The cows were in their first to fifth lactation. Number of cows per lactations is shown in Table 1.

Table 1: Distribution of cows per realised lactations

Lactation	Number of heads
I	50
II	42
III	35
IV	60
V	12

Following milk yield traits were analysed:

- length of lactation (days),
- milk production in standard lactation (PMSL - kg),
- production of milk fat in standard lactation (PMMSL - kg),
- content of milk fat in standard lactation (SMMSL - %),
- production of protein in standard lactation (PPSL - kg),
- content of milk protein in standard lactation (SPSL - %),
- production of 4% fat corrected milk in standard lactation (4%MKMSL - kg).

The yield of 4% fat corrected milk (4%MKM), was calculated by use of Gaines and Davidson formula such as follows:

4% FCM = 0.4M + 15F

M – quantity of milk (kg)

F – quantity of milk fat (kg).

Main parameters of descriptive statistics were calculated for these traits. By the method of variance analysis (F-test) the effect of lactation in order and of origin of animal (I- imported animals; II- animals born in Serbia) on expression of these milk yield traits was studied.

In certain number of first calf heifers originating from Austria (imported from Austria as pregnant heifers) and those originating from the farm (born on farm in Central Serbia) production traits in first lactation were compared as well as the age at first insemination and age at first calving. Out of total of 78 first calf heifers the 39 animals were included in each group, the first and second one.

Results and Discussion

A determined length of lactation was higher compared to the results obtained by Pantelić et al. (2014) in their research. During standard lactation the analysed cows produced 6659.9 kg milk with 4.09% milk fat and 3.40% protein on average.

Much lower values than these were obtained by Pantelić et al. (2008), Kučević et al. (2005) and Bolacali and Öztürk (2018) in their research.

In Table 2 the results of descriptive statistics for studied traits of milk yield of Simmental lactating cows during 2013/2014 are shown.

Table 2. Parameters of descriptive statistics for studied traits of cow's milk yield

Traits	N	Average	Minimum	Maximum	Std. Dev.
TL (length of lactation, days)	205	351.4	253.0	660	79.9
PMSL (kg)	205	6659.9	3342.0	9473.0	1300.5
PMMSL (kg)	205	272.6	135.3	388.3	53.8
SMMSL (%)	205	4.09	4.03	4.13	0.02
PPSL (kg)	205	226.36	111.90	322.00	44.78
SPSL (%)	205	3.40	3.35	3.43	0.02
4%MKMSL (kg)	205	6752.8	3366.3	9613.7	1327.1

Tables 3 and 4 show the results for studied traits of milk yield in cows.

Table 3. The effect of lactation in order on the milk yield traits

Lactation	Length of lactation	Length of lact. Std.err. (days)	PMSL	PMSL Std. err. (kg)	PMMSL (kg)	PMMSL Std. err. (kg)	SMMSL	SMMSL	N
1	325.6	10.97	5976.9	174.51	244.2	7.22	4.08	0.003	50
2	361.5	11.97	6996.3	190.41	286.4	7.88	4.09	0.003	42
3	374.9	13.11	7068.6	208.58	289.3	8.63	4.09	0.003	35
4	361.8	9.55	6852.7	151.89	280.6	6.29	4.09	0.002	66
5	298.3	22.39	6076.9	356.22	249.5	14.74	4.1	0.005	12
F exp.	4.071**		6.642***		6.597***		4**		205

*** (p<0.001), **(p<0.01), *(p<0.05), n.z (p>0.05)

The lactation statistically significantly affected all traits analysed within the milk yield traits what corresponds to the results obtained by Pantelić et al. (2014) and Petrović et al. (2015).

Table 4. The effect of lactation in order on the milk yield traits

Lactation	PPSL (kg)	PPSL Std.err. (kg)	SPSL (%)	SPSL Std.err. (%)	4% MKM SL (kg)	4% MKM SL Std.err.(kg)	N
1	202.6	6.01	3.39	0.002	6053.8	178.1	50
2	238.0	6.55	3.40	0.003	7095.1	194.3	42
3	240.3	7.18	3.40	0.003	7167.7	212.9	35
4	233.0	5.23	3.40	0.002	6950.0	155.0	66
5	207.1	12.26	3.41	0.005	6172.6	363.6	12
F exp.	6.667***		6***		6.615***		205

*** (p<0.001), **(p<0.01), *(p<0.05), n.z (p>0.05)

In Table 5 the parameters of descriptive statistics for studied milk yield traits in first calf heifers are shown. It can be seen from table 5 that average length of lactation in first calf heifers was longer compared to the results obtained by Pantelić et al. (2014). A much higher milk production in standard lactation was determined also by calculating 4% fat corrected milk compared to the results obtained in the trial conducted by Pantelić et al. (2014). On the basis of the results shown it can be seen that on average the content of milk fat was higher compared to the results obtained by Pantelić et al. (2014).

Table 5. Parameters of descriptive statistics for analysed milk yield traits in first calf heifers

Traits	N	Average	Minimum	Maximum	Standard deviation
Age at first insemination, days	78	595.1	420	841	89.1
Age at first calving, days	78	877.7	705	1122	89
Age at first calving, months	78	29.3	24	37	3
Length of lactation (days)	78	345.3	251	660	86.9
PMSL (kg)	78	5968.1	2652	9404	1247.8
PMMSL (kg)	78	243.166	106.1	385.5	51.485
SMMSL (%)	78	4.08	3.92	4.9	0.1
PPSL (kg)	78	201.75	90.2	319.7	42.57
SPSL (%)	78	3.39	3.35	3.5	0.03
4%MKMSL (kg)	78	6034.7	2652.3	9544.1	1271.1

Table 6 shows the effect of the origin of animal on the age at first insemination and on the age at first calving.

Table 6. The effect of the origin of animal on the age at first insemination and at first calving

Origin of animal	Age at I insemination (days)	Age at I insemination (Std.err. days)	Age at I calving (days)	Age at I calving Std. err. (days)	Age at I calving (months)	Age at I calving Std.err. (months)	N
Austria	561.4	13.28	845.8	13.42	28.2	0.45	39
Serbia	628.8	13.28	909.5	13.42	30.3	0.45	39
F exp.	12.867**		11.248**		11.248**		78

*** (p<0.001), **(p<0.01), *(p<0.05), n.z (p>0.05)

It was determined that an average age at first calving in cows imported from Austria was 845.8 days, what is similar to the results (847 days) obtained by Bolacali and Öztürk (2018). The effect of origin of animal on length of whole lactation as well as on the milk yield traits in standard lactation is displayed in tables 7 and 8.

Table 7. The effect of the origin of animal on the length of whole lactation and milk yield traits in standard lactation

Origin of animal	Length of lactat. (days)	Length of lactat. Std.err. (days)	PMSL (kg)	PMSL Std. err. (kg)	PMMSL (kg)	PMMSL Std.err. (kg)	SMMSL (%)	SMML Std. err. (%)	N
Austria	344.4	14	5674.4	195.4	230.6	8.043	4.08	0.016	39
Serbia	346.2	14	6261.9	195.4	255.7	8.043	4.08	0.016	39
F exp.	0.008n.z.		4.521*		4.892*		0.0 n.z.		78

*** (p<0.001), **(p<0.01), *(p<0.05), n.z (p>0.05)

Table 8. The effect of origin of animal on milk yield traits in standard lactation

Origin of animal	PPSL (kg)	PPSL Std.err. (kg)	SPSL (%)	SPSL Std.err. (%)	4% MKM SL (kg)	4% MKM SL Std. err. (kg)	N
Austria	190.8	6.627	3.38	0.004	5728.5	198.77	39
Serbia	212.7	6.627	3.40	0.004	6341.0	198.77	39
F exp.	5.475*		13***		4.747*		78

*** (p<0.001), **(p<0.01), *(p<0.05), n.z (p>0.05)

Production of milk in first calf heifers in Austria in 2014 was 6483 kg milk with 4.11% milk fat and 3.38% protein (Zuchtdata, 2014). These results show that there is no great difference in production of milk in the first calf heifers of Austrian Simmental in the region of Austria and the animals imported to the region of Serbia.

Conclusion

A determined length of lactation was higher in relation to the results obtained by other authors in their research. During standard lactation analysed cows on average produced considerably high-

er quantities of milk compared to the results obtained in previous research. The effect of lactation in order was statistically highly significant for length of lactation and content of milk fat, while for production of milk in standard lactation the yield of milk fat, yield of protein, content of protein and 4% fat corrected milk had a statistically very high significant effect. The effect of origin of animals had a statistically highly significant effect on the age at first insemination and on the age at first calving. The effect of the origin of animal showed no statistical significance on the length of lactation and content of milk fat in standard lactation while it had a statistical significance on production of milk in standard lactation, 4% FCM and production of protein in standard lactation. In animals born on the farm, the first insemination, and therefore the first delivery, occurred much later compared to the animals originating from Austria. In these two groups of animals a statistically significant difference was determined in the production of milk in standard lactation wherein it can be seen that higher yield was realised by the animals originating from the farm, that is, the group in which insemination was conducted later. This confirms the hypothesis that animals that have the time of the first insemination prolonged, i.e. parturition planned to take place in later period, can realise higher milk production.

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THE EFFECT OF LONGEVITY ON AGE STRUCTURE IN DAIRY COW HERDS

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Abstract: The research was conducted with an aim of assessing the effect of longevity on Black and White cows herd replacement rate and age structure. The research included 16539 Black and White breed cows raised in the period from 1981 to 2013 on 7 farms of the Agricultural Corporation of Belgrade. Data regarding longevity, productivity and origin of these animals were known. The animals remained in production for 1300 days on average during which time they realised only 3.04 lactations averagely. Survival rate, average annual replacement rate as well as average herd structure in analysed period have been calculated within the scope of the research. The largest number of cows (almost 50%) is being culled during the first two lactations what has a highly adverse effect on the structure of breeding stock. On average, 28% of total number of cows is being culled which is deemed a high culling rate. The result of all these factors produced an unfavourable structure of analysed breeding stock in given period the consequence being that 59% of cows in herd were in their first two lactations. The results obtained indicate a key effect of longevity on some of the most important population characteristics which can have the most direct impact on profitability of milk production.

Keywords: longevity, herd structure, replacement rate, Black and White breed

Introduction

The process of intensification of milk production due to increased market demands resulted in prevailing one-way selection and high selection pressure on milk yield traits. One-way selection based on milk yield traits has led to impairment of fertility traits, longevity and resistance of organism which all play an essential role in profitable milk production. The consequences thereof were increased production costs on the account of decreased fertility, resistance and general functionality of dairy cow organism. In addition, this assertion has been supported by the results of the research conducted by Dunklee et al. (1994) who determined that superior cows in milk production require higher level of care, their medical treatment is much more expensive while disorders, such as ketosis, mastitis and reproductive problems, occur more often in such cows.

In the last decade of the XX century and the first decade of the XXI century a number of studies (Allaire and Gibson, 1992; Smith et al., 2000; Harder et al., 2006; Orpin and Esslemont, 2010) showed that a high degree of involuntary cullings had a negative effect on economy of milk production on farms, so the authors focused on fertility traits as the traits that may have a key effect on milk production profitability. Well pronounced fertility traits are highly desired in dairy cow

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breeding stocks. These traits are in positive correlation with the profit realised per cow and/or per day of cow's productive life (Ducrocq, 1987).

When we speak about longevity and its impact on milk production, and on the economy of this branch of livestock production, there are several key aspects which should be paid attention to (Boettcher, 2005):

- Decrease in the number of involuntary culling contributes to the change of structure of breeding stock what ensues the increase in the percentage of animals which are in their later lactations thus realising higher production compared to the animals in the first lactations.
- By reducing the number of involuntary culling the expenses regarding replacement of culled heads are also reduced. These expenses are defined as a difference of the cost of raising (or market cost of high-stage gestation heifers) and the cost of sold culled animals. Many experts do not agree with such derived calculations because the loss created by premature culling, i.e. by the inability of animals to reach lactations in which they can realise highest production, is being neglected, what in turn leads again to herd structure.
- By improving the functional traits the medical treatment costs are being reduced as a consequence of lower incidence of mastitis, laminitis, metabolic disorders and other health problems.
- By reducing the number of involuntary culling the space is created for increase of voluntary (selection or planned) culling, i.e. we can set more severe criteria when making a choice of animals to be the parents of the next generation. In this way the share of voluntary culling in total number of culled animals is being increased.

Material and Methods

The trial included Black and White breed cows raised on 7 farms of the Agricultural Corporation Belgrade in period from 1979 to 2011. Original data set contained the records regarding longevity, production, and origin of 25301 cows. Upon culling the animals that were, up to that point, still in production, along with the heads with illogical and unknown dates of birth and weaning, a final data set encompassed records of 16539 cows, which were first calved on Agricultural Corporation Belgrade farms from 1981 to 2013.

All the cows that calved at least once were taken into account at calculating the survival rate, mean length of productive life and herd average structure. The cow was considered "to have survived" a given lactation if it finished studied lactation normally and had the date of the next calving, i.e. the date of the beginning of the next lactation recorded. Number of first-calf heifers was represented by 100%. A survival rate for succeeding lactations was calculated as a relationship of the number of cows which realised given lactation and number of cows which realised first lactation, i.e. potential number of cows that could realise given lactation.

An average number of calving was calculated as a sum of average survival rates for each lactation. The average length of productive life was expressed in months and calculated by means of following formula suggested by Nieuwhof et al. (1989):

$$H = \frac{\Sigma_1^7 \left[(S_{i+1}) * C_i + (S_i - S_{i+1}) * D_i \right] + S_8 * 365{,}25}{30{,}4375}$$, where:

H- is an average length of productive life expressed in months,

Si- survival rate for i-lactation,

Ci- average length of calving interval expressed in days for i-lactation,

Di- average number of days in lactation for the cows with the next calving unrecorded.

The average percentage of replaced animals at an annual level was calculated by means of a following expression:

$$R = \frac{100}{H/12}$$

The average structure of herd was calculated by use of formula suggested by Nieuwhof et al. (1989):

wherein Pi is a percentage of cows in the herd in i- lactation.

$$P_i = \frac{[(S_{i+1}) * C_i + (S_i - S_{i-1}) * D_i]}{30{,}4375 * H}$$

The percentage of cows in the herd in the eighth lactation was calculated according to the following formula:

$$P_8 = \frac{12 * S_8}{H}$$

Results and Discussion

The cows encompassed by the analysis were first calved in an average age of 809 days (26.6 months), while an average share of genes of Holstein Friesian breed in examined population accounted for 81.9%. The average length of productive life for all cows included in analysis was 1300 days (42.7 months), and during this time the cows realised on average 3.04 lactations and produced 21016 kg milk.

Survival rate is an indicator of the intensity of culling. This parameter was calculated by presenting the number of first calf heifers as a potential number of animals that might have all 8 lactations. Survival rate for each next lactation represents the relationship of the number of cows which realised that parity and of potential number of cows which might realise given parity. Values of survival rate are displayed in Table 1.

Table 1: Values of survival rates

Lactation	I	II	III	IV	V	VI	VII	Average annual replacement rate (%)
Survival rate (%)	75.82	53.32	35.12	21.58	11.46	5.21	1.76	28.1

The values of survival rate coefficients indicate that the largest number of dairy heads is being culled during the first three lactations, i.e. much earlier than the time when they could realise their maximum production, i.e. much earlier than the moment when the funds invested in their breeding could have been returned and expected profit realised. Nearly half cows in examined population are being culled during the first and second lactation, while only somewhat less than 2% cows finish the eighth lactation. Similar culling trend was determined also in the trials conducted by Nieuwhof et al. (1989), Caraviello et al. (2004) and Hare et al. 2006. who studied the survival rate of dairy cows in the USA.

The results obtained in the research suggested that averagely 28.1% cows out of total number of cows were culled in the course of one year. A determined average culling rate during one year in examined population is very close to an optimal share of 25% suggested by Rogers et al. (1989) for population of Holstein cows in the USA.

Determined value for average annual culling rate is much lower than the value determined by Dürr (1997) in his research. He determined average replacement rate in population of Holstein cows in Canada to be over 35%. Such a high replacement rate was explained by the system of quotas in milk production that were in effect in Canada at that time.

The calving interval (Ci) was calculated in cows that had the next calving and lactation recorded, while the number of days in lactation from calving to culling (Di) was calculated for cows that had no next calving recorded. The average values of these parameters are presented in Table 2.

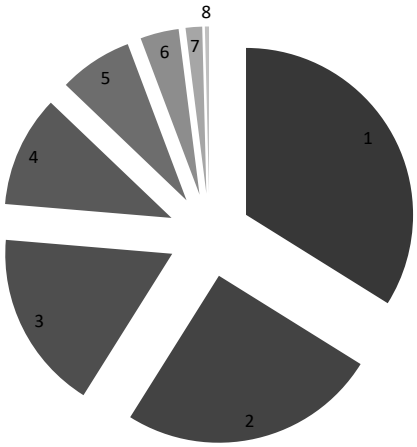
Table 2: The average values of calving interval (Ci) and interval from calving to culling according to realised lactation (Di)

Lactation	I	II	III	IV	V	VI	VII	VIII
C_i (days)	445	433	429	425	429	422	418	-
D_i (days)	422	418	415	420	428	422	415	396

Significantly lower values for calving interval were determined by Dürr (1997) in his research. He determined the values of 390.3 days for calving interval between the first and second calving to up to 403.3 days for calving interval between the sixth and seventh calving. Considerably lower length of calving interval determined by mentioned author is a consequence of shorter length of service period. Similar results for mentioned trait were determined also in the research by Nieuwhof et al. (1989). Mentioned authors also determined the difference between the two set of data used in analysis. Namely, the data set which they used in analysis consisted of two sub-sets, one which was the part of official evidence and the other which consisted of data recorded by the breeders themselves. Higher values of calving interval and interval from calving to culling within given parity were determined in an official sub-set of data. The authors explained the difference as a consequence of different approaches in management of reproductive traits in breeding stock.

A slightly negative trend was determined regarding the length of calving interval, what is contrary to the results reported by Dürr (1997). Namely, he determined the increase of the length of calving interval in later parities. He explained this phenomenon as a consequence of a negative impact of the level of production on reproductive efficacy of cows since in later lactations a higher production is being reached, therefore a negative impact of production on reproductive traits is higher. When the length of period from calving to culling within given parities is in question, the values determined in this research were relatively constant and significantly higher than the values determined by Nieuwhof et al. (1989) and Dürr (1997) in their research.

The average structure of herd in analysed population was calculated on the basis of survival rate and length of calving interval and interval from calving to culling. The shares of cows per lactations from the first to the eighth lactation, in average herd structure, were 34%, 25%, 17%, 11%, 7%, 4%, 1.6% and 0.4%, respectively. The average herd structure is shown in Graph 1.



Graph 1: Average structure of herd in examined population

Almost 60% of cows were in the first two lactations, i.e. the herd structure was not optimal due to decreased number of cows in later lactations in which animals can realise higher production, i.e. herd structure was made predominantly of young animals. Similar herd structure in his research was determined by Dürr (1997). A lower share of first and second lactation cows was determined by Nieuwhof et al. (1989) in Holstein breed population in the USA.

Conclusion

Relatively low values of longevity traits determined in analysed population have caused that nearly half cows should be culled in the first two lactations, i.e. they do not live to reach lactations with maximum production. Long calving interval indicates cow's disturbed reproductive ability, primarily a long service period, what has negative consequences on economy of milk production as well. In addition, all this has led to unfavourable herd structure consisting predominantly of the first and second lactating cows in which realised milk production is significantly lower. In order to solve this complex problem it is necessary to bring longevity traits into sharp focus, to include them into breeding programmes and conduct a genetic evaluation of animals for these traits because the longevity seems to be the key to solving these problems.

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THE CURRENT STATE IN BUSHA POPULATION OF THE REPUBLIC OF SERBIA

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Abstract: The size of Busha cattle breed population, as a direct descendant of *Bos brachyceros Adametza* in the Republic of Serbia, was, by the end of the 20th century, reduced to minimum. According to FAO criteria it was classified as a highly endangered population. Great efforts regarding a conservation of Busha have been conducted since 2000 including the increase of the size of a current population, implementation of planned reproduction, studies on productive and reproductive breed characteristics and elaboration of breeding programmes for current population. The objectives of all activities imply preserving of Busha cattle as a sustainable resource in genetic, economic, cultural and historical sense.

For the purpose of preserving Busha gene fund the *in situ* and *ex situ* conservation is being conducted. In addition, an integrated preservation of Busha, is carried out, through forming ethno complexes where protection of Busha, besides genetic, also has economic and cultural and historical significance. The trend in Busha population was positive in the last ten years and current size of the population encompassed by the programme of protection counts about 1000 heads.

Keywords: Busha, conservation, genetic resource

Introduction

Busha belongs to the group of shorthorn cattle which originates from *Bos brachyceros Adametza*. It is also known as a Balkan mountain or Illyrian cattle. It belongs to primitive cattle breeds which had survived over a very long time in the regions of extensive cattle production wheree the effect of man on the cattle breeding was very weak. It is presumed that even before the arrival of Slavs to the Balkans this short-horn cattle, of small dimensions and body mass had already been raised here.

Busha is always one coloured, either light grey, red, black or yellow. The variant with tiger-like pattern (narrow tiger-like stripes along a whole body) can also be seen. By the middle of the 20th century, the Busha strains in these colours, were territorially separated and raised in certain characteristic regions. For instance, grey (Polimska) Busha was raised predominantly in south-west Serbia and northern Montengro, grey Herzegovina and improved grey Busha (Gatačka Busha) was bred in Herzegovina, red Busha was raised in southern Serbia and in Metohija, black Busha in Kosovo and western Macedonia, yellow Busha along the coast of Montenegro and blue Busha in Macedonia, near the river Vardar. The varieties similar to Busha were represented also in Karpates and other Balkans countries (Bulgaria, Greece, Albania).

Typical characteristic of Busha is a roe deer muzzle, i.e. dark pigmented mucous nose membrane with the wreath of white (light) hairs around it. Along dorsal line there is a striped (eel-like)

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line which is always contrasted to the major colour of the hairs. The colour of horns and toes is always dark to black. The horns are short, crowned, with light colour around the base and black tips. The head bones are fine, forehead is wide, deeply set, eyepits prominent. The neck is thin, poorly covered by muscle tissue. The backs are narrow, short and flat, (hips) buttocks are pointed and slanted. The chest is narrow, but deep and spacious. Legs are relatively short, with large and firm toes. Position of legs is ¨cow-like¨ (x)¸ which is one of breed characteristics. The udders are poorly developed, but normal.

Body dimensions in some Busha strains (Table 1), reported by *Belić* and *Ognjanović* (1961), citing the results obtained by other authors by the middle of the 20th century when Busha was significantly larger in number.

Table 1. Body dimensions of Busha strains according to various authors (cited by *Belić* and *Ognjanović,* 1961)

Traits	Marković		Đurić				Rako				Belić M.	
	Polimska Busha		Dark strain		Blue strain		Black strain		Neretvanska Busha		Metohijska Busha	
	cm	%	cm	%	cm	%	cm	%	cm	%	cm	%
Withers height	103.1	100	107	100	106	100	107	100	102.8	100	105.5	100
Loins height	109.7	106.4	108	100.9	109.3	103.1	109.3	102.1	105.1	102.2	108.1	102.5
Chest width	32.7	31.7	31.2	29.1	28.9	27.2	27.5	25.6	28.1	27.2	29.2	27.6
Chest depth	54.2	52.5	58.2	54.4	59.5	56.1	57.2	53.4	53.1	51.6	55.5	52.6
Chest circumference	151.1	146.5	150.5	140.6	145.5	137.5	145.5	135.9	138.6	134.8	151.4	143.5
Trunk length	128.1	124.1	117.5	109.8	123	116	124	115.8	121.5	118.2	122.6	116.2
Tibia circumference	14.7	14.2	15.5	14.4	14.4	13.5	13.5	14.6	13.3	12.9	13.6	12.9

These results show that in Busha, in all its strains, a regular trait, is that the loins are higher than withers. Similar results were obtained also by *Memisi et al.* 2000. but much later. Exterior measures varied considerably, so the height of withers in cows was in the interval of 105 to 120 cm, and in bulls 120 cm and more. The mass of adult animals varied depending on the conditions of breeding being from 220-300 kg for females and 250-400 kg in males.

Busha is a late maturing breed. It becomes sexually mature in the age of 18 to 24 months, depending on raising conditions, when they mate for the first time. However, in improved conditions of nutrition and care, it can sexually mature much earlier. The mass of calves at birth is 14-20 kg. Fertility is quite good, and calving interval is often shorter than one year. Calvings are regular in older cows (approximately once a year). Regular calvings are particularly characteristic in the cows which are being calved in spring when more favourable conditions of nutrition (grass) have a positive effect both on milk production and raising of calves and on short duration of service period (45 days), and therefore a shorter length of calving interval.

Busha is a durable breed and females remain in the production 12-15 years and very often up to 20 years of age. Busha is more superior than noble breeds due to its resistance and frugality regarding the demands of nutrition and housing, so that even in the ultimate extensive conditions it

can produce some quantity of milk. Majority of other breeds cannot survive the conditions which are endurable by Busha. Since raising of Busha was mostly extensive the effect season-season via nutrition was to a high degree present. The best period for calving – beginning of lactation from the aspect of milk production and lenght of lactation is late winter and early spring.

Busha's production results are very modest what is a consequence of small dimensions and body mass on one hand and extensive raising conditions on the other. Milk yield in some strains of Busha (Table 2) was very modest as reported by *Belić* and *Ognjanović* (1961), citing the results of other authors determined by the middle of the 20th century.

Table 2. Milk yield of various Busha strains (cited by *Belić* and *Ognjanović*, 1961)

Busha strain	Average milk yield, kg	Lactation lenght, days	Cow body mass, kg	Author, year
Croatian Busha	1004.5	-	250	Frangneš, 1990
Polimska Busha	915	229	247	Ogrizek, 1936
Red Metohijska	911.21	244	248.5	Mitrović, 1948-1950
Improved Metohijska	1006.8	261.2	290	Mitrović, 1948-1950
Pešterska Busha	1043.0	271	286	Milosavljević-Antić, 1948
Busha of Croatian karst	1338.0	-	230	Horvat,1936

Busha's milk production was very modest (about 1000 liters in lactation shorter than standard one). Milk contained 4 to 6 % milk fat, depending on the stage of lactation. It was thought previously that Busha has a potential for relatively high milk yield and high % fat in milk. It is true that Busha reacts positively to improved conditions of nutrition and care but not in an expected volume. This is confirmed by research results cited by *Belić and Ognjanović* (1961), (Table 2) for animals analysed on the state farms with considerably better production conditions. Obtained milk yield was threefold and fourfold in relation to body mass what is certainly insufficient, since for example, Simmental breed has 8 to 10 times higher milk production in relation to its own body mass, observed at the level of lactation.

The current state in Busha population
The size of Busha population

The activities on Busha preservation have been conducted since 2000 and are directed towards increasing the size of actual population. The *in situ* and *ex situ* conservation is being conducted. Busha breeding stocks, except in the regions where it had been traditionally raised in the past (hilly and mountainous regions), were formed in low-land areas as well. It is illustrated by data regarding the number of Busha heads and distribution of agricultural estates – farms which raise Busha per individual districts of Serbia (Table 3)

Table 3. Number of Busha in a previous period 2007 – 2017 and distribution of farms which raise Busha

Year	2007	2008	2009	2010	2012	2013	2014	2015	2016	2017
Total number of protected Busha animals	450	550	650	750	600	550	550	669	815	916
Districts of Serbia	Number of farms which raise Busha (2018)	Total number of Busha animals per individual Districts of Serbia								
		Heads older than 2 years			Heads from 6 months to 2 years old		Calves up to 6 months old			
Belgrade City	4	178	125	51						
Jablanički	3	36	33	7						
Southern Banat	4	145	26	11						
Kolubarski	1	10	5	7						
Mačvanski	2	23	18	8						
Moravički	5	21	8	12						
Pirotski	5	127	74	27						
Raški	34	173	53	44						
Šumadijski	2	31	14	4						
West Bačka	1	11	5	1						
Zlatiborski	2	22	4	0						
Zaječarski	1	4	3	0						
Total	64	781	368	172						
Total	1321									

Source: Data base of the Ministry of Agriculture of the Republic of Serbia

The largest breeding stocks of Busha are found in the territory of Pirot and Southern Banat district. In some breeding stocks the production is organised per principles of organic production (*Knežević,* 2015). An integrated protection of Busha is also conducted through forming ethno complexes, where protection of Busha, besides genetic, has economic and cultural and historic significance as well. Likewise, integrated protection is conducted also in population of Podolska breed, but mostly in the regions where Podolska breed has always raised (*in situ protection*) as reported by *Perišić et al*. (2004).

Busha population in Serbia has had a positive trend in the last ten years and current size of population encompassed by the protection programme is about 1000 heads. Taking into account that Busha is still regarded as one of endangered populations it is very important to conduct planned reproduction in order to evade inbreeding.

Scientific researches on Busha population

Study on phenotypic characteristics

Upon activities conducted on identification and forming of Busha breeding stocks individual studies have been written. In addition, within the project entitled *Defining a breeding programme for Busha as a sustainable genetic resource* (*Perišić et al*., 2005) a morphometric traits of Busha were studied. In one of studied Busha population the exterior measures were determined and they are shown in Table 4.

Table 4. Exterior measures of cows and bulls in Grey Busha strain (*Perišić et al*., 2005)

Busha strain	Sex	Withers height	Loins height	Trunk lenght	Chest width	Chest depth	Thigh bone distance	Distance of ischium	Chest circumference	Tibia circumference	Temple distance	n
Grey Busha	♂	120.5	123	137.5	34	60.5	37	12.5	175	19	22,5	3
	♀	113.4	116.2	131	30.8	56.1	37.9	12.7	158.4	15.7	18.9	26

On the basis of body dimensions of Grey Busha, shown in Table 4, it can be concluded that exterior measures (particularly height of withers and loins) were on average 5 to 10 cm larger compared to dimensions determined by the authors in previous researches cited by *Belić* and *Ognjanović* (1961). Compared with the results of the other authors the characteristic that loins in Busha are higher than withers was also determined in this research what confirms that this trait is a breed characteristic of Busha cattle.

In Busha breeding stocks in Serbia, besides usual colours which are characteristic for some strains and which were in the past associated with certain territories of raising, today, brown coloured animals are quite common. In scientific and technical literature from previous period, there is no mention of Busha animals of this colour. The reason for this should be sought in the fact that in modern Busha populations there are animals of different colours in the same breeding stocks and that often there is no possibility that mating should be conducted between the animals of the same colour.

Genetic research

In breeding stocks of Busha encompassed by a protection programme a genetic research was conducted. Đ*edović et al*. (2015) studied the dependence between genetic polymorphism for κ- casein and quantitative traits of milk yield in Busha and other breeds.

In a selected sample of a Busha breeding stock, studying the polymorphism of genes for κ-casein, the frequency of genotypes of AA, AB and BB in the ratio of 41.7% : 50.0% : 8.3% was determined. Frequencies of A and B alleles, estimated on the basis of genotypic frequencies accounted for 0.667 and 0.333, respectively. In order to determine the phenotypic variability of milk yield in standard lactation, yield of milk fat and content of milk fat per genotypes of κ-casein within the breeds, a fixed model was used which involved the effect of breed, lactation in order and effect of genotype for κ-casein (AA and BB). The milk yield statistically significantly ($p \leq 0.05$) depended on genotype for κ-casein, yield of milk fat highly significantly ($p \leq 0.01$) depended on κ-casein genotype, while the content of milk fat did not statistically significantly ($p > 0.05$) varied under the effect of animal genotype for κ-casein.

Maletić et al. (2016) studied polymorphism for κ-casein and β-lactoglobuline in Busha and Holstein Friesian cows in Serbia. The aim of research was to determine a distribution of genotypes of κ-casein (κ-CN) and β-lactoglobuline (β-Lg) in autochthonous (Busha) and dairy (Holstein-Friesian, HF) cattle population. The results showed that κ-casein (κ-CN) gene in Busha represented by AA genotype, accounted for 44.44% and AB genotype accounted for 55.56%. Genotypes for β-lactoglobuline gene were also represented by 44.44% for genotype AA and 55.56% for genotype AB. Genotype BB was not found.

Research on fattening traits

Perišić et al. (2008) studied fattening traits in Busha in semi-intensive conditions. The traits were monitored by measuring the body mass in the animals of both sexes, included into fattening. Measuring of body mass was conducted in the beginning of fattening (at the age of three months), in the age of 6 months, 12 months and 18 months of age (finishing of fattening). The average daily gains in some stages of fattening were determined as well as average daily liveweight gains (Table 5).

The average values obtained for fattening traits for all studied animals were as follows: average body mass in the beginning of fattening (3 months of age) was 69.32 kg, at 6 months 106.63 kg, at 12 months 162.32 kg and at the end of fattening (with 18 months) 203.62 kg. The average daily gain in the period up to 3 months was 0.562 kg/day, from 3 to 6 months 0.415 kg/day, from 6 to 12 months 0.310 kg/day, from 12 to 18 months 0.229 kg/day. The average daily weight gain for period from birth to fattening finish (in the age of 18 months) accounted for 0.339 kg/day.

The highest daily gains were obtained during a suckling period (averagely 0.562 kg/day), and the lowest in the finishing stage of fattening, in the period from 12 to18 months of age (0.229 kg/day). There are certainly a number of reasons for this phenomenon the most important being weak genetic potential of Busha animals for creating muscle tissue and changes in the structure of weight gain which occur in older animals. With aging process the unit of gain (kg of gain) has higher energy value, thus, ensuing a higher consumption of feeds for kg of weight gain. Valorisation of production with autochthonous breeds is possible only with low investments in production systems especially if the production of quality food is conducted under strict standards of ecological production and organic livestock breeding.

Table 5. Fattening parameters in Busha bullcalves (Perišić et al., 2008)

Fattening parameter	n	Average	Std.Dev.
Mass at birth, kg	16	18.75	1.653
Mass at 3 months, kg	16	69.32	9.428
Daily gain up to 3 months, kg	16	0.562	0.091
Mass at 6 months, kg	16	106.63	16.464
Daily gain by 6 months, kg	16	0.473	187.382
Daily gain at 3-6 months, kg	16	0.415	0.113
Mass at 12 months, kg	16	162.32	21.975
Daily gain at 6-12 months, kg	16	0.310	0.090
Mass at 18 months, kg	16	203.62	28.209
Total gain by 18 months, kg	16	184.75	27.506
Daily gain at 12-18 months, kg	16	0.229	0.060
Total gain at 3-18 months, kg	16	134.312	23.751
Daily gain at 3-18 months, kg	16	0.295	0.052
Daily gain at 0 – 18 months, kg	16	0.339	0.051

Having as an aim the increase of production of milk and meat per cattle head during the 20th century a suitable breeding and selection measures were conducted in a Serbian cattle breeding. High-yielding breeds were favoured (Simmental, Black and White lowland cattle, Holstein-Frie-

sian), which gradually replaced the autochthonous, primitive cattle breeds, characterised by low productivity. Thus the size of population of autochthononous cattle breeds more and more decreased in size and often there occurred inbreeding. Certain breeds of cattle (Kolubarska, some Busha strains) have completely disappeared by crossbreeding mostly with Simmental breed. The size of population of Podolska and Busha breeds were reduced to minimum (Perišić et al, 2004; Perišić et al, 2007). The protection of these breeds has a multifold importance, primarily genetic (preservation of natural variability), geographic, cultural, historical, economical, etc. Conservation of endangered breeds already had some positive results what is reflected, in the first place, in gradual recovering of populations of Busha and Podolska breed.

Conclusion

The activities conducted on protection of autochthonous cattle breeds in Serbia yielded some results what is reflected, primarily, in a positive trend of numeral state of endangered species (Busha, Podolska breed). The number of Busha heads encompassed by the protection programme has increased from 450 in 2007 to 916 heads in 2017. The *in situ* and *ex-situ* conservation is also being conducted.

The research is carried out on Busha breeding stocks on whose basis it can be concluded that body condition scoring of adult animals in Busha contemporary populations is higher in relation to body condition scoring measured by the middle of the 20th century. The withers and loins heights are by 5 to 10 cm higher in modern Busha populations. Besides usual colours characteristic for certain strains and in the past associated with certain breeding territories today the animals in brown colour are often seen what is a consequence of mating of animals of different colours. In the future period, after attaining sufficient size of Busha population, we should pay the attention to the choice of parental pairs (breeding should be performed within the animals of the same colour).

Genetic research – determination of the frequency of genes and genotypes for κ-casein and β-lactoglobuline, as well as studies on the effect of genotype on production traits (quantitative traits of milk yield) in Busha population were also conducted.

In semi-intensive fattening of Busha bullcalves which lasted up to 18 months of age the highest daily gain in suckling period (up to 3 months) accounted for 0.562 kg/day on average and the lowest gain was observed in the finishing stage of fattening (period from 12 to 18 months of age) and accounted for 0.229 kg/day.

For the purpose of integrated protection of Busha (as genetical resource and the breed which can be economically usefull), the breeding of Busha is justified for fattening and production of meat in the regions (zones) in which conventional breeds cannot be successffully raised. Those are most certainly mountainous regions of higher altitudes and extensive grazing since only in this way very low daily gains which Busha generally attains can be economically justified.

Valorisation of production with autochthonous breeds is possible only with low investements into production systems particularly if the production of quality food is conducted under strict standards of ecological production and organic livestock breeding. Such integrated protection by way of organising etno-complexes could attain its complete valorisation through rural tourism.

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EXTERIOR CHARACTERISTICS OF DUBIAN PRAMENKA SHEEP

Dokso A.[1], Zecevic E., Rustempasic A., Brka M.

Abstract: The aim of the present research is to study the external features and the morphological parameters of the Dubian pramenka sheep. Research were carried out on 95 adult sheep (80 ewes and 15 rams) on area Una-Sana. In order to establish the main features of the exterior, basic body measurements were taken: the height of withers, the body length, the chest depth and circumference of the chest. The average height of withers of adult Dubian sheep was 75.51 cm, the body length 86.92 cm, the depth of chest 35.10 cm and the circumference of the chest 108,42 cm for ewes and, 81.52 cm, 88.10 cm, 37.40 cm and 119.50 cm for rams. Data were analyzed using procedure UNIVARIATE of SAS statistical package. The results obtained show somewhat higher values than previous research on this breed, which can be explained by good breeding and environmental conditions. To obtain a more complete picture of the exteriors characteristics of Dubian sheep, it is necessary to continue the research, including a significantly larger number of animals with the wider breeding area.

Keywords: Dubian sheep, exterior, features.

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MEASURES OF HEALTH PROTECTION OF BEES FROM VAROOSIS IN MONTENEGRO

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Abstract: Varroa is a disease of bee litter and bees caused by ectoparasite, a tick (acarine) *Varroa destructor*. This parasite is present throughout the year in the bee colonies feeding with haemolymph of bees, larvae and dolls. If the bee colonies does not get rid of these parasites, it will die after a year or more. Varoosis is also present in the beekeeping of Montenegro. Measures to prevent varoosis consist of beekeeping on favorable terrains, early detection of diseases, do not merge weak and strong bee colonies without control, control of newly procured colonies, control of treatment efficiency etc. It is very important to select a queen bee from bee colonies that are more resistant to *V. destructor*. Diagnostic examination at varoosis is carried out once a year - until the end of March on all bee colonies. If it is suspected that the bee colonies is invaded by this parasite, the diagnostic examination is carried out also in other months. For the destruction of the ticks, only registered products should be used. In Montenegro, natural preparations are registered - Apigard (based on timol) and Api Life Var (based on timol, eucalyptus oil, camphor and levomentol). Preparations should be used in accordance with the instructions and advice of the veterinarian. The tick is most effectively destroyed in the period when there is no or very little bee litter - in October and November and in favorable days in December. Varoa quickly receives resistance to synthetic chemical preparations based on compounds, such as amitraz, coumaphos, synthetic pyrethroids - fluvalinate and flumetrin. In addition to gaining resistance, there is a great danger of depositing residues in bee products. Such bee products are a source of contamination for humans through the consumption of bee products (honey, royal jelly, propolis, etc.) and bee wax is a source of contamination through the cosmetic products that contain it. Therefore, these synthetic preparations should not be used for the treatment of bee colonies against varroa.

Keywords: Apis mellifera carnica, bee, Montenegro, Varroa destructor

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FAUNAL AND GAME RICHNESS OF THE NATIONAL PARK PELISTER

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Abstract: National Park Pelister extends to the far southwestern part of the Republic of Macedonia, i.e. on the slopes of Mount Baba, as part of the mountain range of the Mountains Ilinska, Plakenska and Bigla, and ends with the Neredska Mountain in Greece. With an area of 17,150 ha, "Pelister" is the smallest national park in the Republic of Macedonia. The altitude ranges from 891 m to 2.601 m, with a height difference of 1.710 m. By the Law on Hunting, in the Republic of Macedonia there is declared a total of 133 species of wildlife: 23 species of mammals and 110 bird species. Permanently protected are 9 mammal and 76 bird species, i.e. in total 85 species (63.9%). Hunting includes 34 wild game species or 25.6%, of which 7 are mammals and 27 bird species, while the category without protection includes only 14 species, i.e. 7 of the two classes. The richness of the diversity of the game fauna species in the Mavrovo National Park is reflected in the fact that out of the total of 133 declared game species on the whole territory of the Republic of Macedonia, 52 or 39.1% of the species (78.3% of mammals and 30.9% of birds) were registered in this park. Of these, 78.8% (41 species) are in the category "under protection", and 11 species (21.2%) are in the category "without protection". Of the total number of registered species, 27 (51.9%) are permanently protected.

Keywords: National Park, diversity, game species, mammals, birds, protections

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WHY THERE WAS A NEED TO SET THE MANAGEMENT OF GOLDEN JACKAL WITHIN THE RESEARCH PROJECT IN SLOVENIA

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We live in a changing world, both in the external environment and the social one. Through our intense encroachment into environment, we need to have a proper knowledge for successful management. Monitoring of changing conditions is becoming a key activity in various areas, including management of wildlife populations. Since the presence and the increase of the golden jackal population in Slovenia is a recent phenomenon, there is a missing knowledge about the species, its ecosystem role, interspecific interactions, and effects on other species, population dynamics and the ability to spread in the environment. There is a strong need for a wider social discussion and consensus about the expected objectives concerning the species, which is a perquisite for a proper management. From previous experiences, we know that in such cases a scientific approach to do that is very important. The need for immediate start of researches on and particularly of the systematic monitoring of the jackal was recognized by the competent Ministry which, together with Slovenian Research Agency, proposed a research project entitled "Spatial distribution, abundance, and estimation of population trends and the potential spread of the golden jackal (*Canis aureus*) in Slovenia". As for all game species, it is very important that the monitoring of jackal population is based on integration of hunters who are obliged to provide monitoring as a public service; moreover, the monitoring has to be based on scientific backgrounds. It was supported by the Hunting Association of Slovenia and the Slovenia Forest Service, too. The project was carried out in the two-year period and was discussed in 11 work packages, with an emphasis on the determination of population status of the jackal in Slovenia, the establishment of national monitoring, and the preparation of background strategy for the management of the species. In cooperation with the HAS, the on-line available hunting information system (LISJAK) was upgraded by developing a new monitoring module. In the presentation we will present the project, the methods used and its key results.

Keywords: jackal, Canis aureus, monitoring, management, CRP V1-1626, Slovenia

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EFFECTS OF DIETARY TANNIN SUPPLEMENTS ON RUMEN FERMENTATION CHARACTERISTICS AND LACTATION PERFORMANCE IN DAIRY COWS

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*Abstract: Ta*nnins are a group of complex polyphenol compounds, found in many plant species consumed by ruminants. They can have both useful and harmful effects, depending on type and consumed quantity, chemical structure and molecular weight. High concentrations of tannins decrease feed intake and digestibility of nutrients, while low or moderate concentrations can improve digestibility, mostly through protective impact on easy degradable proteins from feed, decreasing their digestion in the rumen and increasing amino-acid flow in the small intestine. Such effects of tannins on diet could indirectly have positive influence on lactation performance in dairy cows.

Keywords: digestibility, lactation performance, tannins, rumen fermentation.

Introduction

The path of nutrient decomposition to easily degradable ingredients, which can be absorbed and used as an energy source or cell's structural components is especially complex in ruminants, due to their complex stomach and vast nutrient degradation by microbs in rumen. Diet protein optimization has an aim to maximize animal growth and milk production (Dey and De, 2014). However, one of the main problems in dairy cows nutrition is a surplus of digestible and deficit of indigestible proteins in the diet, both in absolute and in relative context regarding to content of carbohydrate fractions and rate of their digestion in rumen. Regulation of protein fractions imbalance can be achieved by using various additives of natural origin (Đorđević et al., 2006; Davidović et al., 2012; Stojanović et al., 2016). Tannins have a potential capacity to protect feed proteins from degradation in rumen, to provide better protein exploitation and reduce intensity of ammonia nitrogen accumulation in the rumen. This makes them suitable additives in cow's diet with surplus of digestible protein and non-protein nitrogen (Hervás et al., 2000; Henke et al., 2017; Castro-Montoya et al., 2018).

Tannins are secondary plant metabolites of high molecular weight. They include various oligomers and polymers (Schofield et al., 2001). Hydrolysable tannins (HT) have a carbohydrate core, with hydroxyl groups esterified by phenol acids (mainly gallic and hexahydroxydiphenic acid). Pro-anthocyanidins or condensed tannins (CT) are un-branched polymers of flavonoid units (flavan-3-ol, flavan-3,4-diol) and usually have higher molecular weight when compared to HT (1000-20000 Da compared to 500-3000 Da) (Mueller- Harvey, 1999). This heterogeneous group of polyphenolic compounds are included in numerous plant species consumed by ruminants (Patra and Saxena, 2009). Tannins form both reversible and irreversible complex compounds mainly with proteins, but also with polysaccharides (cellulose, hemicellulose and pectins), alkaloids, nucleic acids and minerals (Schofield et al., 2001; McSweeney et al., 2001). Factors enabling forming of such complex

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compounds are relatively of high molecular weight and high structural flexibility of tannins (Mueller-Harvey and McAllan, 1992). High affinity of tannins towards proteins is a consequence of large number of phenol groups which can bind to carbonyl groups of peptides (Hagerman et al., 1992). Proteins that show the highest affinity towards tannins are relatively big and hydrophobic, they have open, flexible structure and they are rich in prolin (Hagerman et al., 1992; Mueller-Harvey and McAllan, 1992). Complex compounds formed by proteins and tannins are mostly unstable, bonds between them are reversible, they constantly brake and again new bonds are formed: hydrogen bonds (between hydroxyl radicals of phenol groups and oxygen from protein amide groups), hydrophobe interactions (between aromatic ring of phenol compounds and hydrophobe region of proteins) and ionic bonds (between phenol ions and cationic part of protein on HT exclusively). Only the covalent bonds are irreversible, and they are formed through oxidation of polyphenols to hinon and their later condensation with nucleophylic groups of proteins (Kumar and Singh, 1984).

Intake of condensed tannins may have positive or negative effects in ruminants, depending on species, chemical structure, molecular weight and quantity of compound. High concentrations of tannins (more than 50 g/kg of dry matter, DM) significantly decrease voluntary feed intake and nutrient digestibility. These concentrations decrease feed flavor and rate of digestion, leading to lower productivity in dairy cows. Intake of low to moderate concentrations of condensed tannins (10-40 g/kg DM) could improve digestion and protein usage, mostly through decrease of protein degradation in rumen, which is related with lower ammonia nitrogen production and higher availability of essential amino-acids and non-ammonia nitrogen for absorption in small intestine (Min et al., 2003; Patra and Saxena, 2011). Use of such concentrations of tannins in dairy cows leads to higher milk production, higher protein, lactose and milk fat content (Makkar, 2003; Waghorn and McNabb, 2003; Mueller-Harvey, 2006; Soltan, 2009; Dey and De, 2014). The toxicity of tannins is variable and related to their molecular size and concentration. Toxic effects of tannins can be determined only at high concentrations of hydrolysable tannins of lower molecular weight (Zhu et al., 1992). With respect to condensed tannins with high molecular weight very high level of intakes appear to be necessary for animals to suffer serious intoxication (Hervás et al., 2003a). Poisoning by HT are mostly characterized by anorexia, depression, atonic rumen, liver and kidney insufficiency, ulcers in digestive organs and gastroenteritis (Spier et al., 1987; Plumlee et al., 1998).

Effects of dietary tannin supplements on rumen fermentation characteristics

The structure of different tannins is variable, but their common characteristic is their ability to decrease feed digestibility. Modifications in digestibility caused by tannin intake are linked to changes in rumen fermentation, as well as to gut digestibility.

Ruminant foregut micro flora digest feed proteins to amino-acids and finally keto-acids and ammonia, which will be used for synthesis of their own proteins if the amount of available easy digestible carbohydrates as a source of energy is high. In peripartum period, when food intake in cows decreases, the incorporation of NH_3 in microbial proteins is decreased as well. Consequently, the absorption of NH_3 through rumen mucous membrane increases, leading to increased levels of urea in blood, milk and urine (Spek et al., 2013; Brinkhaus et al., 2016). Tannins have high affinity towards proteins, whereby pH value of the content in rumen favors the complex formation between these compounds allowing "ruminal escape of proteins" and delay in protein digestion (Mueller-Harvey, 2006). These compounds modify rumen fermentation by decreasing soluble fractions amount, due

to forming of resistible complex with proteins that are stable within the pH range of 3.5 to 7.0. Formed complex compounds protect proteins from microbial hydrolysis and deamination in the rumen. As a consequence, the concentration of proteins digested in abomasum and small intestine increases (Hervás et al., 2000; Min et al., 2003). Lower degradation of proteins is linked to lower production and absorption of ammonia nitrogen through rumen wall, and hence higher flow of non-ammonia nitrogen towards duodenum (Mueller-Harvey, 2006; Behman et al., 2008; Dey and De, 2014). Pace et al. (1993) determined that CT of quebracho led to higher decrease of soy flour degradation than commercial tannin acid. Hervás et al. 2000. and Frutos et al. 2000. treated the soy feed with different doses of tannic acid (0, 1, 4, 7, 9, 13 i 20%) or commercial quebracho CT extract, and significantly decreased the level of crude protein degradation in rumen, with significant effect even with the lowest doses. These authors did not determine negative effects on intestinal digestibility of non degraded proteins up to the levels of 13% tannic acid and 20% quebracho CT treatments. Supplying condensed (acacia and quebracho) and hydrolysable (chestnut and valonea) tannins at 50 g/kg have the potential to reduce methane production and protein degradation in rumen with minimal detrimental effects on the efficiency of fermentation in the rumen (Hassanat and Benchaar, 2013).

The substitution of a smaller amounts (approximately 10%) of alfalfa by *Onobrychis vicifolia* which contains CT is very useful to prevent bloat, when grazing ruminants consume large quantities of leguminous plants (alfalfa or clover). The gases produced in the rumen during fermentation cannot be released in standard paths since they are arrested in the constant spume caused by the rapid release of soluble proteins during chewing and degradation in rumen (Aerts et al., 1999; McMahon et al., 2000). Addition of tannine binding compounds (polyethylene glycol, polyvinyl-polypyrolidone, calcium hydroxide) prevents forming of tannin and protein complex, and it can also unlock proteins from the already formed complex. Therefore, polyethylene glycol is widely used in research of tannine effects on rumen fermentation (Getachew et al., 2000).

Secondary metabolites can form reversible and irreversible complexes with polysaccharides, alkaloids, nucleic acids and minerals (Schofield et al., 2001; McSweeney et al., 2001). Mechanisms of decreasing rumen degradation of different feed components by tannins are not sufficiently investigated. It is believed that some of following paths may be involved: lack of substrate (McMahon et al., 2000), inhibition of proteolytic, cellulolytic and other microbial enzymes and direct impact on rumen micro flora by changing their morphology or membrane permeability (McSweeney et al., 2001). Chiquette et al. (1988) have determined the presence of dense glycol-calix on walls of rumen bacteria, as an answer to high levels of CT from *L. corniculatus*, which is not formed when concentrations of the same compounds were lower. Tannins prevent or interfere in binding of rumen microorganisms to plant cell walls which is needed to begin degradation (McAllister et al., 1994). At the same time forming of complex compounds between tannins and proteins or carbohydrates makes these nutrients unavailable to microorganisms (Mueller-Harvey and McAllan, 1992). Since tannins are chelate agents, availability of certain metal ions needed to microorganisms could be decreased (Scalbert, 1991). Condensed tannins will easier inhibit activity of extracellular hemi-cellulose, which is more sensitive than cellulose bond to bacterial cell wall, leading to lower degradation of hemi cellulose in presence of tannins (Hervás et al., 2003b). The level of tolerance towards different tannin concentrations is specific in different species of microorganisms. Rumen bacteria with proteolytic and cellulolytic activity, which are sensitive to tannins at first, could after a short period of adaptation respond by changes in their metabolism, and continue with their normal func-

tions, if concentrations of tannins are not too high. Several types of microbial enzymes which can metabolite tannins (especially HT) are identified. Among bacteria capable of using HT is *Streptococcus caprinus* (*S. gallolyticus*), which produces pyrogalol (side product of tannic acid degradation) when activity of gallate decarboxylase is increased (O'Donovan and Brooker, 2001). One of disadvantages for using hydrolysable tannins as an additive in feed rich in proteins, is a possibility of their degradation by rumen micro flora and therefore the treated feeds would be just as vulnerable to degradation in rumen as untreated feeds. McSweeney et al. (2001) considered proantocyanidine (CT) as undegradable in rumen.

Many authors found negative effects of tannins on absorption of nutrients from the small intestine. Although tannin-protein complex are degraded at pH<3.5 (the pH of the abomasum), McNabb et al. (1998) have determined that pH in the beginning of the gut (≈5.5) can enable reforming of the tannin-protein complex, and hence decrease intestinal digestibility. Tannins can inhibit digestive enzymes by binding them to form insoluble compounds, or soluble but inactive compounds. Kumar and Singh (1984) and Silanikove et al. (2001) determined that condensed tannins inhibit digestive enzymes (trypsin and amylase) activity. However, the hypothesis that decrease of protein digestibility in the small intestine occurs due to the ability of tannins to inhibit digestive enzymes is not widely confirmed. After their separation from proteins in the abomasum, tannins can again bind to feed proteins in the gut, before getting in touch with digestive enzymes (Mole and Waterman, 1987; Mehansho et al., 1987). Changes in gut wall permeability caused by interactions of tannins and proteins of the mucosal cell membrane lead to the decrease in gut absorption (Silanikove et al., 2001). Blytt et al. (1988) stated that bile salts are capable for detergent actions and for preventing binding of tannins to digestive enzymes.

Due to the decrease of digestibility by addition of tannins into feed, the excretion of less volatile fecal nitrogen in organic form will be linearly increased. At the same time, excretion of urinary nitrogen in the form of easily degradable urea will be decreased (Aquerre et al., 2016; Henke et al., 2017). In this way, the emission of nitrogen into atmosphere is decreased, exerting positive effects on the environment (Brinkhaus et al., 2016). Addition of tannins into feed induces higher secretion of endogenous proteins such as salivary glycoproteins, mucus and digestive enzymes, and higher rate of gut cell peeling (Mehansho et al., 1987). Increased content of fecal nitrogen can be a result of metabolic endogenous nitrogen increase, and not necessarily the decreased amount of protein absorbed from feed.

Effects of dietary tannin supplements on lactation performance

According to Patra and Saxena (2011) effects of tannins of lactation characteristics in dairy cows depend on quality and quantity of protein in the diet. The influence of condensed tannins on production improvement can be explained by increase of amino-acid exploitation from the protected feed proteins in the gut and their distribution to different tissues (Garg et al., 2005). High levels of condensed tannins in consumed feed (above 50 g/kg DM) have a negative impact on milk production, while use of low to moderate levels of tannins (10-40 g/kg DM) can have a positive influence, since it leads to increase of milk production, higher protein, lactose and fat content in the milk, better fertility and animal health preservation (Wang et al., 1996; Makkar, 2003; Waghorn and McNabb, 2003; Mueller-Harvey, 2006; Soltan, 2009; Dey and De, 2014). Wang et al. (1996) have found a significant increase of milk production efficiency (21% higher in the middle and at the end of lac-

tation), higher protein and lactose production, as well as decrease of milk fat content in ruminants fed with *Lotus corniculatus* (44,5 g/kg BW CT), compared to animals treated with polyethylene-glycol. Milk protein concentration increase can be explained by higher availability of amino-acids in the small intestine, especially methionine and lysine. Higher content of lactose is a result of higher supply of glucose, since the synthesis of lactose in the mammary gland relies directly on glucose content in the blood. In ruminants, gluconeogenesis is mostly undertaken from propionic acid and amino-acids, hence higher availability of amino-acids leads to higher glucose synthesis rate. These authors have shown that increase of lactose concentration appears without changes in volatile fatty acids ratio, confirming the effects of tannins. The decrease in milk fat content was attributed to effect of dilution, due to lactose and protein concentration increase.

In the earliest stages of lactation, the frequency of oxidative stress and metabolism disorders increases. This might lead to decreased intensity of gluconeogenesis and increased keto-genesis. Due to the negative energy balance, mobilization of fatty acids from fat depots is more intense than metabolic abilities of liver cells, causing fatty liver and ketosis. Behman et al. (2008) determined that use of tannins in cow nutrition increases the level of glucose and decreases β-hydroxybutyrate (BHBA) concentration in blood, a parameter that is higher than 2 mmol/L in cows with clinical signs of ketosis. Addition of CT in cow's diet during transition period, can improve animals anti oxidative status by inhibiting lipid peroxidation and increasing anti oxidative activity of enzymes in plasma and in liver (Liu et al., 2013). In dairy cows fed diet containing products of soy flour treated with plant tannins, reduction ($p<0.05$) in blood urea N was documented (4, 9, 6 and 7% compared to control) (Soltan, 2009). The lower blood urea N concentrations reflected improvement in metabolism of nitrogenous components in diet and increased absorption of essential amino acids (Mabjeesh et al., 2000). Milking cows fed with silage containing different levels of condensed tannins from birdsfoot trefoil (*Lotus corniculatus* L.) or with added Quebracho tannin extract at dosages of 15 and 30 g/kg DM had reduced milk urea nitrogen, rumen ammonia and urinary N excretion (Broderick et al., 2017; Henke et al., 2017).

However, Aprianita et al. (2014), Brinkhaus et al. (2016) and Broderick et al. (2017) did not find significant effects of low tannin concentrations in cow diet on feed intake, milk yield, concentration and composition of proteins, concentration of nitrogen, yields of lactose and milk fat, or changes in fatty acids profile in milk. Aquerre et al. (2016) have described a trend of linear decrease in protein and fat content in the milk with increase of tannin levels in cow's diet. Feeding dairy cows with high level of tannins, 100 or 200 g/day of commercial tannin preparations, results in significant decrease of daily milk production from 30.17 kg to 26.34 kg and 25.92 kg in treated experimental cows when compared to the controls (13.0 and 14.2%, respectively).

Conclusion

Appropriate management of natural resources that contain tannins (selective pasture) or addition of tannin preparations to cow's diet in optimal levels, with diet rich in degradable proteins and non-protein nitrogen, has a potential to protect different nutrients from rumen degradation and improves the digestive utilization of feed. Depending on type, source and concentration, tannins can decrease the level of fermentation, microbial growth and feed digestibility.

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EFFECT OF HARVEST TIME ON FORAGE QUALITY OF INTERCROPPED COMMON VETCH (*VICIA SATIVA* L.) AND OAT (*AVENA SATIVA* L.)

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Abstract: Most annual legumes, such as common vetch (*Vicia sativa* L.) are sown with oat (*Avena sativa* L.) to improve growing conditions and forage harvesting. Some studies have examined the effects of varying seeding ratios, but the optimum seeding rates for those combinations in terms of forage quality are not well defined. The aim of these investigations was to evaluate forage quality of common vetch : oat mixtures depending on seeding rates and time of harvest.The experiment was conducted at the experimental field of Institute for forage crops, Kruševac, Serbia. The field trial was arranged in a randomised block design with three replications. In this research, pure stands of vetch and oat and their seed mixtures (60% vetch + 40% oat, 50% vetch + 50% oat and 40% vetch + 60% oat) were studied. The samples were harvested at three different cutting stages (begining of vetch flowering – 10% of flowering; forming the first pods on 2/3 vetch plants and forming green seeds in 2/3 pods). Quality characteristics such as crude protein and lignin content, as well as acid detergent fiber were highest in monoculture common vetch, followed by mixture with the lowest rate of oat. There were no significant differences among vetch:oat mixtures for neutral detergent fiber, hemicellulose and dry matter digestibility. With the advancement of growth stage, lignin content increased progressively, whereas dry matter digestibility decreased from 621.6 to 573.3 g kg^{-1} DM. According to the data obtained from this research work, it was realized that the mixtures with more than 50% vetch contribution could be recomended for high quality forage production.

Keywords: common vetch, oat mixtures, forage quality, forage harvesting

Introduction

Common vetch (*Vicia sativa* L.) is the important legume used for fresh and dry forage production. Cereals are also important contributors to animal feeding, both as grain and forage. Forage plants cultivated to provide good quality forage for farm animals can be grown either in a single or multiple species mixtures. Growing multiple species is generally preferred, because mixtures produce higher quality forage in addition to producing high yields and utilize the natural resources more effectively (Kokten et al., 2009). The incorporation of legumes in forage mixtures with grasses or cereals is an important and well established practice in some regions. Furthermore, oat, barley, wheat and triticale are added to provide a climbing frame for the legumes and to increase the bulk of feed produced (Tuna and Orak, 2007). Dairy cows can only produce high milk yields and beef cows can only reach their maximum potential if their intermediary metabolism is supplied with sufficient nutrients. Thus, high-quality forages have to be produced (Karsli et al., 2005).

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Vetch is grown as a rotation plant and utilized as grain or hay in animal production. Vetch hay contain up to 20% crude protein and can supply a considerable amount of the crude protein requirement of ruminant animals. Cereals like oat are palatable, succulent and nutritious forages with sufficient amount of carbohydrate, but are deficient in protein which is necessary for animal health and productivity (Ansar et al., 2010). Oats form an excellent combination, when fed along with other cold season legume crops like berseem, alfalfa and vetch (Anil et al., 1998).

Some researchers have suggested that grain cereal-legume intercropping has the potential to provide higher grain yield (Haymes and Lee, 1999) and more nutritionally balanced forage (Anil et al., 1998). Common vetch has a vine growing habit and if sown as monocrop, it lodges heavily (Caballero et al., 1995). As a result, forage yield and quality start to decrease due to the decomposition of herbage (Gulcan et al., 1988). Due to this, it is sown with winter-sown small grains, such as oats (*Avena sativa* L.), wheat (Triticum *sativum* L.), barley (*Hordeum vulgare* L.) and triticale (*Trititicosecale* Wittmack). In mixtures, companion cereals provide structural support for common vetch growth, improve light interception and facilitate mechanical harvest, whereas common vetch in mixtures improves the quality of forage (Caballero et al., 1995; Lithourgidis et al., 2006).

For perennial legumes such as alfalfa and some clovers, forage biomass and forage quality are often negatively correlated. This offsetting effect is reached near the bloom stage, which is recommended as optimum for maximizing the export of nutrients (Buxton et al., 1991). In *Vicia spp.* and other annual legumes such as soya bean, the seed fraction accounts for a greater proportion of the harvested biomass, and thus moving the harvest stage to the pod filling period may be desirable (Caballero et al., 1996; Hintz and Albretch, 1994). For this reason, comparative forage quality studies of these annual legumes should include harvesting stages within the seed filling period.

The aim of these investigations was to evaluate forage quality of common vetch : oat mixtures depending on seeding rates and time of harvest.

Material and Methods

The experiment was designed with three replications according to a randomized complete block. Experiment was established in autumn in 2012, on October the 20th and the samples were taken in spring in 2013. Common vetch and oat were grown in binary mixtures at the experimental field of Institute for Forage Crops, Kruševac – Serbia (21°19´35´´ E, 43°34´58´´ N). The vetch and oat were tested at five different mixture rates: 100% vetch + 0% oat; 0% vetch + 100% oat; 40% vetch + 60% oat; 50% vetch + 50% oat and 60% vetch + 40% oat. All mixtures were sown on plots of 20 m^2. Initial soil test from 0-30 cm soil depth before the trial commenced showed 0.16% N, 4.9 mg P_2O_5 / 100 g of soil, 23.1 mg K_2O / 100 g of soil, 3.5% organic matter and a pH of 5.7 in N KCl. One level of fertilizer was applied, 300 kg ha^{-1} NPK (15:15:15) before the seeding. The samples were harvested at three different cutting stages (begining of vetch flowering – 10% of flowering; forming the first pods on 2/3 vetch plants and forming green seeds in 2/3 pods).

Standard procedure described by the AOAC (1990) were used to determine Crude Protein (CP; AOAC 984.13). NDF assayed with heat stable α-amylase (aNDF), acid detergent fiber (ADF) and lignin content were determined according to Van Soest et al. (1991). Two stage pepsin-cellulase method was used for *in vitro* DMD (dry matter digestibility) (De Boevar et al., 1986).

The experimental data were analyzed by a two-way analysis of variance using a model that accounted for the main effects of common vetch:oat mixtures and time of harvest. Effects were

considered different based on significant ($p< 0.05$) F ratio. The significance of differences between arithmetic means was tested by LSD test (STATISTICA 6, Stat. Soft. 2006).

Results and Discussion

Data regarding the dry matter yield recorded during this study, presented in the Table 1 showed statistically significant differences among treatments. Maximum dry matter yield was produced by pure oat and increased with plant growth and development from 13.82 t ha^{-1} to 14.68 t ha^{-1}. The treatments and growth stages means showed that all mixtures gave better performance that pure stands of vetch. The highest dry matter yield of 13.13 t ha^{-1} was produced by 40 : 60 vetch-oat mixture, and with growth and development decreased to 11.50 t ha^{-1}. Ansar et al. (2010) worked on the cereal – vetch mixtures for forage yield and quality under rainfed conditions of Pothwar and recorded that vetch – oat mixtures produced the highest dry matter yield in comparison with any other vetch- cereal mixtures. Tuna and Orak (2007) also found the mixtures produced higher forage yield than the pure stands. Lower results were reported by Assefa and Ledin (2001) in which dry matter forage yield of pure vetch, oat and mixture forage was 4.3, 7.6 and 7.3 t ha^{-1}, respectively.

An analysis of variance found statistically significant differences among mixture rates, and cutting stages for crude protein content. The mixture rates x cutting stages interactions were also significant. As seen in Table 1, the highest crude protein content (199.1 g kg^{-1} DM) was observed in the 100% common vetch, and the lowest crude protein content was obtained from the 100% oat (103.7 g kg^{-1} DM). Droushioitis (1989) indicated that legumes are more palatable to animals and possess a higher crude protein content than cereals. Among the mixtures, the highest crude protein content (152.8 g kg^{-1} DM) was found in the 75% vetch mixture. Since common vetch had a high nitrogen content, crude protein content increased as the percentage of common vetch in mixture increased. It is well known that the crude protein content decreases as pplant growth stage progresses. In this study, the crude protein content increased from the first (142.2 g kg^{-1} DM) growth stage to the second (148.3 g kg^{-1} DM) growth stage, but the crude protein content decreased with the delay of the cutting stages, and 143.0 g kg^{-1} DM was obtained at the third cutting stage.

The highest crude protein yield was obtained from 25% vetch + 75% oat mixture (1571.2 kg ha^{-1}). Whereas, the lowest crude protein yield was found in the 100% common vetch (1262.3 kg ha^{-1}). The 100% common vetch produced the lower crude protein yield than pure oat. Bayram and Celik (1999) found similar results in common vetch + oat mixtures. Cereals constitute forages relatively low in protein, and animals usually need to be fed with some form of protein concentrate supplementation. Legumes produced higher crude protein concentration, but total crude protein yield per unit area was less than cereal-legume mixture as crude protein yield depends upon dry matter production, which is higher for mixtures.

The average values for the NDF content at different stages of growth showed that NDF content increased from flowering stage (568.7 g kg^{-1} DM) to forming the firs pods of common vetch (583.6 g kg^{-1} DM), but did not differ significantly. With the further growth and development a significantly lower NDF content (550.1 g kg^{-1} DM) was determined. The lowest NDF content was found in pure vetch (519.0 g kg^{-1} DM), while the highest NDF content was found in pure oat (623.2 g kg^{-1} DM).

The lowest ADF content was found in the third stage of growth (404.4 g kg^{-1} DM). Despite the NDF content, the highest ADF content was determined in pure common vetch (434.2 g kg^{-1} DM). Results of this investigation showed that lignin content increased with growth and development

from 77.3 to 94.0 g kg^{-1} DM. The highest lignin content was found in pure common vetch (95.2 g kg^{-1} DM). According to the fact that common vetch contained significantly larger amount of lignin, and the mixture with the highest proportion of common vetch contained the highest amount of lignin (89.4 g kg^{-1} DM). Dry matter digestibility of the examined vetch-oat mixtures decreased from 621.6 to 573.3 g kg^{-1} DM with growth and development. Pure common vetch was predominant in DMD (648.3 g kg^{-1} DM) compared to pure oat by 14% (568.5 g kg^{-1} DM), whereas common vetch seeding rate did not contribute to the better digestibility of vetch-oat mixtures.

Table 1. Forage quality of intercropped common vetch (*Vicia sativa* L.) and oat (*Avena sativa* L.)

Factors A	B	DMY t ha^{-1}	CP g kg^{-1} DM	CPY kg ha^{-1}	ADF g kg^{-1} DM	NDF g kg^{-1} DM	Lignin g kg^{1} DM	DMD g kg^{1} DM
a_1	b_1	7.00^{h}	183.7^{c}	1285.9^{j}	440.4^{b}	509.2^{g}	88.0^{d}	649.9^{b}
	b_2	13.82^{b}	99.5^{i}	1375.1^{h}	404.5^{e}	627.0^{b}	61.3^{b}	589.7^{d}
	b_3	13.13^{c}	125.3^{g}	1645.2^{ab}	432.9^{c}	575.8^{d}	75.8^{g}	619.2^{c}
	b_4	11.06^{ef}	147.2^{e}	1628.0^{b}	415.0^{d}	562.2^{d}	74.8^{g}	620.9^{c}
	b_5	9.56^{g}	155.3^{d}	1484.7^{f}	441.0^{b}	569.2^{d}	86.5^{e}	628.6^{c}
$\overline{X}_{A_1}$		**10.91^{AB}**	**142.2^{B}**	**1551.4^{B}**	**426.8^{A}**	**568.7^{A}**	**77.3^{C}**	**621.6^{A}**
a_2	b_1	6.21^{i}	213.4^{a}	1325.2^{i}	434.0^{c}	527.7^{f}	96.9^{b}	656.7^{a}
	b_2	13.97^{b}	114.5^{b}	1599.5^{c}	441.4^{b}	663.8^{a}	81.1^{f}	579.8^{d}
	b_3	12.81^{cd}	121.6^{g}	1557.7^{d}	448.9^{a}	597.0^{c}	91.1^{c}	567.3^{e}
	b_4	12.12^{d}	134.3^{f}	1627.7^{b}	413.8^{d}	569.9^{d}	85.2^{e}	574.7^{e}
	b_5	10.60^{f}	157.6^{d}	1670.5^{a}	419.5^{d}	559.5^{e}	88.3^{d}	566.5^{e}
$\overline{X}_{A_2}$		**11.14^{A}**	**148.3^{A}**	**1652.0^{A}**	**431.5^{A}**	**583.6^{A}**	**88.5^{B}**	**589.0^{B}**
a_3	b_1	5.81^{j}	200.1^{b}	1162.5^{k}	428.0^{c}	520.1^{f}	100.8^{a}	638.6^{b}
	b_2	14.68^{a}	97.0^{i}	1423.9^{g}	396.6^{f}	578.8^{d}	89.9^{d}	536.1^{g}
	b_3	11.50^{e}	130.9^{f}	1505.3^{e}	397.1^{f}	548.0^{e}	90.9^{c}	565.4^{e}
	b_4	9.86^{g}	141.6^{e}	1396.2^{h}	399.2^{f}	551.5^{e}	95.1^{b}	552.2^{f}
	b_5	9.06^{g}	145.5^{e}	1318.2^{i}	401.2^{e}	551.9^{e}	93.4^{c}	574.4^{e}
$\overline{X}_{A_3}$		**10.18^{B}**	**143.0^{B}**	**1455.7^{C}**	**404.4^{B}**	**550.1^{B}**	**94.0^{A}**	**573.3^{C}**
$\overline{X}_{B_1}$		**6.34^{E}**	**199.1^{A}**	**1262.3^{C}**	**434.2^{A}**	**519.0^{C}**	**95.2^{A}**	**648.3^{A}**
$\overline{X}_{B_2}$		**14.15^{A}**	**103.7^{E}**	**1467.4^{B}**	**414.2^{BC}**	**623.2^{A}**	**77.4^{D}**	**568.5^{C}**
$\overline{X}_{B_3}$		**12.48^{B}**	**125.9^{D}**	**1571.2^{A}**	**426.3^{AB}**	**573.6^{B}**	**85.9^{C}**	**584.0^{B}**
$\overline{X}_{B_4}$		**11.01^{C}**	**141.0^{C}**	**1552.4^{AB}**	**409.4^{C}**	**561.2^{B}**	**85.0^{C}**	**582.6^{B}**
$\overline{X}_{B_5}$		**9.74^{D}**	**152.8^{B}**	**1488.3^{B}**	**420.6^{B}**	**560.2^{B}**	**89.4^{B}**	**589.8^{B}**

A – stage of growth; B – mixture rate; DMY – dry matter yield; CP – crude protein; CPY – crude protein yield; ADF – acid detergent fiber; NDF – neutral detergent fiber; DMD – dry matter digestibility; a_1 - beginning of vetch flowering – 10% of flowering; a_2 - forming the first pods on 2/3 vetch plants and a_3 - forming green seeds in 2/3 pods; b_1 - 100% vetch + 0% oat; b_2 - 0% vetch + 100% oat; b_3 - 40% vetch + 60% oat; b_4 - 50% vetch + 50% oat and b_5 - 60% vetch + 40% oat; Different letters denote significantly different means ($P< 0.05$).

Aasen et al. (2004) reported that an increasing the seeding rate of leguminous component in the mixtures with grasses and cereals contributed to the reduction of NDF and ADF content. On

the other hand, Lithourgidis et al. 2006. found that NDF content increased with increasing of the common vetch germinated grain in the mixtures. Carpita and McCann 2000. found that increasing the seeding rate of common vetch in mixture increased the lignin content, which could be explain by the fact that the cell wall of cereals contain lower lignin compared to the cell wall of leguminouses. The results of these investigations are in agreement with the results obtained by Assefa and Ledin (2001).

Conclusion

The results of these investigations suggested that sowing common vetch in mixture with oat could produce as much forage as pure common vetch, while forage quality in terms of crude protein content would be far superior to pure common vetch. DMY and CP concentration of vetch-oat mixture depend more on the mixture structure than on growth stage of harvest. Growth stage, however, had a significant effect on lignin concentration and DMD. It was also determined that high quality hay production could be possible from all investigated vetch-oat mixtures and would be adequate to support high levels of animal production.

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THE INFLUENCE OF INOCULATION ON FERMENTATION INTENSITY AND PROTEOLYSIS IN ANNUAL LEGUME SILAGES

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Abstract: The overview of domestic and foreign investigations of the influence of various inoculants on parameters of fermentation, proteolysis and aerobic stability in annual legume silages. The use of inoculants based on homofermentative lactic acid bacteria is not achieving better results than the use of chemical conservants (organic acids and their salts) but they are extensively used because they can be easily applied and don't produce residues. That is the reason why they are allowed in organic animal production. Aside from that, they limit proteolysis and have positive effects on nitrogen fraction status, which is especially important in intensive dairy production. Modern inoculants contain also heterofermentative lactic acid bacteria that intensify not only production of lactic but also acetic acid. As a result the smaller amounts of fermentable sugars remain in the silage (as substrate for yeast activity and aerobic degradation of silage), more acetic acid is produced (which has fungicide properties) and better aerobic stability is obtained.

Keywords: annual legumes, inoculants, fermentation, proteolysis, aerobic stability.

Introduction

Annual legumes are important plants species for production of forage and concentrate feeds. They are used as green mass, as hay, haylage, silage and grain. Some of them (peas, vetch) have soft stems and they are mostly produced mixed with cereals, while other (soya, horse beans) are produced as sole crop (for grain) or mixed (for biomass) with millet-type cereals (Đorđević et al., 2018). They have a significant content of protein with high biological value in both biomass and grain, which is why they are important feeds for animals. Considering evident climate changes during the par several years (dry and very hot summers), in our situation especially important became peas and vetch, since they can be produced with autumn sowing and use of winter moisture from the soil. The concept of green forage is mostly abandoned in intensive cattle production so that the most important use of these legumes became conserved form, as hay, silage or haylage (Đorđević et al., 2010). The hay production form sole crops or mixtures of peas and vetch with cereals can be a problem because of high precipitation in the spring (Đorđević et al., 2011). That is why those species are mostly used as silage or haylage (Đorđević et al., 2000 and 2005). Contrary to that, soya is produced mixed with maize or sorghum in order to obtain better energy and protein ratio in the silage (Đorđević et al., 2004). However, sowing such crops is done in the spring of summer and their yields depend on the amount of summer rainfalls.

In the process of annual legume ensiling inoculation has the aim to intensify fermentation of the homofermentative type in order to produce maximal amounts of lactic acid, rapid decrease in pH value, decrease in enzymatic activity of the presents microorganisms and maximal preservation of nutritive value (Đorđević and Dinić, 2003). In recent times especially important became inoculants with heterofermentative lactic acid bacteria which enable better aerobic stability in silages.

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Such silages have longer usable time after the silo is opened and in contact with oxygen, which is very important for small and medium sized farms with inadequate silo objects and irregular extraction of silage (Ivetić et al., 2013).

Discussion

Although the inoculation of ensiled legumes cannot be compared with the use of chemical conservants considering the results (Keles et al., 2014), it is dominantly used technology because its application is simple and they do not produce residues in animal feeds and animal products (Đorđević et al., 2014). For example, Tyrolova and Vyborna (2011) ensiled peas with natural moisture content or wilted and with bacterial inoculants added (*Lactobacillus rhamnosus* and *Enterococcus faecium*, 1×10^{11} CFU/g), or with chemical conservant (formic acid 55%, propionic acid 5%, ammonium formicate 24% and benzoic acid 2.2%) in the amount of 4 l/t (Table 1). They determined that chemical conservant has bigger influence on pH value and reduced complete fermentation and proteolysis. Compared with control treatment, inoculant produced decrease in pH value and increase in lactic acid production. In wilted material of all treatments the lower level of proteolysis is determined, expressed as percent of ammonia N compared to total N. All this emphasizes the necessity to wilt grasses and legumes when ensiled, in order to obtain moisture content below 70% (Đorđević et al., 2001). Wilting is enabling suppression of unwanted micro flora, favoring lactic acid bacteria and decreasing activity of proteolytic enzymes (Đorđević et al., 2013).

Table 1. Fermentation parameters in field pea silage as affected by wilting and the addition of inoculant or chemical additive, % (Tyrolova and Vyborna, 2011)

Parameters	WS	T			Significance		
		C	I	CH	T	WS	T×WS
DM	U	22.03	21.80	22.24	n.s.	***	n.s.
	W	32.83	33.29	33.49			
pH	U	4.89^{a}	4.02^{ab}	3.94^{b}	***	n.s.	***
	W	4.31^{a}	4.10^{b}	4.08^{b}			
Lactic acid	U	2.56^{a}	2.87^{a}	2.02^{b}	***	n.s.	n.s.
	W	2.30	3.29^{b}	2.54^{a}			
Acetic acid	U	0.45^{a}	0.51^{a}	0.34^{b}	n.s.	**	***
	W	0.54	0.44	0.52			
Butyric acid	U	0.09	0.07	0.07	n.s.	n.s.	n.s.
	W	0.16	0.10	0.09			
Lactic/acetic	U	5.79	5.71	5.84	***	n.s.	***
	W	4.31^{a}	4.10^{b}	4.89^{a}			
NH_3N/Σ N	U	1.79	1.82	1.58	n.s.	***	n.s.
	W	1.37	1.02	1.00			

WS: wilting status (U - unwilted; W- wilted); T- treatment; DM: dry matter; C: control; I - bacterial inoculant; CH - chemical additives.

The other way to increase the dry matter is in the natural process of plant maturation. During plant maturation the fiber content is increasing and the degree of its lignification, which is decreasing its digestibility and utilization of such feeds (Blagojević et al., 2017). However, chemical

composition of annual legumes and cereals is not changing in the same way as in perennial legumes and cereals, because of husk and grain presence in the total plant mass (Salawu et al., 2001).

In the nutrition of high yielding dairy cows today the nitrogen matter status is very important, which is highly influencing their production, health and reproduction (Grubić et al., 2014). The characteristic of ensiling technology is intensive proteolytic processes which may significantly decrease protein utilization (Đorđević et al., 2004). Slottner and Bertilsson 2006. cite that in living plants 75-90% of the total nitrogen is in the form of true proteins while in silages it is 30-50% only. Modern recommendations for ruminant feeding call for detailed information about precise content of protein and carbohydrate fractions in the diets which enable maximal utilization of present nutrients. In companion crops consisting of legumes and cereals the contents of protein and carbohydrate fractions depend on their ratio in the mixture, moment of cutting and fermentation quality. CNCPS system (Fox et al., 2003) is taking into account those variations in protein and carbohydrate components when calculating metabolizable energy and in order to maximize microbial protein synthesis when formulating rations for dairy cows. Following the contemporary trends Blagojević et al. (2017) investigated the influence of inoculation (*Lactobacillus plantarum, identified faecium* and *Lactobacillus brevis*) on protein fractions in companion crops of peas and oats (Table 2). Authors detected that the amount of true protein was significantly higher and the amount of soluble protein and NPN significantly lower when inoculants were used. Also, inoculation of companion crops resulted in significant decrease in PA protein fraction (soluble protein mostly of non-protein origin), from 575.8 to 523.7 g kg^{-1} TP and significant increase in PB_1 fraction (soluble true protein which is rapidly digestible in the rumen) and PB_3 fraction (true protein that is not very degradable in the rumen).

Table 2. Protein fractions by CNCPS of pea:oat bi-crop silages (Blagojević et al., 2017)

Protein fractions, g kg^{-1} CP	Pea:oat mixture						
	Treatments	A_1	A_2	A_3	A_4	A_5	$\bar{x}_B$
PA	B_1	687.1^{b}	440.0^{f}	481.3^{e}	623.4^{d}	647.2^{c}	575.8^{a}
	B_2	705.2^{a}	447.6^{f}	365.6^{g}	451.7^{f}	648.2^{c}	523.7^{b}
	$\bar{x}_A$	696.2^{a}	443.8^{d}	423.4^{e}	537.5^{c}	647.7^{b}	
PB_1	B_1	17.0^{e}	79.8^{bc}	65.7^{c}	21.7^{de}	2.8^{e}	37.4^{b}
	B_2	18.2^{e}	39.3^{a}	105.3^{a}	87.1^{ab}	8.5^{e}	51.6^{a}
	$\bar{x}_A$	17.6^{c}	59.6^{b}	85.5^{a}	54.4^{b}	5.6^{c}	
PB_2	B_1	$210.^{cd}$	311.4^{a}	283.0^{b}	236.8^{c}	258.5^{bc}	260.0^{ns}
	B_2	186.5^{d}	278.2^{b}	276.8^{b}	282.4^{b}	231.7^{c}	251.1^{ns}
	$\bar{x}_A$	198.5^{c}	294.8^{a}	279.9^{a}	259.6^{b}	245.1^{b}	
PB_3	B_1	10.6^{d}	8.5^{d}	26.2^{bc}	17.0^{c}	11.2^{d}	14.7^{b}
	B_2	12.2^{d}	35.2^{b}	101.5^{a}	19.4^{c}	14.5^{c}	36.5^{a}
	$\bar{x}_A$	11.4^{c}	21.8^{b}	63.9^{a}	18.1^{bc}	12.8^{bc}	
PC	B_1	74.7^{e}	160.2^{b}	143.8^{c}	101.1^{d}	80.1^{e}	111.9^{b}
	B_2	77.8^{e}	199.6^{a}	150.7^{bc}	159.4^{b}	97.1^{d}	136.9^{a}
	$\bar{x}_A$	76.2^{e}	179.9^{a}	147.2^{b}	130.2^{c}	88.6^{d}	

A_1, 100% pea + 0% oat; A_2, 0% pea + 100% oat; A_3, 25% pea + 75% oat; A_4, 50% pea + 50% oat; A_5, 75% pea + 25% oat; B_1, control treatment without bacterial inoculant; B_2, treatment with bacterial inoculant; different letters in a row denote significant differences between means ($P<0.05$); ns, not significant.

One of the biggest problems for practical silage (mostly maize) use is in secondary fermentation which occurs later, when silage is not regularly used and is exposed to the air for some time. Lactic acid as the main product of homofermentative sugar fermentation in the ensiled mass has strong anti bacterial effects but not very strong fungicide. On the contrary acetic, butyric and propionic acid have strong fungicide effects, which is why those acids are welcome in silages (Lynch et al., 2012; Arriola et al., 2011; Tabacco et al., 2011). However, larger amounts of acetic acid have negative effects on silage intake and decrease its energy value. When homofermentative lactic acid bacteria dominate in the ensiled material (in classic inoculation) the faster decrease in pH value id obtained with less sugars used. The higher amount of residual sugar is very favorable for yeast development after the silo is opened and higher aerobic degradation is occurring. This is the reason why today the inoculants are used which also contain some heterofermentative lactic acid bacteria (like *Lactobacillus buchneri*), which are increasing acetic acid synthesis and with that the higher aerobic stability is achieved. Such inoculants are important also for companion crop silages of annual legumes and cereals, because of residual sugars (from cereals) and lower aerobic stability. Keles and Demirci (2011) ensiled mixture of triticale and vetch with the addition of homofermentative bacteria (*Lactobacillus plantarum*), mixture of homofermentative bacteria and enzymes (*L. Plantarum, Enterococcus faecium, Pediococcus acidilactici, Lactobacillus salivarius*, and alpha-amylase, cellulase, hemicellulase and pentonsanase), and heterofermentative bacteria (*Lactobacillus buchneri*). The authors discovered that the treatment with homofermentative bacteria (*Lactobacillus plantarum*) produced higher amount of lactic acid and lowest amount of acetic acid, while the most unused sugars remain (Table 3). That "surplus" of sugars can be the main reason for later secondary fermentation. Treatment with homofermentative bacteria and enzymes had significantly reduced residual sugars (because of enzymes) and the ratio of lactic acid to other fermentation products was narrower (0.66). With the use of *Lactobacillus plantarum* the amount of residual sugars was significantly lower and the amount of acetic acid produced was higher than lactic acid. All differences were significant. In the second part of the investigation the silages were used in lamb fattening. Lambs preferred to consume silage treated with *Lactobacillus buchneri.* This shows that inoculants with heterofermentative bacteria have significant effects on ensiling process and animal intake of produced silages.

Table 3. Fermentation characteristics of baled triticale-Hungarian vetch silage (Keles and Demirci, 2011)

Variable	Treatments				SEM	P
	C	LP	HM+E	LB		
Dry matter, g/kg	441	407	440	445	22.9	0.636
pH	4.6	4.5	4.6	4.6	0.07	0.601
Lactic acid (g/kg DM)	53	62	52	39	4.13	0.015
Acetic acid (g/kg DM)	23	16	24	44	5.70	0.024
Propionic acid (g/kg DM)	0.8	1.9	4.8	9.5	1.26	0.002
Butyric acid (g/kg DM)	0.3	0.3	0.5	0.5	0.15	0.662
FP (g/kg DM)	77	81	81	94	7.45	0.441
LA/FP	0.69	0.77	0.66	0.43	0.04	0.000
WSC	49	61	56	29	6.09	0.014
Ammonia-N (g/kg N)	113	115	104	110	13.6	0.994

C = control; LP = *L. Plantarum*; HM+E = homofermentative LAB + enzyme; LB = *Lactobacillus buchneri*; LA/FE = proportion of lactic acid (LA) in total measured fermentation products (FP); WSC = water soluble carbohydrates.

Conclusion

The use of inoculants became regular measure when various feeds are ensiled. That way the fermentation process is maximally controlled, the proteolytic processes are limited and nutritive value is preserved. For silages made from companion crops of annual legumes and cereals the inoculants with heterofermentative lactic acid bacteria. Such silages have more sugars, originating from cereals, and aerobic degradation of nutrients may occur. When inoculating with heterofermentative bacteria the more intensive fermentation is achieved, more sugars are utilized and more acetic acid which is fungicide is produced. The result is better aerobic stability of produced silages.

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ECOLOGICAL LIVESTOCK IN REPUBLIC OF CROATIA

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Abstract: Ecological agriculture is a special form of agricultural production that takes into account agriculture as a whole system in terms of water-soil-plant-animal and takes care of the balance of all key elements. The objectives of ecological agriculture are to preserve soil fertility, nutrient circulation, animal welfare, environmental protection and biodiversity preservation, energy and raw materials preservation, and production of healthy food. The ideal ecological farm is the mixed type farm with the plant and livestock production organized in a way that represents a harmonious whole that meets most needs from its own resources. The main characteristics of ecological agriculture is the avoidance of artificial materials (artificial fertilizers, herbicides, pesticides) in the production and use of fertilizers and preservatives on a natural basis.

Keywords: ecological agriculture, ecological livestock, healthy food, environmental protection, Croatia

Introduction

Ecological agriculture is a special form of agricultural production that takes into account agriculture as a whole system in terms of water-soil-plant-animal and takes care of the balance of all key elements. Special emphasis is on preservation of soil fertility with more humus and organic fertilizers. The objectives of ecological agriculture are to preserve soil fertility, nutrient circulation, animal welfare, environmental protection and biodiversity preservation, energy and raw materials preservation, and production of healthy food. The ideal ecological farm is the mixed type farm with the plant and livestock production organized in a way that represents a harmonious whole that meets most needs from its own resources.

It is important to emphasize that ecological agriculture is not a return to the old, return to our grandparents' agriculture, but is part of modern agricultural production, trade and agronomic science, and is based on its latest knowledge and achievements. Ecological agriculture is also a sustainable agriculture because the production processes are trying to restore what it has taken. At the same time, it is an integral part of sustainable agriculture because it contains certain characteristics that are reflected in the reduction of all forms of pollution, sustainably use natural resources, preserves indigenous breeds / varieties, preserves agro-eco system, maintains and increases the fertility of agricultural land, crop and animal production are linked, or rounded their process. Water for irrigation and livestock feed requires high quality water. Along with certain conditions in the transitional period, some changes need to be made to the farm as well, such as optimizing the number of goats on the farm, introducing new plant crops into production, modifying the facilities or building

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new animal facilities, abandoning the use of artificial fertilizers and other chemical resources and abandoning routine animal treatment with chemical agents (Grgić, 2013).

The application of ecological agriculture in the world

Today, around 37 million ha are under ecological agriculture, of which 53% of the area is cultivated, while the rest are meadows and pastures (Figure 1). In recent years there has been a large increase in the area under ecological farming, and the largest increase was recorded in Europe and Oceania. Depending on climatic and relief conditions, ecological production differs on the continents, so a similar structure is found in North America and Europe, where most of the oranges and gardens and grassland are represented, while the Latin American and Oceania areas are mostly represented by permanent pastures. Most of the area of ecological pasture in the world is in Oceania (50%), Europe (20.7%) and South America (20.7), and most of the arable land is located in North America (20.7%) and Europe (about 70%). Approximately two-thirds of the areas in the ecological farming (23.7 million hectares) are used as pastures (97% of Australian ecosystems are pasture in Australia), while around 17% (6.1 million hectares) of world's land is used in ecological plant production. Kisić (2014) states that in 2010 there were about 1,600,000 ecological farms around the world, of which 280,000 in Europe. Of that number, in Africa it is 34%, Asia 29% and Europe 18%. States with the most eco-growers are India, Mexico and Uganda, while the European countries most ecological farms are Italy and Spain.

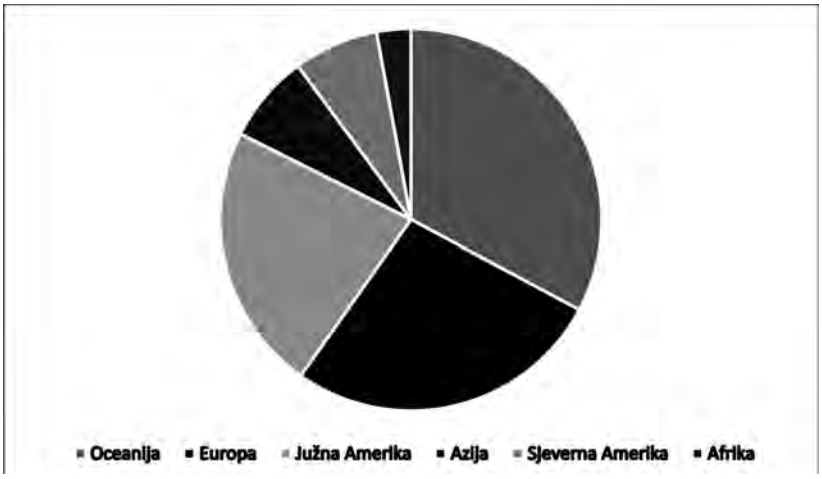

Figure 1. Global distribution of land use in ecological agriculture (Kisić, 2014).

Ecological agriculture in Croatia

The official statistical status of ecological agriculture in the Republic of Croatia is being carried out since the year 2000 when it was only 12 ha since the situation improved significantly as shown in Table 1 (Kisić, 2014). The number of farms that apply ecological principles increased from 1125 (the year 2010) to 3,546 (2016).

Table 1: Plant Production in Ecological Agriculture in the Republic of Croatia from 2010 to 2016 (MPRH, 2016)

Plant production (ha)	2010.	2011.	2012.	2013.	2014.	2015.	2016.
Arable land	17.066	22.156	17.815	19.183	23.802	30.444	44.147
Orchards	1.770	2.058	2.851	3.239	3.790	5.638	7.814
Vineyards	400	614	634	791	931	913	1 119.16
Olive groves	322	600	860	1.330	1.472	1.334	1.536
Meadows and pastures	2.452	4.934	7.635	14.279	16.403	33.612	39.089
Fallow	156	452	720	293	477	-	1.868
Uncultivated land	444	352	69	-	-	-	20
Vegetables	284	143	160	165	304	343	323
Medicinal herbs	388	718	1.159	1.368	2.876	3.494	4.226
Nursery gardens and other permanent crops	-	-	-	-	-	103	41.03
Number of farms	1.125	1.494	1.528	1.609	2.194	3.061	3.546
Total areas	23.282	32.036	31.904	40.660	50.054	75.883	93.814

During the period from year 2010 to 2016 can be seen positive trends in the representation area of plant production under ecological farming. In the total area under ecological farming in year 2010 was dominated by arable land, accounting for 73.30%.

Table 2: The share of ecological areas in relation to the total utilized agricultural land (MPRH, 2016)

Year	Utilized agricultural land	Areas under ecological farming	Share of ecological areas in relation to the total utilized agricultural land (%)
	ha	ha	
2010.	1.333.835	23.282	1,75
2011.	1.326.083	32.036	2,42
2012.	1.330.973	31.904	2,40
2013.	1.568.881	40.660	2,59
2014.	1.508.885	50.054	3,32
2015.	1.537.629	75.883	4,94
2016.	1.546.019	93.814	6,07

Ecological livestock

Ecological livestock farming is part of the ecological agriculture that seeks the livestock production in an environmentally friendly way, while preserving the environment and producing high-quality foods in the human diet. Ecological livestock, as part of ecological agriculture, has a number of disadvantages, both in the production of plants that feed animals and in animal breed-

ing. Contamination of soil, air and water, hazardous substances found in food, decrease the quality of food, the extinction of species and breeds, the reduction of biodiversity and damage caused to the landscape are just some of the features of conventional agricultural production. By contrast, there is ecological agriculture or as we call it organic or biological agriculture within which ecological livestock is found. The aim of ecological livestock breeding is not just to obtain high-value food for human consumption, but is partly a goal of this production and to ensure animal welfare and environmental protection. Domestic animals use only a portion of the feed to produce, while the rest of the feed, is cast in the form of manure. The use of manure that is the fundamental factor of soil fertility in ecological agriculture closes the cycle of nutrient and energy circulation in the agroecological system. Manure has a very important role in improving the soil and some of them improve the water-air mode, improved soil structure, better soil microbial activity, and thus better fertility.

In ecological farming, in breeding of domestic animals, it is important for animals to stay in the open because they are moving in the fresh air and the sun, which is beneficial for fertility, circulation, metabolism, digestion and many other advantages. In this way of animal breeding it is possible to provide social relations between them, while in conventional farming in the majority of cases it is not possible because the animals are kept in pens or cages which are certainly not humane conditions for keeping animals. Mixed farming, that is plant production with livestock farming is ideal for ecological farming because it has a cycle between these two production that complement each other. Ecological livestock production in the Republic of Croatia has a small share, but from year 2010 to 2016 growth of individual branches of livestock was recorded, while some noted the decline can be clearly seen in Table 4.

Table 4: Quantity of ecological livestock in the Republic of Croatia (MPRH, 2017)

Species	**2010.**	**2011.**	**2012.**	**2013.**	**2014.**	**2015.**	**2016.**
Cattle	9.796	7.646	5.640	6.540	7.308	7.002	14.442
Equidae	452	920	507	874	291	265	1.753
Sheep	9.349	14.773	17.601	19.411	21.690	23.774	50.135
Goat	1.545	1.206	1.477	1.769	1.552	2.163	3.080
Pigs	130	448	1.361	1.122	961	1.114	1.083
Poultry	1.137	2.107	1.947	2.036	2.540	2.093	3.388
Bees, number of beehives	2.381	1.804	2.462	2.678	3.649	3.418	2.065
Rabbits	50	-	23	47	5	-	5
Aquaculture (tonnes)	5	-	250	810	340	300	100

In the Republic of Croatia, sheep, goats and cattle are mostly represented in ecological livestock, while poultry, pigs and bees are bred in a smaller number, with the smallest number being equidae, rabbits and aquaculture products. In year 2010 in the Republic of Croatia 9796 cattle was recorded, and by 2012 that number decreased to 5,640 cattle. Another increase was recorded in year 2016 with records of 14,442 cattle. The number of sheep have been growing steadily, for instance in year 2010 there were 9,349 sheep in ecological farming, and by 2016 this number increased significantly when it was 50,135 sheep, which was a considerable increase. In other species, it has been recorded an increase and fall, or vice versa, which means that the number of species in ecological livestock farming varies from year to year.

For animal breeding, all breeds of animals are not suitable, and for breeding is used breeds that are adapted to local conditions of posture that are naturally resistant and which have the ability to be fed with as much volumes of voluminous feed. Naturally resistant animals are the most modest in terms of keeping, feeding and health care, and those that are suitable for outdoor use, and such animals are mostly pigmented and resistant to sunlight. One of the main principles of ecological animal husbandry is a strict ban on the use of antibiotics, coccidiostats, sulphonamides and other chemotherapeutic agents, growth promoters and other substances that stimulate the growth or production. Animals should be fed with ecologically produced feed from their own farm or if it is not possible, feed should be purchased from another ecological breeding farm (Senčić et al., 2011).

The impact of ecological animal breeding on the environment

In construction of facilities for animals in ecological animal breeding, every animal should be kept in a more natural environment. Housing conditions should correspond to their ethological needs to express natural behaviour peculiar to their species in order to prevent behaviour disorders, that is animal welfare should be taken into account. "Animal welfare" can generally be defined as the state of total mental and physical health in which the animal is in harmony with the environment (Senčić et al., 2011).

In conventional animal breeding, animals are bred in unnatural and inhumane conditions where they are held on a slatted or partially slatted floors, on small box areas or cages, where they are restricted in movement and do not have social contact with each other. The consequences of such breeding conditions are unusual animal behaviour, frustration and stress, less pronounced and in some cases omitted estrus in animals, then animal cannibalism and poorer quality meat. In ecological facilities it is necessary to have adequate temperature and relative humidity, gas pollution and airflow that will not harm the animal's health, and it is necessary to ensure the more natural ventilation and lightening (Safundžić, 2015).

The livestock production is a producer of large quantities of gases that go into the air (methane, ammonia and nitrogen oxide) and soil (nitrates, phosphates and heavy metals). This is a key problem in areas of high population density and population of animals and affects legislation and sustainability of livestock production. According to research, raising livestock or ecological farming is not as damaging as industrial, where animals accumulate in industrial farms where large amounts of CO2 are produced. If animals are kept on open natural areas it is necessary to provide them with a space for protection against precipitation, sun, wind and high and low temperatures. For the above reasons, animals should be provided with access to open areas, i.e. pastures, whenever weather conditions allow, unless prohibited for health reasons. In animal treatment, it is necessary to use as much medicine as possible on a natural basis (plant, animal and mineral resources). If it is necessary to use drugs, animals must not be slaughtered, and their meat is not for use until after the double wait period of the drug, which for a particular drug prescribed by the manufacturer.

Conclusion

One of the main advantages of ecological livestock in the Republic of Croatia is that there are agro ecological resources for engaging with such production. The significant increase in land area and the number of farms involved in ecological plant and animal production indicate increasing

demand for ecological products by the market, but also on the possibility of export of the same. Trends in tourism which favour the development of rural tourism as well as the resources available throughout EU funds give opportunities for many family farms, which can go through ecological farming and production of healthy feed.

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ATTITUDES OF TABLE EGG CONSUMERS/PURCHASERS FROM THE CITY OF BELGRADE TOWARD ORGANIC EGGS

Tolimir N.*[1], Maslovarić M.[1], Škrbić Z.[2], Rajković B.[3], Radišić R.[1], Lukić M.[3]

Abstract: In order to examine the habits of consumers to buy eggs from organic production and their willingness to pay more for eggs from this production system, 278 consumers in the City of Belgrade were surveyed. Part of the research also involved investigation of the supply of the organic eggs on the Belgrade market, as well as the price ratio between eggs from conventional and organic production systems. Through the survey, respondents were asked whether they buy organic eggs and are they willing to allocate more money for eggs from the organic system. Based on the monitoring of the organic eggs supply in six most common super/hyper-markets in Belgrade, it was found that in three of them organic eggs were offered, with a price that was 104.7 to 207.7% higher compared to eggs from conventional production. According to the results of the survey, it can be concluded that most of the surveyed consumers do not have the habit of buying organic eggs (only 4% do), 58.8% of respondents expressed willingness to pay up to 30% more for organic eggs while 12.4% are willing to buy organic eggs which price is more than 30% higher compared to the price of eggs from conventional production system. The results of the study suggest that only a smaller number of consumers in Belgrade chooses to buy organic eggs, that the supply in terms of the number of producers or suppliers on the market of the City of Belgrade is limited. Furthermore, consumers' willingness to pay more for this kind of eggs is low, which could be expected considering that the price of organic eggs is significantly higher compared to those from conventional production system.

Keywords: organic eggs, consumer, survey, super/hyper-market, Belgrade

Introduction

Consumers/purchasers can no longer be regarded as passive observers, but as active participants in creating an environment in terms of production, markets, environment, etc., whereby their habits and attitudes are becoming increasingly important. Investigation of consumer attitudes in Serbia has been the subject of many studies (Pavlovski and Mašić, 1994; Pavlovski et al., 2002; Tolimir et al., 2016). However, in Serbia there is a small number of studies aimed at determining the opinion and attitude of consumers towards eggs from organic production. In this regard, this paper can be considered as the preliminary one and one of the first aimed to determine the starting point for the development of the organic eggs' market, and, in broader sense, for the development of organic egg production, respectively.

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Out of the 380.5 million hens in the EU, the smallest number is in the organic production (around 4%), then in the so-called "free range" - about 14%, in the floor system about 26% and in the cage system about 56% (Committee for the Common Organization of the Agricultural Markets, 2016), with a tendency of increasing the number of poultry in organic production (Willer et al., 2017). In the Republic of Serbia the production of organic agricultural products is characterized by a growing trend (Zarić and Mijajlović, 2014), that is also present in organic egg production. Observed at the level of the three-year period, the number of laying hens in organic production status increased from 1079 (2015) to 3122 (2016), or 4415 (2017), according to the organic producers database maintained by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, on the basis of the reports from authorized control organizations that carry out the control and certification of this production (Directorate for National Reference Laboratories, 2018). However, regardless of this growing trend, organic egg production is still at the beginning, with a modest volume compared to the total egg production in Serbia. According to the data by the Statistical Office of the Republic Serbia, in 2016, 1.853 million eggs were produced in Serbia (Statistical Yearbook of the Republic of Serbia, 2017). As a consequence of the small volume of organic production, the share of organic eggs on Belgrade market is small. Organic eggs are present in specialized stores for organic products, at green markets in the section for organic products and in super/hyper-markets, in addition to eggs from conventional cage systems, traditional production systems and eggs from the functional food program (enriched with selenium and ω-3 acids). According to Rodić et al. (2010a), in the case that they could choose, 9% of the consumers in Serbia would choose eggs from conventional production, while 26% would choose organic eggs. The same authors pointed out that the majority of the surveyed consumers were willing to pay 10-20% higher price for eggs from non-cage systems, while Rakonjac (2016) stated that consumers more often decide to buy products obtained in a "natural way", with the willingness to pay the greater amount of money for them. One of the main reasons for the consumer's preference for organic eggs is that they consider them to be better for their health than the eggs from conventional production (Perić et al. 2015).

The aim of this paper is to determine consumer/purchaser' attitudes in the City of Belgrade when buying eggs, i.e. how often they buy the eggs from organic production, as well as their willingness to allocate more money for them. At the same time, the aim of the paper is to enable the insight into the supply of organic eggs in super/hyper-markets on the Belgrade market, as well as the price ratio between the eggs from organic and conventional production.

Material and Methods

Examination of supply of organic eggs on the Belgrade market was conducted from May until August 2018, by monitoring the supply in six most common super/hyper-markets. The supply was recorded monthly, during the first week of the month.

Monitoring the prices of eggs from organic and conventional production has been conducted within the four-month period, on the basis of which their average values were calculated, and then compared in order to obtain their relations, also expressed as a percentage.

The survey amongst the consumers on the territory of the City of Belgrade was conducted on the sample of 278 table eggs consumers, who were randomly selected. In order to achieve full objectivity of the data, respondents filled in the questionnaires without the presence of interviewer.

The structured questionnaire consisted of the following questions: 1) Do you buy organic eggs? 2) How much more are you willing to pay for the organic eggs? - proposed answers: I am willing to pay 10% higher price, 20% higher price, 30% higher price, and more than 30% higher price.

Answers from the respondent consumers were processed using the standard analysis method in Microsoft Excel programme.

Results and Discussion

Table 1 shows the results related to the monitoring of egg supply from organic production in the most prominent super/hyper-markets in the City of Belgrade.

Table 1. Supply of eggs from conventional and organic production on the market of the City of Belgrade

Super/hyper-market						
Number of egg producers/suppliers						
Production type	1	2	3	4	5	6
May 2018						
Organic	0	0	0	1	1	0
Conventional	3	2	**4**	2	3	2
June 2018						
Organic	0	0	0	1	1	0
Conventional	3	2	**4**	2	2	3
July 2018						
Organic	0	0	1	1	1	0
Conventional	3	2	**7**	2	3	3
August 2018						
Organic	0	0	1	1	1	0
Conventional	3	2	**6**	2	3	3

Based on the present market investigation in terms of egg supply in Belgrade's super/hyper-markets (Table 1), it can be concluded that organic eggs were offered only in two (May and June 2018) and three (June and July 2018), out of six of the super/hyper-markets. Moreover, within a single super/hyper-market eggs originated from one producer. At the same time, all markets had eggs from conventional production, and viewed within a single market, the number of different producers/suppliers ranged from two to seven.

The obtained results indicate that the presence of organic eggs in Belgrade super/hypermarkets is considerably lower than that of conventional egg production, which can be linked to a small volume of organic egg production in Serbia, which in the total scope of the certified production in 2017 was only 4415 hens (Directorate for National Reference Laboratories, 2018). Such a small volume of organic eggs production reflects the general situation in the organic agriculture sector of Serbia. According to the data given by Roljević Nikolić et al. (2017), organic production of food is practiced only on 0.4% of the total used agricultural land in Serbia, while the number of certified organic farms accounts for 0.3% of the total number of farms. On the other hand, in the previous ten-year period in the European Union the area under the organic production increased by 70%, the

number of certified producers by 60%, doubling the total market value. Furthermore, the organic production of poultry in the EU recorded the increase of 108% in the period from 2007 to 2015, which was also the largest increase in organic livestock production (Willer et al., 2017).

Based on the monitoring the supply of organic eggs on the Belgrade market, it has been found that, besides certain hyper/super-market, organic eggs can be found in specialized organic products stores and green markets that have a special section for products from certified organic production. The situation in Belgrade, as one of the largest markets in Serbia, suggests that consumers who decide to buy organic eggs actually encounter a limited supply compared to eggs from conventional production. On the other hand, when observing the market situation in many European countries, it can be seen that great attention is payed to hyper/super-market supply of organic eggs and that hyper/super-market chains are considered to be factors that trigger the development of the market of organic products and offer a wider range of organic products. At the same time special attention is also paid to the specialized stores of organic products, with the aim of making organic products available to as many consumers as possible (Rader, 2018). In contrast to our country, in many European countries, organic eggs have one of the most important roles in organic product markets; in Switzerland, Sweden and France they reach market share of over 20% (Willer et al., 2017).

Based on the results of this survey, it was that only 4% of consumers on the Belgrade market claimed to buy organic eggs. According to the obtained results, this is a far smaller number of consumers compared to the results of the research by Rodić et al. (2010b) according to which 26% of consumers in Serbia would choose to buy organic eggs. In some European countries consumer preference for organic eggs is very pronounced, for example, 65% of Polish consumers have claimed to buy organic eggs (Żakowska-Biemans and Tekien, 2017).

Part of the work involved examining the market in terms of price of eggs from organic and conventional production. Table 2 shows the results referring to the relation between the prices of organic and eggs from conventional production.

Table 2. Price ratio of eggs from organic and conventional production

Price ratio of eggs from organic and conventional production			
	Super/hyper-market		
	1	2	3
Price ratio of organic eggs/eggs from conventional production	2.35 – 3.07	2.05 - 2.42	2.43 – 2.74
% by which organic are more expensive than conventional eggs	135.3 – 207.7	104.7 -141.9	142.6 – 173.8

The average price of organic eggs within the analyzed period, observed for all three super/hyper-markets was from 37 (0.31 EUR) to 40 RSD (0.33 EUR). The data for minimum and maximum prices of organic eggs obtained in this research are in compliance with those obtained by Simić, 2017, in the research that was conducted during the period of September – October of 2016, collected from 7 retail stores in Belgrade and Novi Sad, where those values vary form 0.28 up to 0.37.

By comparing the prices of eggs from the organic and conventional production in three hyper/mega stores, it was found that organic eggs had 2.05 to 3.07 times higher price compared to the eggs from conventional production (Table 2). Expressed in percentage, a conclusion can be derived that the percentage for which organic eggs are more expensive than the conventional was between

104.7 and 207.7%. The calculated data can be linked to the research by Patterson et al., 2001, who suggest that the price of eggs from organic production is 2,9 times higher compared to the price of conventional eggs, while according to Hidalgo et al. (2008), price of organic eggs was 95% higher than the conventionally produced eggs. Higher price of organic eggs derives from the higher costs of this production (Patterson et al., 2001), and which according to the experiences of developed countries of Europe can be over 200% compared to conventional production (AGRA CEAS, 2004).

The willingness of Belgrade egg consumers to pay higher price for organic eggs is presented in Table 3.

Table 3. The willingness of consumers to pay a higher price for organic eggs

Answers to the question: "How much are you willing to pay more for eggs from organic production?"					
Answers					
Respondents	I am not willing to pay more	I am willing to pay more			
		10%	20%	30%	More than 30%
%	28.8	25.2	18.8	14.80	12.4

Based on the results of the survey (Table 3), it can be concluded that the dominant share of interviewed consumers (71.2%) are willing to spend more money on organic eggs, with 58.8% of consumers ready to pay up to 30% higher price, and only 12.4% consumers over 30% higher price. These results can be linked to the research by Rodić et al. (2010a), indicating that consumers in Serbia expressed their willingness to give 20% more money for eggs from non-cage production system.

Conclusion

Although organic egg production in Serbia tends to grow, it is still modest in volume, with a small share of organic eggs on the Belgrade market, as one of the largest. Only a certain number of super/hyper-market chains (3) have in their offer organic eggs, originating from a small number of producers/suppliers. In addition to the super/hyper-markets, consumers can also buy organic eggs in Belgrade in specialized organic products stores and at the green markets with specialized section for organic products. Official data on the organic egg consumption do not exist. The results of this survey show that the share of consumers who decide to buy organic eggs is small (4%) and lower than in related research performed in the previous period. Reasons for such situation could be found in a relatively limited offer on the Belgrade market, as only about 12% of consumers expressed their willingness to pay a 30% higher price for organic eggs. On the other hand, the actual price of organic eggs is 104.7 to 207.7% higher compared to those from conventional production. The research can be considered as preliminary and can be considered as a guideline for the development of the market and organic production of eggs in Serbia.

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AFRICAN SWINE FEWER – SPREADING THE DISEASE IN EUROPE AND PREVENTIVE MEASURES TAKEN IN THE REPUBLIC OF SERBIA

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Abstract: African swine fever (ASF) is a viral, contagious disease of domestic pigs and wild boars of all breeds and ages, members of *Suidae*. The causative agent is DNA virus, genus *Asfivirus*, a member of family *Asfarviridae*. African swine fever is enzootically present in sub-Saharan Africa, where the virus for decade back circulates in the wild boars and domestic pig population. On the European continent, taking into account the past few decades, the presence of the disease has been confirmed in the Iberian Peninsula and Sardinia. Eradication of ASF in Europe was done in the late 1990s and until 2007, the disease was not detected. In the following year, the disease was diagnosed in Georgia from which ASF spread to the Russian Federation, and the surrounding states (Armenia, Azerbaijan, Ukraine and Belarus). In 2014, the disease has been reported in Lithuania, Latvia, Estonia and Poland, 2016 in Moldova. In mid-2017, the disease was diagnosed in the Czech Republic and in Republic of Romania and lately in 2018 in Hungary, Bulgaria and Belgium. Having in mind that ASF was in 2017 and 2018 for the first time diagnosed in the domestic swine and wild boar population of the neighborhood countries (Romania, Hungary), the Veterinary Directorate of the Republic of Serbia ordered the implementation of a set of control, preventive measures to be applied in the country but also at border crossings. The special Expert Group, National Center and Decision-making Team for Movement Tracking, Prevention and Control ASF were established. The aim of the paper is to review the spreading disease in Europe and to present the applied control measures in the Republic of Serbia in order to try to prevent disease occurrence and protect swine production sector.

Keywords: African swine fever, control, Serbia

Introduction

African swine fever (ASF) is a viral, contagious haemorrhagic disease that affects domestic pigs and wild boars of all breeds and ages, all members of *Suidae* species (Blome et al., 2013; Gallardo et al., 2018). The causative agent is African swine fever virus (ASFV), a large enveloped double stranded DNA virus, the genus *Asfivirus*, the only member of the family *Asfarviridae* (Dixon et al., 2005). African swine fever is considered as one of the most serious diseases of pigs that can severely affect and disrupt regional and international trade with animals and animal products with a serious socio-economic impact on pig farming (Bellini et al., 2016; Iglesias et al., 2017; Cappai et al., 2018).

African swine fever was first identified in East Africa in the early 1900s as a disease causing high mortality in domestic pigs (Guinat et al., 2016). It was established that warthogs (*Phacochoerus*

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africanus) are important source of infection and that this host, along with a species of soft ticks (*Ornithodoros spp*.) which live in warthog burrows, could be persistently infected with ASFV without showing signs of disease (Penrith et al., 2013; Sánchez-Cordón et al., 2018). Currently, ASF is enzootically present in sub-Saharan Africa, where the virus for decade back circulates in the sylvatic cycle that includes: wild and domestic pig population, and *Ornithodoros* ticks (Costard et al., 2013). In the past, transcontinental disease spread occurred to Europe (Spain and Portugal) in 1957 and 1960, and from there to other European countries, South America and the Caribbean. With the exception of Sardinia, disease was successfully eradicated from outside Africa in the mid-1990s (Cappai et al., 2018; Sánchez-Cordón et al., 2018) and until 2007 the disease was not detected in continental Europe. The disease in Europe remained confined to the Italian island of Sardinia until the introduction from East Africa of a different genotype of ASF virus in Georgia in 2007 (Rowlands et al., 2008; Gogin et al., 2013). In the following year, the disease was diagnosed in Georgia from which ASF spread to the Russian Federation (2007), and the surrounding states: Armenia, Azerbaijan, Ukraine 2012. (Malogolovkin et al., 2012) and Belarus 2013., the Baltic countries: Lithuania, Latvia, Estonia and Poland (2014) (Nurmoja et al., 2017), Moldova (2016), Czech Republic (2017) and Romania (2017) (Gallardo et al., 2018). The last three new confirmed outbreaks were detected and officially recognized in Hungary, Bulgaria and in Belgium in 2018 (OIE, 2018). Certainly, a recently confirmed ASF in China (2018) will have disastrous consequences, recognizing that this country contains more than half of the world's pig population (Gallardo et al., 2018; Sánchez-Cordón et al., 2018).

Starting from a single introduction of ASFV into Georgia from southeast Africa in 2007, ASF has spread northwards and westwards into Eastern Europe and has affected many countries (Rowlands et al., 2008; Nurmoja et al., 2017; Sánchez-Cordón et al., 2018). Wild boars have been identified as the main actors and have played an important role in the introduction and subsequent local spread of the disease in the Baltic countries and Poland (Iglesias et al., 2017; Nurmoja et al., 2017). However, the population of domestic pigs and pig production sector is also severely affected (Guinat et al., 2016; Nurmoja et al., 2017).

After infection, the ASFV causes immune suppression and very high mortality rates in susceptible domestic swine and wild boars, so that the effect of disease outbreaks is severe (Gogin et al., 2013; Gallardo et al., 2015). The virus encodes 150 – 165 proteins, which have 'essential' functions in virus replication, as well as 'non-essential' roles in host interactions, including evasion of host defenses; for example, many proteins inhibit the early innate responses, including type I interferon and cell death pathways (Dixon et al., 2013). By the sequencing of the gene encoding the major capsid protein, 23 different genotypes of ASFV have been defined (Achenbach et al., 2016). Genotype I is spread through West and Central Africa, was introduced to Europe in 1957 and 1960, and is currently in Sardinia (Iglesias et al., 2017). Genotype II was introduced to Georgia in 2007 and has spread through the Russian Federation and Eastern Europe (Rowlands et al., 2008; Gallardo et al., 2014; Gallardo et al., 2018). It has been demonstrated that isolates circulating in Eastern Europe from 2007 to 2011 were almost identical (Malogolovkin et al., 2012). However, sequencing of ASFV isolates from wild boar found dead in Lithuania and Poland in 2014 identified minor variants that were identical to isolates obtained from Belarus in 2013, but different from isolates obtained from Russia in 2012 and Georgia in 2007 (Gallardo et al., 2014; Gallardo et al., 2018).

The aim of this review is to summarize ASF disease spreading dynamics in Europe, and to present the preventive and control measures that had been undertaken in the Republic of Serbia.

The ASFV and disease is not detected in the territory of the Republic of Serbia. So in this review, we used the data and experiences from other countries in Europe. The review is partly based on search in PubMed (http://www.ncbi.nlm.nih.gov/pubmed). Also, the Office International des Epizooties (OIE) official data, European Food Safety Authority (EFSA) Scientific Review and Opinions on ASF were used as source of additional information about disease spreading. Our experiences on contagious swine disease control and active involvement in the control measures that are established in Serbia 2017, are directly connected with our previous activities in Classical swine fever (CSF) control (Prodanov-Radulović et al., 2014 and 2017) and involvement in the work of the special Expert Group for Movement Tracking, Prevention and Control ASF that is established in 2017 by the Veterinary Directorate Ministry of Agriculture, Forestry and Water Economy (http://www.vet.minpolj.gov.rs/srb/africka-kuga-svinja).

African swine fever in Europe remained confined to the Italian island of Sardinia until the introduction from East Africa of a different genotype of ASF virus in Georgia in 2007 (Rowlands et al. 2008; Cappai et al., 2018). Since then, the disease spread through the Caucasus and the Russian Federation, affecting both wild boar and domestic pig populations (Gogin et al., 2013; Nurmoja et al., 2017; Gallardo et al., 2018). So, starting from a single introduction of ASFV into Georgia from southeast Africa in 2007, ASF has spread northward and westward into Eastern Europe and has affected many countries (Rowlands et al., 2008). In mid-2017, the disease was diagnosed in the Czech Republic and in the Republic of Romania. In Czech Republic, human activities were the most probable the route of the ASF introduction because the nearest reported ASF cases were about 400–500 km away. Moreover, most of the dead ASF-positive wild boars were found in inhabited areas. Generally, the outbreaks in domestic pigs of the EU were rapidly eradicated, remaining the majority of notifications in the wild boar population (Gallardo et al., 2018). It is now recognized that wild boar have an important role in the spread and maintenance of ASFV in these regions (Sánchez-Cordón et al., 2018). The ASFV strains circulating in eastern EU countries since 2014 belong to the p72 genotype II, (Dixon et al., 2013; Gallardo et al., 2014; Gallardo et al., 2018).

The disease is mainly transmitted by direct contact between infected and susceptible pigs and/or through the ingestion of ASFV contaminated pork (Costard et al., 2013; Guinat et al., 2016) or contaminated products (swill and garbage waste) (Gogin et al., 2013). Pigs are infected mainly through the oro-nasal route after contact with infected pigs or after feeding on virus containing pork or contaminated products (Blome et al., 2013). It should be pointed out that all excretions and secretions of infected pigs (blood, faeces, urine, saliva) can contain virus, and ASFV may remain viable in blood and tissues for long periods (EFSA, 2015; Gallardo et al., 2015). As regards ASFV genotype II, in pigs experimentally infected, it has been observed that the excretion of the virus started during the incubation period and lasted for the entire period of the disease (Gallardo et al., 2015; Bellini et al., 2016).

Historically, in Europe different courses of ASF have been described in domestic pigs in the field (Blome et al., 2013; Gomez-Villamandos et al., 2013). Disease manifestations include peracute, acute, subacute and chronic forms. In the peracute form, pigs die within 4 days post-infection (dpi) without gross lesions. The acute form results in the death of a high number of infected pigs (90 – 100%) 4–21 dpi, with characteristic pathological changes: skin erythema, pulmonary oedema, hyperaemic splenomegaly, haemorrhagic lymphadenitis, and petechial haemorrhages in the lungs,

urinary bladder and kidneys (Blome et al., 2013). In the subacute form, the mortality is 30–70%, incubation periods are longer (pigs die after 20 dpi) and clinical signs tend to be less marked. The chronic form of the disease is characterized by low mortality rates, but clinical signs such as delayed growth, emaciation, joint swelling, skin ulcers and lesions associated with secondary bacterial infection are frequently present (Sánchez-Cordon et al., 2018). The existence of inapparent infections has also been suggested in survivor pigs, which are infected but do not display clinical signs or lesions. Virus can persist for prolonged periods in tissues and blood from recovered pigs, which might contribute to virus transmission and ASFV introduction into disease-free zones (Costard et al., 2013; Gallardo et al., 2015). Recent studies in some infected countries in Africa have identified ASFV sequences in apparently healthy pigs, suggesting that reduced virulence isolates may be circulating in these regions (Achenbach et al., 2016; Sánchez-Cordón et al., 2018).

Following the introduction of ASFV genotype II to the Trans-Caucasus and Russian Federation in 2007, most reports have been of the acute form of disease with high lethality in domestic pigs and wild boar (Gogin et al., 2013). In the Russian Federation from 2007 to 2014, epidemics of ASF in wild boar and domestic pigs were independent from each other (Malogolovkin et al., 2012). In contrast, in the Baltic States, ASFV spread extensively in wild boar, with only occasional spill-over to domestic pigs. In Estonia, there is evidence for two separate introductions of ASFV into wild boar population, possible of different virulence. One of suggested explanation for these differences is that a moderately virulent isolate is circulating in this region and a greater number of animals survive (Nurmoja et al., 2017). The transmission mechanisms of ASFV in wild boar in the Baltic States still remain unclear, but may include the movement of infected wild boar, infected wild boar carcasses and/or environmental contamination. A role for persistently infected carrier animals has also been suggested. Spreading and transmission mechanism in domestic pig population may be a result of spill-over infections from wild boar or through the well-established routes involving movement of pigs, pork or infected fomites (Guinat et al., 2016; Sánchez-Cordón et al., 2018). The occurrence of less virulent virus isolates will certainly increase the prevalence of subacute and inapparent infections (Blome et al., 2013; Gallardo et al., 2014). Pigs that survive natural infection usually develop antibodies against ASFV from 7 to 10 dpi which persist for long periods (Dixon et al., 2013). However, ASF diagnosis is complicated by the varying pathogeneses and epidemiological scenarios, as well as by its similarity to other hemorrhagic diseases, such as Classical swine fever (Prodanov-Radulović et al., 2014; Prodanov-Radulović et al., 2017). In experimental and field samples from the Russian Federation, 3.7% of serum samples from wild boar were positive for antibodies against ASFV, indicating that few pigs survived infection, thus confirming the high virulence of circulating isolates (Malogolovkin et al., 2012; Gogin et al., 2013).

Although ASF was first described almost a century ago, controlling the disease has proven to be a challenge, because no vaccine is available. The only control measures available are still strict quarantine and biosecurity, animal movement restrictions and slaughtering affected/exposed animals (Bellini et al., 2016; Sánchez-Cordón et al., 2018). Prevention and early detection play a key role in the control strategy for ASF and enhancing early detection would also improve the efficacy of the disease control measures (Bellini et al., 2016). Biosecurity measures in pig farming are recognized as a most important control measure (Prodanov-Radulović et al., 2018). The basic elements of biosecurity derive from the knowledge of the epidemiology of the disease, the duration of pathogen excretion in infected animals, the main routes of excretion, survival of the pathogen in the environment and its routes of infection (Bellini et al., 2016). It should be stressed that backyard

holdings with poor biosecurity in place are currently playing an important role in the maintenance and spread of ASFV in the infected eastern neighboring countries of the EU (Prodanov-Radulović et al., 2018 and 2018a). Biosecurity measures on commercial pig farms are of great importance for preserving the health status population as a whole, as they often carry out further distribution of pig breeding material to different type of family farms (Prodanov-Radulović et al., 2018).

ASFV has a remarkable ability to survive for long periods in a protein rich environment. The virus is not affected by meat maturation processes and meat from pigs slaughtered in the infective stages of ASF or that die spontaneously of the disease provides a good source of virus. ASFV persist in tissues for up to 6 months and can be infectious in susceptible animals fed with such meat (Bellini et al., 2016). Fresh and frozen pork, as well as smoked, salted and dried pork may contain infective quantities of ASFV. Virus is also able to survive for long periods in some tissues such as bone marrow, in spite of putrefaction (Penrith al., 2013). Stability of the ASFV virion in a protein environment exacerbates disease spread, and high levels of environmental contamination facilitate spread via contaminated vehicles, equipment, instruments, and clothing. European wild boars are equally susceptible to ASF, making it very difficult to eliminate once it has become endemic in these wild populations (Gogin et al., 2013; Prodanov-Radulović et al., 2016). It is very important to stress that the virus can remain viable in tissues and after natural death of wild boar can re-initiate a new epidemic cycle of ASF if carcasses are not properly disposed and the virus gets in contact with a susceptible animal (Costard et al., 2013). The large amount of virus shed during the infectious period of the disease (Blome et al., 2013) and the ability of ASFV to survive for long periods in a protein rich environment, even under adverse conditions (Costard et al., 2013) mean that infectious live wild boar and their carcasses should be considered in any control measures (Bellini et al., 2016).

African swine fever (ASF) is continuing to spread across Europe and in the 2017 for the first time the disease has been confirmed in domestic pig population in the neighborhood country of Serbia (OIE, 2018). Since then, the Veterinary Directorate ordered the implementation of a set of control, preventive measures in the country but also at border crossings for passengers, vehicles, ships in the river ports and at the international airports. The special Expert Group, National Center and Decision-making Team for Movement Tracking, Prevention and Control ASF were established (Prodanov-Radulović et al., 2018a). At the country borders, in order to inform all passengers, the special informative posters written in several languages were set. The passive and active surveillance of dead and hunted wild boars was intensified during the last hunting season. The risk analysis was conducted to assess the possible risk of disease introduction from neighborhood, infected country. The special form of Veterinarian Manual about ASF was written in Serbian in order to provide the latest information about disease. In order to increase awareness, the series of lectures for all involved subjects (veterinarians, breeders, hunters) was organized in all country districts. In the risk profile, different routes or events which could lead to ASF entering country were identified. The most important are: habits of people living in border areas, infected meat or meat product, catering waste from international transport. In the event of a worst-case scenario (ASF outbreak), the existing types of swine holdings (backyards and free-range) and low level of biosecurity in certain number of commercial pig holdings were recognized as a significant risk factor. In the case of outbreak, the backyard pig operations might be a potential risk for further spread of diseases from wild boar to commercial pig holdings. The identified key measures to protect swine population from ASF are improving existing biosecurity of farms and effective and aimed information on the disease risk (active awareness campaign) (Prodanov-Radulović et al., 2018 and 2018a).

The high lethality of ASFV in domestic pigs, the introduction of massive culling campaigns and pig movement restrictions all contribute to the high socio-economic impact of ASF on pig production, global trade and people's livelihoods (Bellini et al., 2016). In Eastern Europe and the Russian Federation, the greatest numbers of outbreaks in domestic pigs have been on small scale or backyard farms, which generally have lower biosecurity standards and, in some regions, have closer contact with infected wild boar (Gallardo et al., 2014; Guinat et al., 2016). In contrast, in the Baltic States, relatively few farms have been affected and most notifications have been in wild boar (Nurmoja et al., 2017). However, the cases of ASF in domestic pig population result in the death or destruction of substantially larger numbers of pigs. From 2014 to 2017, almost 800,000 pigs have died or been destroyed as a result of ASF in Eastern Europe and the Russian Federation (Gallardo et al, 2018). ASFV infection in wild boar can have an impact on the hunting industry, but the greatest impact is the threat and economic impact on commercial pig farms (Sánchez-Cordón et al., 2018). The lack of biosecurity, presence of asymptomatic carrier pigs in the wild, illegal pig breeding in free-range grazing, the presence of wild boars, contact between livestock and wild boar and poor sanitary measures for pig breeding increase the chances of ASF persistence (Bellini et al., 2016; Prodanov-Radulović et al., 2016).

Conclusion

From the first occurrence in 2007, ASF had epidemiological characteristics that appear to be different in the various countries where the disease appeared. For this reason, it is important to link the disease spread to the characteristics of the territory/country. In the current European situation, the risk of ASF spreading to other pig producing countries has increased, and since there is no vaccine, rapid and specific diagnostic procedures is an essential component to control plans in the affected countries. Further spread of ASF in continental Europe seems very likely, since attempts to control the disease have not been effective.

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THIN SOW SYNDROME IN COMMERCIAL FARMS

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Abstract: The syndrome of thin sows is a disease of complex etiology. Acording to literature data in 75% of cases, the main causes are qualitative and quantitative malnutrition, and also unfavorable housing conditions for sows. Less frequently, the syndrome can be caused by presence of endoparasites *Hyostrongylus rubidus*. The aim of this research was to observe the observation of a thin sow syndrome sows on a commercial farm. During the period of one year, the occurrence of weight loss in sows during lactation at one commercial farm was observed. The loss of body weight was mostly detected in sows in second lactation. Corrections in the feeding technology and better conditions for sows during lactation period are crucial for managing the thin sows' problem at the farm.

Keywords: farm, nutrition, thin sow syndrome

Introduction

The "thin sow syndrome" is a condition characterized by anorexia and weight loss. Usually, it appears as a consequence of some deficiencies in sows' nutrition especially during pregnancy and lactating period, as well as different failures in farm's production technology. This condition may results as a combination of parasitism, low environmental temperatures and inadequate feed intake, particularly during lactation. In East Balkan (i.e. Serbia) climatic conditions, a more frequent occurrence of the syndrome is observed during the winter months as a result of inadequate environmental conditions (temperature less than 21 °C and inappropriate ventilation rate in pig barns. The syndrome may also occur during or after recovery period from some infective diseases, such as influenza (Lipej, 2015).

Some authors suggested that some parasitic infections may also play role in pathogenesis of the thin sow syndrome (Šamanc, 2009, Lipej, 2015). From the group of endoparasites, the most frequent causes are gastric parasites *Hyostrongylus rubidus*, and from the group of ectoparasites the causative agents may be itch mites (Šamanc, 2009). However, the parasitism is less important when adequate prophylactic measures and therapy are routinely carried out in the commercial swine production.

"Thin sow syndrome" is most often clinically observed after first and second farrowing or lactation. This phenomenon is one of the main reasons for the exclusion of a large number of sows

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from reproduction after first or second farrowing. Clinical signs of suboptimal condition include increased weaning to service intervals, small litters and low weaning weights. Piglets of sows in suboptimal condition may be restless and demand milk more frequently (Petrujkić et.al. 2011). During lactation, the nutritional needs are high, and inappropriate diet is one of the most frequent reasons of significant decrease of sow's body condition. If this phenomenon lasts longer, and if the deficit of nutrients is more pronounced, a "thin sow syndrome" occurs. Thin sows may be identified by observation and systematic condition scoring of the herd. Pressure sores in sows at weaning also indicate the poor condition. Some production parameters can also be used to detect thin sows. The particular susceptibility of young gilts is due, among other things, their unequivocal use of large quantities of food during first lactation and small body reserves, which should be considered that they body is still developing. In extreme cases, the weight loss can involve 30- 90% of sows in one herd (Šamanc, 2009, Lipej, 2015).

The "thin sow syndrome" is a significant welfare problem and some data can be found in paper by Relić et al. (2016). In this paper, situation concerning body and health condition of lactating sows at one commercial Serbian farm is discussed.

The aim of this research was to observe the observation of the thin sow syndrome sows on the commercial farm.

Material and Methods

The material for this research included animals from one commercial swine farm (capacity 500 sows), where health disorders i.e. clinical and pathomorphological signs resembling to the problem of thin sow syndrome and/or sudden body loss in large number farrowed females were detected. The animals were observed and inspected for clinical signs of disease and abnormal behavior.

Results and Discussion

The regime of preparing the sows for farrowing and the feeding is not regulated. Decreases of feed intake and weight loss in the lactation period were noticed in animals after farrowing. In the cases when gilt condition and nutrition in the lactation period was inadequate, the second litter was smaller than the first. Certainly that low number of newborn may reflect overall sow condition at service. The extended weaning to service intervals and low weaning weights were connected to the poor body condition. In some cases, problems with conception or abortion in pregnant sows were also noticed. On the sows' body, some skin changes (wrinkles and different types of lesions) and swellings were noticed, as well as clogged and dirty hair. The lesions were formed dominantly at the point of bone compression (Fig.1). Most often, thin sows were found in a position lying on the sternum and do not showed any interest for the environment (Fig.2). In some cases, it was very difficult for these animals to take a standing position in the box. The detected post mortal lesions were grossly classified as poor body condition, low fat thickness and as an increased incidence and extent of skin lesions, especially over shoulders and hips where pressure sores can develop.

Fig 1. Swelling on the body of thin sow

Fig 2. Skin changes and irritation (caused by mange) on the thin sow

Fig 3. Visible spinous processes and lack of interest for the environment

Anorexia (loss of appetite) in sows develops after farrowing as part of a "thin sow syndrome", and as a result there is an intense loss of body weight. The clinical signs of this syndrome, observed in the examined cases, show an unusual similarity to the clinical signs of anorexic nerve (*Anorexia nervosa*). In addition to losing appetite and body weight, sows limit the intake of normal foods and consume large quantities of straw. Animals spend more time on non-intrusive hyperactive behavior, constantly moving inside the box. The sows affected by anorexic nervousness are easily recognized by the prominent backbone of the spine and their rough and long hair (Treasure and Owen, 1995), which is also in line with the observed changes in our investigation experiment.

Many factors affect the appetite of sows in lactation, and the most important are: consumption of the food during pregnancy, air temperature and ventilation in the pig barn, energy level in the meal and the number of feeding per day (Kovčin, 1993). The most powerful effect on the level of consumption has the level of energy in the meal, so if lactating sows are not allowed to eat *ad libitum* or close to it, than production of milk, body weight and level of body reserves decreases. On the other hand, the needs in nutrients during lactation vary and depend on the concentration of energy in the meal and the previous feeding of the sow. In practical nutrition, the highest efficiency of energy consumption from meals is achieved by controlled diet during gravidity in order to minimize the mobilization of body depots of fat during lactation (Jovanović et al., 2001). Cases of severe constipation can be avoided by increasing the amount of dietary fiber during the last phase of suppression (Oliviero et al., 2009; Tabeling et al., 2003). Ensuring optimum levels of dietary fiber improves the functioning of the bowel and reduces the degree of constipation. It seems that the use of high-fiber meals in the form of coarse humpy 128 nutrients is a useful strategy for improving the health of pigs (Peltoniemi et al., 2016).

Conclusion

"Thin sow syndrome" on commercial farms can be prevented by correction in the feeding technology and feeding sows during the lactation period. In the critical period, at the beginning of lactation, the health control of sows should be performed regularly on a daily basis in order to spot and detect the earliest symptoms of the disease (long sleeping periods, reduced appetite and constipation).Certainly that improved sow nutrition at key stages in the breeding cycle will help improve the number, birth weights and piglet vitality. This breakthrough in sow nutrition can help the sow in supporting larger litters, from birth to weaning. Our findings stress the need for further studies in the etiology and prevention of this condition.

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RELEVANCE ANALYSIS AND SELECTION OF KEY INDICATORS FOR ASSESSING THE WELFARE OF DAIRY COWS

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Abstract: In the last few decades, numerous researches have been carried out on the methods of assessing dairy cows' welfare. These methods take into account a number of indicators, which are usually divided into direct and indirect, depending on whether they relate to individual manifestations in animals or they are derived from the environment. Considering the large number of parameters that can point to the welfare of animals, existing welfare assessment protocols are generally complex for easy and reliable on-farm assessment. For this reason, interest in standardizing the welfare assessment methodology has been growing in recent years. This paper considers the relevance of welfare indicators and the choice of their key parameters. Further work on welfare assessment protocols and some indicators is still needed.

Keywords: dairy cows, indicators, relevance, welfare assessment

Introduction

Intensive farm production reveals necessity of systematic scientific analysis of farm animal welfare and problems that accompany it (Harison, 1964). In addition, Brambells' Committee report (1965) for the British Parliament included major farm animal welfare problems and indicated key animal needs. Fruitful scientific and public deliberation about necessity of dairy cows welfare assessment have begun in the middle of XX century. Dairy cattle welfare became an essential issue of livestock production in the beginning of the XXI century.

Fundamental changes in attitude towards animals were shaped in the second half of XX century in Europe, particularly English speaking countries. Since the agriculture tended to mass production, the automation of the technological process and the reduction of spatial conditions, arose many problems that had not been observed in extensive production at that time. Over the time, criticisms that have indicated that animals do not have a happy and healthy life in such production conditions has been started, aiming the necessity to overcome obvious and fundamental problems. These problems resulted as a consequence of denying basic animal needs and preventing the manifestation of basic forms of natural behaviour. The studies soon imposed to identify indicators for assessing animal welfare.

The aim of this paper is to review the relevance of dairy cattle welfare indicators and the choice of their key parameters, which can serve to define a comprehensive, simple, standardized methodology for assessing welfare in dairy farms.

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Definitions, concepts and methods for assessing the welfare of dairy cows

Animal welfare assessment methods take into account a number of indicators (Hristov et al., 2007a). The basis for their formulation is found in numerous definitions of animal welfare. One of the most significant definitions suggests that the animal welfare is its ability to adapt to physiological, behavioural, cognitive and emotional physico-chemical and social conditions in the environment (Fratrić et al, 2007; Sejian et al., 2011; Relić et al., 2012). According to Broom (1986), the welfare of an individual is a state related to its attempts to cope with the environment. The initial considerations of the concepts and the measurement of animal welfare can be found in paper by Broom (1991), and many methods of assessing farm animal welfare at the herd level in the paper of Johnsen et al. (2001).

In defining the general concept of welfare key issues are adaptation, stress, needs (freedom) of animals and their rights. The most important issue in terms of providing welfare is certainly a question of animal needs (Hristov and Stanković, 2016). According to Broom and Johnson (1993), a requirement is a part of a biological basis of an animal that seeks to provide the appropriate resource or response to certain stimuli from the surrounding environment or its body. Needs include obtaining basic resources or responding adequately to particular environmental or bodily stimulus that are essential for the survival or fitness of the individual (Webster, 2005). The range of functional systems controlling basic physiological mechanisms (body temperature, nutritional state, etc.) in conjunction with the behavioural response, will together allow the individual to control its interactions with the environment (Broom, 1981; Hristov et al, 2007b; Hristov et al., 2012). Therefore, it can be indicated that when an animal must carry out an action to adjust to its physiological state or environmental situation, it could be defined as a need (Broom and Fraser, 2007; Toates and Jensen, 1991). For example, all animals need food, or water, and they would have to perform a series of behaviours to ultimately obtain the required resources. However, even in the presence of the ultimate objective of the activity (feed and water), the welfare of an animal can still be compromised (Broom, 2002). Unsatisfied needs are often, but not always, associated with bad feelings whilst satisfied needs may be associated with good feelings. When needs are not satisfied, welfare will be poorer than when they are satisfied.

In the literature were presented several methods for assessment of dairy cows welfare and some of them are mentioned in this paper. For example, the animal needs index (ANI) for the assessment of animal welfare in the housing systems for Austrian livestock products and legislation was created by Bartussek (1999), which was great step towards animal needs relevance identification. A method of assessing welfare in loose housed dairy cows was developed by Capdeville and Veissier (2001). Rushen (2001) suggested that health problems represent some of the main threats to the welfare of dairy cattle. Therefore, health status is used in assessing the welfare of dairy cattle, together with other important issues that may influence welfare, such as housing and exploitation (Stanković et al., 2014). This was pointed out by Fregonesi and Leaver (2001), who focused on behaviour, performance and health indicators of welfare for dairy cows housed in straw-yard or cubicle systems. Bracke and Hopster 2006. described system of welfare assessment taking into account the importance of natural behaviour for animal welfare. Today, it is a key element in current Dutch policy-making on animal welfare. In addition, Boissy et al. (2007) emphasized practical applications for assessing and promoting positive emotions that may help in providing animals with a better quality of life.

In time, the patterns of influence for the most factors on dairy cows welfare were explained. For instance, the study of Laven et al. (2008) was demonstrated that the welfare impact of lameness in dairy cattle in New Zealand is long lasting, even when treated effectively. The study of Webster et al. (2008) applied a set of stress measures to dairy cows exposed to extreme cold and wet conditions. Together, these measures indicated activation of the stress axis, physiological and behavioural adaptations to cold and a reduction in welfare quality (Hristov et al., 2007c). A number of these measures could be used to assess dairy cattle welfare under cold conditions on farms**.**

The idea of providing five basic needs to animals was presented for the first time in the Brambell report (1965) that was shaped and developed towards widely accepted concept of animal welfare "Five Great Freedoms" (FAWC, 1992):

1. The freedom from hunger and thirst, which is achieved by providing the animal with sufficient quantities of quality and hygienically proper food and water, and enabling the animal to consume it according to the will and the necessity, that is, in accordance with its needs, conditioned by the age and production category and in a natural way characteristic of the type to which it belongs;
2. The freedom from discomfort, which is achieved by providing the animal with adequate housing conditions;
3. Freedom from pain, injury and illness, which is ensured by the implementation of preventive, prophylactic and rapid diagnostic methods and animal treatments;
4. Freedom of expression of natural forms of behaviour and social contact with members of the same kind; and
5. Freedom from unpleasant emotional experiences such as fear, conditions similar to fear, boredom, suffering, etc.

This concept relates to all animals whose survival depends on man, and the degree of welfare of each of these freedoms can be determined by numerous physical, anatomical, physiological, pathological and ethological indicators.

Based on the Five Freedoms, Welfare Quality - criteria identifies four main welfare principles (good feeding, good housing, good health and appropriate behaviour), each communicating a key welfare issue. Each of the principles comprises two to four welfare criteria, 12 welfare criteria in total (Table 1). These principles and criteria form the basis for the Welfare Quality assessment protocol for cattle (Welfare Quality, 2009).

Table 1. Welfare Quality Principles and Criteria (Welfare Quality 2009).

Welfare Principles	**Welfare Criteria**
Good feeding	1 Absence of prolonged hunger
2 Absence of prolonged thirst	
Good housing	3 Comfort around resting
4 Thermal comfort	
5 Ease of movement	
Good health	6 Absence of injuries
7 Absence of disease	
8 Absence of pain induced by management procedures	
Appropriate behaviour	9 Expression of social behaviours
10 Expression of other behaviours	
11 Good human-animal relationship	
12 Positive emotional state	

This combination can be found in welfare assurance schemes as: GLOBAL- GAP Control Points and Compliance Criteria for cattle, sheep, pigs, and poultry (http://www.globalgap. org).

Furthermore, it should be mentioned the EFSA methodology for welfare risk assessment in dairy cows and calves (2009), and Serbian methodology for dairy cows welfare which has been developed by the national project TR 20110 (Zlatanović, 2015).

The relevance of indicators and the choice of their key parameters

The majority of animal welfare scientists take multiple measurements of parameters that are likely to be relevant to the welfare of the animals (Dawkins, 1980; Fraser, 1995; Mason and Mendl, 1993). The measures or 'indicators' will depend on existing knowledge regarding the species, the question of interest or the context in which it is being assessed (Ostojić-Andrić et al., 2013; Relić et al., 2016).

According to Main et al. (2001), methods of animal welfare assessment can generally be divided into so-called input methods that are based on environmental resource information (i.e. indicators) and output methods using animal information - indicators. Indicators from both groups tend to be evaluative, should be precise and usually quantitative.

Resource-based indicators establish a set of minimum requirements in the physical and social environment of the housed animals and predefine minimum management standards (feeder space, drinkers, space allocation, etc.) that are usually based on current scientific knowledge for the species, or on consolidated practical experience. Their advantage is that they should prevent most severe welfare problems from occurring as the conditions are predefined to assure minimum welfare standards.

Animal-based indicators can be generally divided into four major categories, according to their nature, such as: pathological, physiological, behavioural or productive indicators (Seijan et al., 2007). They can be collected on-farm, either by direct inspection of the animal or by indirectly assessing the effects of a response on the environment (e.g. loose faeces on the floor is evidence of diarrhoea in the group, although further investigation may be necessary to identify the affected individual). Data can also be collected at a slaughterhouse by the use of disease reporting systems (surveillance), or by consulting production records (EFSA, 2012).

Welfare assessment that relies on an evaluation of environmental resources such as type of bedding, feeding, hygiene, etc. is often simple and quick, but it is only a prerequisite for well-being, and not its absolute guarantor. Animal based indicators are more demanding in terms of collecting and processing data as well as the time needed, but they certainly provide more reliable results for the quality of well-being (Johnsen et al., 2001). The same authors conclude that the best benefit assessment method should include both types of indicators (input and output).

Aggregating relevant welfare indicators into welfare protocols involves evaluating suggested indicators step-by-step concerning their independent welfare relevance, their marginal welfare value and finally their applicability for on-farm studies. On-farm welfare-assessment protocols should be based on valid, reliable and feasible indicators which reflect the animal's state in the context of the housing and management system. Three groups of parameters are described: (1) parameters which can readily be included, such as lameness, injuries, body condition score, cleanliness, getting up/lying down behaviour, agonistic social behaviour, oral abnormal behaviours, human behaviour toward the animals and measures of the animal - human relationship; (2) parameters which require

more information on reliability, such as indicators of good welfare and housing factors; and (3) parameters which are regarded as important but so far lack reliability in most countries, such as the incidence of clinical diseases and mortality (Winckler et al., 2003). An issue that is indirectly assessed by available welfare assessment protocols is the level of pain that animals might be experiencing, which is an issue that to date has not received the necessary attention due to difficulties encountered in determining reliable pain indicators suitable of application at farm level. Behavioral and physiological indicators of both short- and long term pain either caused by the animals´ physical or social environment are basic measures of welfare, that, however, still need to be systematically addressed under commercial conditions (Broom and Fraser, 2007).

Identification of pain sings is particularly challenging in some species such as cattle, since they have developed throughout the evolution process effective mechanisms to hide signs of pain (Vučinić et al., 1993; Rutherford et al., 2002). Therefore, there is a large need for valid and practical pain indicators that can be apply during on-farm welfare assessment together with other welfare indicators.

Standardizing the welfare assessment methodology

There is still no established standardizing methodology for welfare assessment; however, various frameworks have been put forth. For example, already mentioned ANI system for welfare assessment based on resource measures, created by Bartussek (1999) as Tier-Gerechtheits-Index in German (TGI) has been further developed to version TGI 35 L, with an index system for cattle, laying hens and fattening pigs (Bartussek, 1995a,b,c). Considering resource-based assessment alone can fail to fully answer questions about the real state of the animals lately there has been increasing interest in the development of animal-based methods of animal welfare assessment (Webster, 2009). The RSPCA and the University of Bristol have pioneered the incorporation of animal based welfare assessment techniques into farming systems by assessing the impact of the Freedom Food Scheme in terms of welfare outcomes. The first protocol developed, tested, implemented and published was for dairy cattle (Whay et al., 2003). The protocol was based on direct indices of welfare derived from a combination of direct observations, recordings and farmers' estimates.

Although there are obvious advantages in increasing the use of animal-based welfare assessment methods, there are also difficulties related to it (Rushen and de Passillé, 2009). Main shortcomings are related to the complexity of the protocols and the intrinsic difficulties to standardize the evaluation criteria by the assessors. In addition, they are time demanding in taking measurements and conducting behavioral observations according to the protocol, resulting in prolonged farm visits disrupting animals and farmers' daily activities.

Challenges in on-farm animal welfare assessment

Welfare assessment protocols not only have to be science-based and reliable, but practical for on-farm application as well. That means that protocols that are simple to apply and easy to understand by farmers and industry technical staff would have better possibilities of being adopted, ultimately becoming a relevant tool to support the companies´ decision making process. Up to date, animal-based welfare protocols have been developed mostly considering their use by assessors, with limited attempts to make them use-friendly by farmers or stockpersons despite their

central role in improving animal welfare on a daily basis (Wiseman-Orr et al., 2011). Protocols must be easy to understand by farmers as only publishing regulations and controlling their fulfillment are insufficient (Ofner et al., 2003). They should be repeatable, so the end users can trust the results and precise to monitor improvements or, on the contrary, compromise welfare. They also should prioritize the most serious welfare issues to be first addressed and allow farmers to position their farm within others, to make them aware of the conditions which they provide to their animals (Botreau et al., 2007; Hristov et al., 2014a,b) and what the repercussions might be at the health and economic level. Many designed animal welfare assessment programs follow standards that make a meaningful difference to welfare, however, they might be too complex and difficult to meet for a broad spectrum of producers, thereby preventing many from engaging at all (Duncan et al., 2012).

Conclusions

In assessment of the welfare of dairy cows, the following indicators and parameters are most commonly used: (1) parameters which can readily be included, such as lameness, injuries, body condition score, cleanliness, getting up/lying down behaviour, agonistic social behaviour, oral abnormal behaviours and measures of the animal - human relationship; (2) parameters which require more information on reliability, such as indicators of good welfare and housing factors; and (3) parameters which are regarded as important but so far lack reliability in most countries, such as the incidence of clinical diseases and mortality.

The most significant problems in the use of indicators and parameters for on-farm dairy cattle welfare assessment are related to resource-based assessment alone can, however, fail to fully answer questions about the real status of the animals; although there are obvious advantages in use of animal-based welfare assessment methods, there are shortcomings related to the complexity of the protocols and the difficulties to standardize the evaluation criteria. In addition, they are time demanding in taking measurements and conducting behavioral observations according to the protocol, resulting in prolonged farm visits disrupting animals and farmers' daily activities.

Further work on protocols should enable accurate use of management and resource based animal welfare indicators through expanding knowledge related to management and environmental conditions impact to the welfare, as well as animal-based welfare assessment methods. Attention has to be paid to the level of pain that animals might be experiencing, which is an issue that to date has not received the necessary attention due to difficulties encountered in determining reliable pain indicators suitable of application at farm level.

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DAIRY FARM BIOSECURITY RISK ASSESSMENT METHODOLOGY - A REVIEW

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Abstract: Animal health risks are common and serious cause of financial failures of livestock production. Awareness of all threats and realistic appraisal of their possible impact are crucial in farm production planning, since every oversight or omission in planning may cause financial failure. The most of accessible methods for health risks assessment are questionnaires with generally empirically assembled questions. This may cause variations in interpretation of obtained results and therefore obtaining incomplete and inaccurate image of undertaken biosecurity measures real value and effects. In order to avoid mistakes and incorrect appraisal regarding threats or its misinterpretation, the most important biosecurity risks indicators have to be taken into account, having in mind their significance for certain type of production and technology organization, such as capacity and openness of dairy cows production. All planned farm biosecurity measures should be part of farm technology and should be observed *in situ*, making so called critical control points. The components of farm biosecurity should be taken into account and assessed as: general idea of farm biosecurity, spatial and technological organization of farm, undertaken measures regarding segregation, technological and protective procedures, pest control, and movement of animals, staff, visitors, equipment and vehicles, as well as impact of farm production on environment. For more precise evaluation, obtained data should be quantified and numerically expressed. The aim of the paper is to illustrate the relevance of indicators and within them to define a comprehensive, simple standardized methodology for assessing the level of biosecurity in dairy farms.

Keywords: assessment, biosecurity, dairy farm, methodology, risk

Introduction

Animal health and food safety systems are facing new and intensified challenges as a result of globalization and trade liberalization. Recent economic, institutional and environmental changes are creating new systemic disease risks, due to combination of rapid structural change in the livestock production, presence of intensive livestock production facilities near urban population centres and movement of animals, people and pathogens between intensive and traditional production systems. Additionally, climate change is altering patterns of livestock disease incidence, as pathogens and the insects and other vectors enter new ecological zones. Increasingly stringent food safety and animal health regulations and private standards aimed at promoting consumer welfare are creating challenges for producers, especially smallholders (FAO, 2009). Furthermore, the risks from diseases and pest that influence the viability of farms can reduce productivity, impact animal welfare, increase veterinary and labour costs, affect consumer confidence, reduce prices that producers receive for their animals and products, close export markets, reduce farm incomes, and reduce the value of farmland (Nold, 2007; Wells, 2011).

Human-health threats from livestock come in two basic forms: (a) zoonotic diseases, and (b) food-borne illnesses. Potentially pandemic viruses, such as influenza, are the most newsworthy of zoonoses, but many others exist, including rabies, brucellosis and anthrax. Food-borne illness can come from disease agents such as salmonella and *E. coli* or contaminants that enter the food chain during the production and processing of animal-based foods. The way these diseases are managed

create problems for all stakeholders and consumers, but smallholders are often particularly vulnerable, because they are more exposed to the risk and have less capacity to respond and recover (FAO, 2009).

There are strong economic reasons to prevent infectious diseases. Each year, many breeders spent significant amounts of money to suppress diseases which were already outspreaded in herd. Also, animal welfare, awareness and way of thinking of stockman should also be important motivation to assess and undertake measures in order to reduce diseases occurrence (Hristov et al., 2007, 2009).

Awareness of all threats and realistic appraisal of their possible impact are crucial in farm production planning, since every oversight or omission in planning may cause financial failure. Investigations and farm biosecurity assessment showed that during production, omissions occurrence allow infectious agents to penetrate and endanger the entire herd health and production (Stanković et al., 2010). Such phenomena occur not only as a result of ignorance or lack of information, as is often the case in developing countries, but sometimes producers include certain risk when protective measures cost "too much" as well (Boklund et al., 2004).

Models of risk analysis

The most of accessible methods for health risks assessment are questionnaires with generally empirically assembled questions. This may cause deviations in interpretation of obtained results and therefore resulting in incomplete and inaccurate image of undertaken biosecurity measures real value and effects.

According to Matei et al. (2007), at the world level, there are four models to approach risk analysis (updated versions cited):

- European model or World Organisation for Animal Health model promoted by this international veterinary organization by its two major publications, "Terrestrial Animal Health Code" (Anon., 2011) and "Aquatic Animal Health Code" (Anon., 2014),
- American model, promoted by Codex Alimentarius Commission (Anon., 2013),
- An international model promoted by World Trade Organisation through "Agreement for the Application for the Sanitary and Phytosanitary Measures" (Anon., 2016) and "Agreement on Removal the Technical Barriers" (Anon., 2003), and
- Model proposed by the Food and Agricultural Organisation through "International Plant Protection Convention" (Anon., 2011).

The first three approaches risk analysis are of interest for the fields of the competence of sanitary veterinary and food safety services. The European model approaching a risk analysis is based on the model proposed by Covello and Merkhofer (1993), which brought together all the methods for assessing risk into a common framework, demonstrating how the various methods relate to one another, providing a comprehensive reference for risk assessment. This one source offers readers concise explanations of the many methods currently available for describing and quantifying diverse types of risks with consistent evaluation and comparison of available risk assessment methods and identifies their specific strengths and, even more important, their limitations. The American risk analysis approach model is based on the model imposed by "National Research Centre" of United States National Academy of Sciences. Risk analysis model proposed by World Organization for Animal Health consists in the following major steps: risk and hazard identification, risk appreciation,

risk management, and risk communication. The Codex Alimentarius Commission model consists only in three stages: risk appreciation, risk management and risk communication. The World Trade Organization model consists in four stages: risk and danger identification, risk assessment, risk management and risk communication, and Food and Agricultural Organization consists in four stages: risk initiation, risk appreciation, risk management and risk communication (Matei et al., 2007).

These models of questionnaires could be used to establish risk for health and production in general, or due to single disease as hazard, or even certain aspects of production process.

A detailed analysis of the steps for each approach on a risk analysis applied for veterinary and food safety domains that was done has revealed that, generally, these four approaches comprise similar steps for doing risk analysis, but their executants competences are different in some extent. This aspect has particular importance, if it took into consideration the request of international organizations in the field of veterinary and food safety, that the structure having the responsibility for doing risk and hazard identification or danger identification or risk initiation and its evaluation must be separated from the structures involved to realized the management and monitor the risk and to lay down the options for corrective measures and at the same time separated from the structures that have select these options and put them in practice (Matei et al., 2007).

General risk assessment questionnaire scrutinize several aspects of farm biosecurity practices, such as in one presented by Smith (2015). The questions in this assessment are grouped by the three main ways diseases could be introduced to a farm: animal health, animal additions, commingling with other herds, visitors and agri-service personnel, and wildlife, birds, and insects, whose results are referred in Farm Biosecurity Risk Assessment Scorecard, and as sum of more similar answers finally establish low, moderate or high risk.

Berghaus et al. (2005) performed factor analysis of a Johne's disease risk assessment questionnaire with evaluation of factor scores and a subset of original questions as predictors of observed clinical paratuberculosis for: 1. bred heifers and cows share water troughs; 2. adult cow manure contamination of feed storage areas/equipment; 3. manure spread on weaned calf pasture; 4. weaned calves and cows share water; 5. pre-weaned calves housed near adults; 6. total number of cows in the herd; 7. pasteurization of pooled milk fed to calves, 8. cows allowed to nurse calves; 9. multiple cows in the calving area; 10. extent of manure contamination in the calving area; and 11. rearing or treatment sick cows in the calving area. These authors concluded that the length of the current risk assessment instrument could be reduced considerably without a substantial loss of information by removing or combining questions that are strongly correlated.

Good example of particular aspect of livestock production questionnaire is part of the Farm Biosecurity Program of Animal Health Australia partial visitor and staff risk assessment (Anon., 2012). Exposure assessment, regarding other farm visiting, ownership of same species animals, contacts with possibly sick animals and travel abroad, as well as mitigating activities regarding use of equipment, working clothes, contact with animals and biosecurity knowledge contribute are included in final assessment as low, moderate or high risk.

Indicators of farms health and production - "*Status praesens*"

Many diseases, such as bovine tuberculosis and foot and mouth disease are likely to be spread by movement of animals (Gilbert et al., 2005; Woolhouse et al., 2005, Humblet et al., 2009), which was

confirmed during the 2001 foot and mouth disease outbreak in the UK (Ortiz-Pelaez et al., 2006). Other contacts may also result in transmission of infectious agents (Relić et al., 2006; Stanković et al., 2015).

According to Hoe and Ruegg 2006., most management practices were associated with herd size and probably reflected differences in facilities and resources among responders from various herd size strata. Producers from large herds adopted more biosecurity practices than those from small herds. Biosecurity risks were prevalent. Almost half indicated that they purchased cattle, but few performed diagnostic testing of those cattle. Larger farms were either more aware of biosecurity risks or more able to undertake preventive measures because the frequency of diagnostic testing and examination of purchased cattle increased with herd size compared with smaller herds. Producers with larger herds performed mastitis control procedures frequently, while, for example, 49% of producers of very small herds had never submitted a bulk tank milk sample for microbiological analysis. Some widely believed opinions of producers were not in agreement with their practices. Raw milk was consumed by more than 60%, but consumption of raw milk decreased and perception of the risk of raw milk increased with increasing herd size. Larger farms had more knowledge of personal health risks related to zoonotic pathogens. Overall, most management practices were associated with herd size, but many beliefs of responders were consistent (Hoe and Ruegg, 2006). Many studies have characterized the prevalence of certain zoonotic agents in individual animals on farms or at slaughter (Chapman et al., 1997; Stanley et al., 1998). These data have obtained a good indication of the overall infection status of farmed livestock. Regarding faecal waste as potential risk, information describing the levels of pathogens present in contaminated manure is required. The levels and incidence of zoonotic agents within British livestock wastes were determined for the first time by a recent national survey (Hutchison et al., 2004). There are currently found in livestock wastes measurable prevalence (Hutchison et al., 2005) of the five pathogens responsible for most of the cases of gastroenteritis in the United Kingdom (Adak et al., 2002).

The health problems associated with farming have not yet been fully evaluated. Subjective records (on-farm surveys or expert opinions) are available for most farm species, but their reliability depends on the survey and type of disease concerned, and good records are available only for the most easily diagnosed diseases (Cabaret, 2003). Pre-harvest food safety is the complex of measures related to farm supply and on farm procedures that aim at preventing and/or minimizing the amount of food borne health risks to humans carried into the food chain *via* animals and animal products (Blaha, 2005).

In order to identify the zones of potential risks on farms with different capacities, ways of keeping and sources for restocking of the stock/herds, it is necessary to look at various biosecurity weak spots regarding possible consequences, facility location and layout, operational routines and animal health management. No matter of level of reference (individual, herd or farm level), infectious diseases transmission is related to any form of contact, direct or indirect (Brennan et al., 2008). In addition, this concept covered the three major components of biosecurity: (*a*) isolation, (*b*) traffic control, and (*c*) sanitation (Buhman et al., 2005), resulting criteria or indicators which are being used for laboratory, feed factory, or farm biosecurity risk assessment and all the effects of general and special biosecurity measures to prevent introduction of infectious agents in a dairy farm or its spreading have to be taken into account (Stanković et al., 2011; Relić, 2014). Therefore, appropriate farm biosecurity questionnaire have to cover as much as possible criteria that could be used as indicators, and measured more accurate as possible.

Serbian farm biosecurity experience

As already pointed out, in order to identify potential risks on farms with different capacities, ways of keeping and sources for restocking of the stock/herds, it is necessary to look at various biosecurity weak spots regarding possible consequences, so called critical control points.

According to all obtained data, intentions and purposes, Hristov and Stanković (2009) suggested questionnaire which fulfilled listed demands, previously mentioned (Buhman et al., 2005). The questionnaire included the 18 most important indicators concerning farm biosecurity. The questionnaire covers general criteria for biosecurity, presented by Stanković et al. (2015), such as: (1) insights into biosecurity management: planning and control of application of biosecurity measures, farm isolation, determination of special zones, control of movement in and between the identified zones, control of movement and traffic (employees, foreigners, newly-born animals, fodder, equipment, devices, etc.), attitude towards visitors, and quarantine; (2) animal health management: control of the movement of animals, monitoring the animals on signs of the disease, defining a response plan for situations of potential disease outbreak, and herd health status; (3) operational farm management: proper disposal of carcasses procedure, disposal of carcasses, proper safe and use of manure procedures, manure removal, maintenance of hygiene of barns, facilities, equipment and vehicles, maintenance of facilities, sanitation, provision of production inputs, control of nutrition and water supply, presence of other animals on the farm, rodents control, arthropods control, birds control, relations and attitude of the farm and farm management to the environment, and planning and staff training.

For each indicator is supposed to be indicated good sides, bad sides, opportunities to overcome the bottlenecks, risks that can impede or prevent the overcoming of bad sides. For each indicator the level of achievement in the indicator criteria has to be evaluated (use the grade from 0 to 5, where 0 is inadequate, there are no resources for improvement; 1 - insufficient, there are resources for improvement, 2 - sufficient, 3 - good, 4 - very good; 5 - excellent level). After accomplishing evaluation of all indicators, obtained marks have to be summarized and divided by number of indicators in order to get average mark, which could be: inadequate: 0-1.99 (high biosecurity risk level); sufficient: 2.00-2.49 (serious biosecurity risk level); good: 2.5-3.49 (moderate biosecurity risk level); very good: 3.5 - 4.49 (low biosecurity risk level); and excellent: 4.5 - 5.00 (minimal biosecurity risk level).

This evaluation is referred to farm biosecurity level and proportional biosecurity risk level in the moment of assessment. To make it clearer, SWOT analysis is required, that is point out all positive qualities of farm biosecurity plan and undertaken procedures (S*trengths*), all negative in planning and/or (not) undertaken procedures (W*eaknesses*), possible answers to minimize anticipated existing and anticipate and minimize (O*pportunities*) existing and future problems in animal health and production (T*hreats*).

After completing this questionnaire, the farmer, veterinarian and veterinary inspector should have a better insight into the existing biosecurity risk on the farm. Completion of the questionnaire answers enables to recognize the source of potential risks, so a new biosecurity plan could be defined or an existing biosecurity plan modified in order to include all relevant risks for the farm production.

The questionnaire was developed as result of the National technology project TR 20110 "Development and implementation of welfare and biosecurity standards in order to improve the

technology of cattle and pig production" (2008-2011), presented in "Biosecurity standards for dairy cattle farms" (Hristov et al., 2011) prepared for Veterinary Directorate of Serbian Ministry of Agriculture, Forestry and Water Management, and have been used as risk assessment tool on dairy farms in Tempus project "Academia - Industry Links in Food Safety and Quality – FOODLINKS, JP 158714 - 2009" (2009-2012). Also, it has been used in investigations in Project TR 31086 "Optimization of technological processes and animal husbandry resources on farms to improve sustainability milk production" of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

The questionnaire has been amended two times since its creation, and further work on it should be directed towards the quantification of the significance of individual indicators and the creation of a mathematical model, which would greatly increase the accuracy of the assessment.

Conclusions

According to presented data, it could be concluded that at least three stages have to take place in farm biosecurity risk assessment: risk appreciation, risk management and risk communication. Appropriate farm biosecurity questionnaire have to cover as much as possible relevant criteria that could be used as indicators, and measured more accurate as possible. Analysis of obtained data should not only give a realistic view of health and production risks in real time, but to point out possible solutions for potential and real problems. In our country the questionnaire which included the 18 most important indicators concerning farm biosecurity was defined. The further work on questionnaire should be directed towards the quantification of the significance of individual indicators and the creation of a mathematical model, which would greatly increase the accuracy of the assessment.

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FREQUENCY OF BEHAVIOURAL DISORDERS OF CALVES IN THE FIRST MONTH OF LIFE

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Abstract: The manifestation of various forms of behavioural disorder in calves up to 30 days old was monitored on two farms for a year during four seasons in one year. On both farms calves were separated from mothers immediately after birth. On the farm A calves were tied in the first week of life, while at the farm B they were kept in individual boxes.

The following forms of behavioural disorder have been reported: apathy, twisting of the tongue, licking of the substrate and intersucking, while the appearance of aggression between calves has not been recorded.

Apathy was recorded in 16.28% of calves at farm A, most commonly in the age of 0-7 and 15 days, and in 84 calves (14.69%) at farm B, most often in the period immediately after birth. The occurrence of tongue twisting was recorded in 0.50% calves at farm A (2 at the age of 22 days and 1 at the age of 30), while no cases were reported at farm B. Calves were more susceptible to the licking of substrates (walls, fences, truncheons and other equipment) on both farms, since this phenomenon was observed in 8.89% of calves at farm A and 8.39% of calves at farm B at different age; most often at the age of 30 days at Farm A, and at the age of 22 days at Farm B, while in the youngest age was the most common occurrence of this form of behaviour. Also, during the examined period on both farms there was a phenomenon of mutual calves sucking, 2.35% of calves at farm A, most often at the age of 22 days and 3.32% of calves at farm B, most often at the age of 8 and 22 days. The occurrence of the behavioral disorders in calves was the most frequent during the winter period, and the least frequent during autumn.

The observed behavioural disorders indicate poor welfare quality. It is thought to have been caused by failures in the technology of keeping and accommodation, as well as early separation of calves from mothers. They are caused by disabling calves to satisfy basic physiological needs in behaviour and contact with other animals or as a result of exposure to pain, fear and stress.

Keywords: welfare, behavioural disorders, calves

Introduction

The behaviour of calves is a conscious or unconscious response to the stimuli that come from the environment, causing voluntary or involuntary movement, actions, changes in body position, etc. The presence of physiological forms of behaviour and the good emotional state of the animals are a good indicator of the quality of well-being and is one of the principles of its assessment.

Calf welfare is largely related to animal behaviour. The positive emotional state of the animals depends on the ability to freely express physiological forms of behaviour in each stage, along with the unimpeded realization of social contact with other individuals (Rushen and de Passille, 2009). In addition, it is extremely important that there is a good "human-animal" relationship. By optimis-

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ing these factors that define the emotions of animals, it can be expected that the exposure to stress, fear and other negative emotions will be minimized, which is the goal of animal welfare care.

Behavioural disorders and negative emotional states (pain, fear, frustration, etc.) in calves in the first month of life often arise as an indicator of poor quality of welfare and low degree of adaptability to the environment (Botreau et al., 2007). It is therefore necessary to monitor the behaviour of calves and the appearance of disturbed behaviours in order to eliminate adverse factors that affect both the quality of welfare and overall production results. The occurrence of abnormal forms of behaviour is the result of certain failures in calving (Færevik et al., 2008; Sutherland et al., 2014), nutrition (Hepola et al., 2006; Vieira et al., 2008) and production technology at the farm. In addition, various procedures by breeders, such as the early separation of calves from mothers, also lead to their occurrence (Stöhulova et al., 2008; Fröberg and Lidfors, 2009; Veissier et al., 2013). Interventions that cause pain, stress and fear, such as dysfunctional anaesthesia and analgesia (Kieland et al., 2010; Gottardo et al., 2011; Bergman et al., 2014) and poor breeders attitude toward animals (Lundvall and Saras- Johansson, 2011; Shütz et al., 2012) also contribute to the occurrence of calving disorders in calves. These abnormal behaviours can be manifested differently, such as apathy, aggression and manipulation of the substrates or parts of the body, of one's own or another's (licking and sucking each other).

The aim of this study was to identify the most significant and most frequent behavioural disorders of calves in the first 30 days after birth.

Material and Method

The study of the effects of rearing conditions and the season of birth on the occurrence of calf behaviour in the first 30 days of life was carried out on two farms (farm A and farm B) within the same production system, so that the conditions of feeding and keeping all categories of animals were similar with the only exception relating to accommodation of the youngest categories. The production capacities of the farms are similar and amount to about 1500 dairy cows with accompanying categories.

On both farms calves were separated from mothers immediately after birth. At the farm A calves were tied on beds for the first week, while at farm B they were kept in individual boxes. At the age of 8 to 30 days, on both farms calves were placed in group boxes, at farm A 10 calves per box, and at farm B half 5 of them. Tattooing of calves was performed on both farms at the earliest age (from 0-7 days). Putting stamps with an identification number was also performed at this age, rarely in older animals without the use of analgesia and anaesthesia.

On farm A only female animals were dehorned earlier than 30 days of age, while on farm B this procedure was performed after the moving to the older category. Dehorning was performed by thermocauter, without prior anaesthesia of the calf and with the use of antibiotic ointment after the completion of the procedure.

Recording of various forms of calf behaviour, in the period from birth to 30 days old, was performed for a year in four seasons (I-Autumn, II-Winter, III-Spring and IV-Summer), with 596 calves on the farm A (nA) and 572 calves at farm B (nB). The research was carried out in the first two hours after the morning feeding of the calves and the observed anomalies were recorded in the defined ethogram. Different forms of behavior in calves were observed on the basis of individual observation of calves, as well as analysis of data recorded by the video camera (Sony DCR – SR75E).

Results and Discussion

Tables 1 and 2 show the established behavioural disorders of calves on farms A and B, depending on the age of the calves and the test season.

Table 1 Expression of behavioural disorders depending on the life of calves (nA = 596, nB = 572)

Age, days	Apathy		Twisting of the tongue		Licking of the substrate		Mutual sucking	
	Farm A N, %	Farm B N, %	Farm A N, %	Farm B N, %	Farm A N, %	Farm B N, %	Farm A N, %	Farm B N, %
0-7	24 4.03	21 3.67	0 0.00	0 0.00	3 0.50	3 0.52	2 0.34	0 0.00
8	22 3.69	18 3.15	0 0.00	0 0.00	10 1.68	10 1.75	4 0.67	8 1.40
15	27 4.53	16 2.80	0 0.00	0 0.00	11 1.84	9 1.57	0 0.0	3 0.52
22	19 3.19	16 2.80	2 0.34	0 0.00	11 1.84	14 2.45	5 0.84	8 1.40
30	5 0.84	13 2.27	1 0.17	0 0.00	18 3.02	12 2.08	3 0.50	0 0.00
Total	97 16.28	84 14.69	3 0.50	0 0.00	53 8.89	48 8.39	14 2.35	19 3.32

Apathy implies lack of reacting calves to environmental stimuli. The number of calves of apathy behaviour was somewhat higher on farm A (16.28%) than farm B (14.69%).

The highest number of calves with this disorder was observed at farm A at the age of 15 days, 4.53% of the all calves; although at the age from 0 to 7 days this number was still considerable, which is 4.03% calves. Observed by season, most apathetic calves at farm A were in the winter season (9.06%). At farm B distribution of calves with manifest apathy problem was more uniform in categories (from 3.67% in the youngest age group to 2.27% in the oldest). The largest number of apathetic calves was registered in the spring season (6.64%). Expressed apathy of calves, especially at the earliest age, can be consequence of the action of more powerful stress factors. The first is separation from the mother, with which Daros et al. (2014) agree, but there may also be inadequate housing conditions, as well as pain (Broom and Fraser, 2007).

In the study on both farms, A and B, has been established that the area for accommodating calves at the earliest age was inadequate because they did not allow them to move unhindered. The confined area of individual boxing (Farm B) and limited movement (farm A) with insufficient social contact act negatively on calves, which most often causes trouble and apathy.

Table 2: Expression of behavioural disorders depending on the season of birth (nA = 596, nB = 572)

Season	Apathy		Twisting of the tongue		Licking of the substrate		Mutual sucking	
	Farm A N, %	Farm B N, %	Farm A N, %	Farm B N, %	Farm A N, %	Farm B N, %	Farm A N, %	Farm B N, %
I	4 0.67	12 2.09	1 0.17	0 0.00	23 3.85	12 20.9	12 2.01	1 0.17
II	54 9.06	12 2.09	2 0.34	0 0.00	17 2.85	10 1.75	0 0.00	12 2.09
III	23 3.85	38 6.64	0 0.00	0 0.00	2 0.34	8 1.40	2 0.34	2 0.35
IV	16 2.68	22 3.85	0 0.00	0 0.00	11 1.85	18 3.15	0 0.00	4 0.70
Total	97 16.28	84 14.68	3 0.50	0 0.00	53 8.89	48 8.39	14 2.35	19 3.32

Twisting of the tongue is a type of behavioural disorder when calf tears tongue out of mouth and then returns to swallow the air. This behaviour most commonly occurs in the conditions where calves are being exposed to inadequate nutrition, that is, because of the diet that excludes sucking, but also in the restricted movement (Broom and Fraser, 2007). No large number of tongue twisting cases in calves was recorded on the investigated farms. Only three such cases were recorded at farm A, namely 2 (0.34%) at age of 22 days and 1 (0.17%) at the age of 30, and 2 cases during the second and one during the first season.

In contrast to the previously described stereotypical behaviour that occurred in rare cases, more frequent behavioural disorders were detected in the older age of the calves, for which the contact with other calves is necessary (intersucking) or the place of accommodation with water heaters, feedlots and fences (stereotypical behaviour of substrate manipulation).

Licking of walls, box fences and drinkers was recorded on farm A with 8.89% of calves, and on farm B with 8.39% calves. At the farm A the most noticeable of stereotypical behaviour was in the category of calves aged 30 days (3.02%), and the smallest were between 0 and 7 days (0.50%). At farm B recorded situation was identical as on farm A in the youngest category, while the number in other categories did not vary much, i.e. from 1.57% (15 days) to 2.45% (22 days). At farm A the highest number of calves with this behavioural disorder was recorded in autumn (3.85% calves) and at farm B the worst situation was in the summer with 3.15% of cases of licking of the substrate. The manifestation of this form of abnormal behaviour of calves was consequence of premature separation from mothers (Broom and Fraser, 2007).

There is a significant influence on the manifestation of abnormal forms of behaviour and the length of stay of calves with mothers and the possibility of sucking. Calves that are able to satisfy innate reflex of sucking for a longer period of time are less likely to manifest anomalies in behaviour, although at the time of separation, they react more turbulent than those who spent shorter times with mothers (Weary and Chua, 2000; Flower and Weary, 2001; Fröberg and Lidfors, 2009; Veissier et al., 2013).

Premature separation of calves from mothers results in an unsatisfied primary need for sucking, which leads to the occurrence of intersucking of calves at different ages. Usually they suck in the area of the navel, scrotum, prepuce, udder or ears (Broom and Fraser, 2007), which can lead to digestive disorders, various infections or damage of the organs.

Analyzing this at farms A and B in calves up to 30 days old, it was notable that it was not widespread, 2.35% and 3.32%, respectively, of observed intersucking. There were no cases at farm A at the age of 15 days, 0.34% of cases were recorded at 0-7 days, 30 days at 0.50%, 8 days at 0.67% and 22 days at 0.84 %. There was a slightly more frequent intersucking at farm B: 1.40% of cases at the age of 8 and 22 days and 0.52% at the age of 15 days. Observed by seasons, the highest number of recorded mutations at both farms was equal to 12 heads in the first season at farm A (2.01%) and in the second season at farm B (2.09%).

The occurrence of mutual aggressive behaviour of calves has not been noticed in this study. As already emphasized in the introduction, the behaviour of calves is a conscious or unconscious response to the stimuli that come from the environment surrounding them by causing voluntary or involuntary movement, actions, changes in the body's position, etc. Botreau et al. (2007) defined twelve criteria for assessing the welfare of calves, including behaviour.

Calves are in various ways exposed to stressful environmental factors from birth. Therefore, it is necessary that they be provided with conditions in which they can exercise all forms of their

physiological behaviour and that the level of stress, fear, and other negative emotions is reduced to a minimum. Behavioural disorders are caused by failures in the process of rearing and treatment of animals, which deprive them from satisfying basic needs in behaviour and contact with other animals or are exposed to pain, fear and stress. One of the most powerful stressors in calves at the earliest age is separation from mothers. This is particularly noticeable in dairy cows, where calves are for economic reasons separated from mothers after a few hours after birth, rarely after a few days, and only in exceptional cases later. This way of separating calves from mothers is reflected in the manifestation of stress reactions, and has long-term consequences on the growth of calves and the appearance of some abnormal forms of behaviour (substrate manipulation, intersucking).

Comparing the behaviour of calves that could suckle mothers (sometimes other cows) and calves that were fed from automatic feeds from the beginning, Fröberg and Lidfors (2009) concluded that calves allowed to suck use solid foods less, lie longer, move less and exhibit exploratory behaviour, have less social contacts than calves fed artificially, but at the same time exhibit less abnormal forms of behaviour, such as intersucking or rolling and twisting of the tongue.

This was supported by findings of Stehulova et al. (2008) and Veissier et al. 2013., who investigated whether mothers influence the reduction of non-nutritive oral activity of calves (licking of other calves, floors and walls, twisting of tongues, etc.). They found that calves that spend more time with mothers are less prone to abnormal behaviour than those who are early separated and artificially fed. Negative response to stress due to separation can be mitigated by allowing physical contact (separation by fence) and avoiding separating calf from mothers with solid walls (Johnsen et al., 2015). One of the important risk factors for the welfare of calves is the endangerment of their comfortable accommodation, movements, resting and other activities that calves practice in the box. Hepole et al. 2006. state that group accommodation has affected that calves consume more concentrate and chew longer in the group than calves that are placed in individual boxes, but are prone to hair licking, other calves or box walls.

In order to avoid the appearance of pathological forms of non-nutritive behaviour (intersucking, twisting of the tongue and licking of substrates), the calves' diet after separation from the mothers should be similar to the milk as much as possible. It is desirable to provide artificial feeders which prevent shortened feeding time. Feeding should take approximately the duration of normal mother's sucking. Additionally, it is possible to prevent movement and prevent contact with other calves for 30 minutes to an hour immediately after feeding. The high biological and nutritive value of rations for calves is a precondition that is implied. In addition to the nutrition recommendations, other recommendations should also be taken into account to reduce the risk and improve the overall well-being of calves, which relate primarily to rearing conditions for the calves. It is necessary to avoid the tying of calves and the placement in individual or group boxes of inadequate size. Calves in the first 30 days of life should be provided with dry and clean bedding in the box, allow movement and establishment of social contact with other sorts.

Conclusion

Based on the study of the frequency of calf behavioural disorders in the first month of life, the following can be concluded:

- apathy, twisting tongue licking substrate and intersucking were identified, while the occurrence of aggressive behaviour in calves is not recorded;

- the highest incidence of calf behavioural disorders was observed in the ages of 8 and 22 days, while the smallest was recorded in the earliest (0 to 7 days) and the oldest age (30 days);
- the most unfavourable impact on calves behaviour was the winter season; on the contrary, autumn season was most favourable in terms of calves behaviour;
- apathy in the behaviour of calves was most frequently present, and less frequent licking of the substrate; the appearance of intersucking was rare, and the occurrence of tongue manipulation was sporadic;
- behavioural disorders are caused by omissions in the process of keeping and treatment of animals, which prevent them from meeting basic needs in behaviour and contact with other animals or are exposed to pain, fear and stress;
- for the manifestation of physiological forms of calves behaviour, it is necessary to provide adequate nutrition and appropriate accommodation, which would allow for a greater degree of freedom of movement and better establishment of social contact in animals.

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QUALITY INDICATORS OF EGGS ENRICHED WITH DHA

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Abstract: By designing hens' mixtures, it is possible to achieve a change in the fatty acid profile of eggs. The content of omega-3 fatty acids in egg yolks therefore depends on the share and source of oil in hens' mixtures. In recent times, an alternative to fish oil in hens' mixtures are microalgae rich in docosahexaenoic fatty acids (DHA). The aim of the study was to determine the impact of feeding treatments on egg quality. As sources rich in DHA, microalgae (*Schizochytrium limacinum*) or fish oil were used. In the study, 120 hens of Tetra SL line were used. The trial period lasted for three weeks, and the hens were between 42 and 45 weeks of age. Hens were randomly divided into three experimental groups, which were fed with specially prepared mixtures as follows: A=5% soybean oil (SO); B=2% SO+1.2% rapeseed oil (RO)+1.3% linseed oil (LO)+0.5% microalgae; C=2.0% SO+1.2% RO+1.3% LO+0.5% fish oil (FO). Analysis of the results showed statistically significantly higher DHA content per 100 g of eggs in groups B and C compared to group A (C=148.65 mg; B=140.74 mg and A=89.90 mg; $P<0.05$). The use of microalgae in hens' feed has favorably influenced shell quality. The statistically significantly thicker shell ($P=0.025$) was measured in group B (0.461 mm) in relation to groups A and C (0.431 and 0.437 mm). The addition of fish oil to hens' feed had statistically significant influence on the better values of egg weight, albumen height and HU, while microalgae addition had the most favorable influence on the pH values of albumens and yolks. The feeding treatments used had no effect ($P>0.05$) on the share of basic parts in eggs, yolk color and shell strength.

Keywords: eggs, microalgae, fish oil, quality, DHA

Introduction

Increasing the content of omega-3 fatty acids in chicken eggs can be achieved by adding different vegetable oils (flax and rapeseed) or fish oil to mixtures for laying hens. Recently, researchers say that a very good alternative to fish oil is microalgae. Microalgae have excellent nutritional value and may be added as an oil or powder in mixtures for hens. Microalgae are a rich source of essential amino acids, vitamins, minerals, carotenoids and fatty acids (Becker, 2004). The use of microalgae in poultry nutrition can be very effective in terms of better pigmentation of egg yolk and broiler skin (Herber and Van Elswyk, 1996). It also has a positive effect on production and egg weight, and on increasing the content of eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids in egg yolks compared to conventional feeding (Park et al., 2015). Many authors in their research have emphasized that the addition of fish oil to the mixtures for hens can negatively affect the sensory properties of eggs. Thus, when using microalgae, it is also important to take care of the share of microalgae in the mixture (Kos et al., 2018). Saleh 2013. states that the addition of fish oil to hens'

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mixtures in shares of 2.5% to 5% statistically significantly affects egg weight and daily intake of feed. These parameters in the mentioned groups are lower in relation to the control group. The addition of fish oil to the mixtures may affect the eggshell quality, and the results depend to a great extent on the proportion of fish oil in hens' mixtures. Papas et al. (2005) reported that the addition of fish oil to hens' mixtures affect the reduction in eggshell thickness relative to the control group fed diet supplemented with soybean oil, while Salah 2003. reported significantly thicker shell in eggs of hens that consumed mixture with fish oil (3.5%) in relation to the control group (5% vegetable oil). Eggs enriched with omega-3 fatty acids, particularly the essential DHA, are becoming important foodstuff in the food chain, because their consumption can alleviate insufficiency of omega-3 fatty acids in the human diet (McNamara et al., 2010). The aim of this paper was to determine egg quality and DHA content in eggs of hens fed with a mixture of different vegetable oils in combination with fish oil or microalgae.

Material and Methods

Experiment organization and hens feeding

The experiment was conducted on 120 hybrid Tetra SL hens. The trial period lasted for 3 weeks, and hens were aged from 42 to 45 weeks. The experiment was carried out during this hens' age, because in our previous studies on enrichment of eggs with omega-3 fatty acids, we noticed that the use of designed mixtures, especially over a longer period of time, may result in the egg shell weakening. At the beginning of the experiment three groups were formed: control (A) and two experimental (B and C). In each group there were 40 hens. Composition and chemical analysis of mixtures prepared for research purposes are shown in Table 1. The control mixture was of standard composition supplemented with 5% soybean oil, while experimental mixture was supplemented with 4.5% oil mixture (2.0% soybean oil-SO, 1.2% rapeseed oil-RO and 1.3% linseed oil-LO). The experimental mixtures differed by addition of 0.5% algae (*Schizochytrium limacinum* from Alltech® company containing 14% -16% DHA) added to B or 0.5% fish oil added to C group. All groups of hens were kept in the same building, and microclimate conditions matched the requirements for this type of poultry. Eggs were collected manually each day. Feeding and watering of hens was automatic. The egg quality indicators and DHA content in eggs were determined after three weeks of feeding hens with special mixtures, one day after egg collection.

Determination of egg quality

The analysis of the external and internal quality of eggs was determined on 60 eggs of the weight class L, to which belong eggs weighing from 63 to 73 g. where eggs ranged from 63 g to 73 g. Among external egg quality indicators analyzed were egg weight, eggshell strength and thickness. Considering internal egg quality indicators, the following were analyzed: yolk color, albumen height, Haugh units (HU), albumen pH and yolk pH. Shares of basic parts in egg were also calculated. The weight of eggs and basic parts (yolk, albumen and shell) needed for the calculation of the share is determined by the Mettler Toledo BBK 422 – 6 DXS scale. Eggshell strength was measured with automatic device Eggshell Force Gauge Model-II. The eggshell thickness was measured using an electronic micrometer with an accuracy of 0.001 mm in the middle of the eggshell. Egg yolk color, HU and albumen height were determined by the automatic device Egg Multi-Tester EMT-5200. The Haugh unit score (Haugh, 1937) was calculated using the following formula:

$HU = 100 \log_{10} (H - 1.7 W^{0.37} + 7.57)$

where H = height of the albumen (mm) and W = weight of egg (g)

Table 1. Composition and chemical analysis of laying hens' diets

Ingredient %	A[3]	B[3]	C[3]
Corn	49.20		
Soybean meal	21.00		
Roasted soybean	3.33		
Sunflower meal	5.00		
Alfalfa	1.50		
Calcium grain	10.67		
Monocalcium phosphate	1.33		
Yeast	0.50		
Salt	0.33		
SAL CURB- acidifier	0.33		
Nanofeed-zeolite	0.33		
Methionine	0.15		
Premix[1]	1.33		
Soybean oil	5	2	2
Rapeseed oil	-	1.2	1.2
Linseed oil	-	1.3	1.3
Fish oil	-	-	0.5
Microalgae	-	0.5	-
Total	100.00		
[2]Chemical composition of diets (g/kg)			
Moisture	85	82	82
Ash	160	193	144
Raw protein	172.9	176.1	176.7
Fat	75	77	84
Raw fiber	37	34	31

[1]Premix: Calcium 33%, vit. A 833.34 I.U., vit. D_3 208.34 I.U., vit. E 8.350 mg, vit. K_3 170 mg, vit. B_1 150 mg, vit. B_2 375 mg, Panthotenic acid 590 mg, Niacin 2.100 mg, Choline chloride 33.340 mg, vit. B_6 200mg, vit. B_{12} 960 mg, Biotin 7.100 m, Folic acid 70.5 mg, vit. C 1.900 mg, Iron 2.500 mg, Copper 415 mg, Zinc 5.200 mg, Manganese 5.835 mg, Iodine 75 mg, Selenium yeast 35 mg, Antioxidant (Apo-ester 85 mg, canthaxanthin 250 mg).

[2]Referential methods applied for chemical analysis of feed: HRN ISO 6496:200; HRN EN ISO 5983-2:2010; HRN EN ISO 6865:2001, Modified according to instructions of FOSS Fiber Cap manual; HRN ISO 5984:2004; HRN ISO 6492:2001, Modified according to instructions of extraction system ANKOM XT15; RU-5.4.2-11 (internal method)

[3]A= 5% soybean oil (SO); B=2% (SO) + 1.2% rapeseed oil (RO) + 1.3% linseed oil (LO) + 0.5 microalgae, C=2.0% SO + 1.2% RO + 1.3% LO + 0.5% fish oil (FO)

Determination of fatty acids

Fatty acids were determined on a total of 15 egg yolks, i.e. on 5 yolks per each experimental group. Homogenized samples fat content was extracted with the method of Folch et al. (1957). All solvents used were ultrapure-grade by Sigma-Aldrich (Schnelldorf, Germany), and 100 mg/L butylated hydroxitoluene was added to the extraction mixture (chloroform/methanol 2/1 vol/vol) as antioxidant. After this, fatty acid containing lipids were transmethylated by the base-catalyzed sodium- methoxide method of Christie (1982). Gas liquid chromatography was performed on a Bruker 430-GC apparatus (Billerica, MA, SAD), equipped with a FAMEWAX (RESTEK, Bellefonte,

USA) type capillary column (30 m x 0.32 mm internal diameter, 0.25 μm film) and flame ionization detector. Characteristic operating conditions were: injector temperature: 220 °C, detector temperature: 230 °C, helium flow: 25 ml/min. The oven temperature was graded: from 50 to 225 °C: 6.0 °C/min, 21 min at 225 °C. To identify individual FA in the chromatogram, a FA standard mixture (Supelco 37 Component FAME Mix) was used. Content of DHA is shown as mg/100 g egg.

Statistical analysis

The research results were processed using program Statistica for Windows version 13.3. (StatSoft Inc., 2017.). Variance analysis (ANOVA) was used in the analysis of results, and if the P value was statistically significant, the differences between the groups were tested by the Fisher LSD test.

Results and Discussion

The Table 2 presents egg quality indicators showing the statistically significant difference in egg weight (P=0.032) and eggshell thickness (P=0.025) in the examined groups.

Statistically significantly lower egg weight was observed in group of hens that consumed the mixture with the addition of microalgae in relation to the control group and a group of hens that consumed feed supplemented with fish oil (B=67.07 g i.e. A=68.48 g and C=68.72 g). Saleh 2013. also states that there is no statistically significant difference in the egg weight between the control group and the experimental group with the lower share of fish oil, which is consistent with our results. Park et al. (2015) state that there is no statistically significant difference in egg weight between the control group and the group of hens that were consuming feed supplemented with 0.5% microalgae powder (*Schizochytrium*), which is not in accordance with our results.

Table 2. Egg quality indicators

Indicators	Treatment			P value
	A	B	C	
Egg weight (g)	68.49±2.78^{a}	67.07±2.27^{b}	68.72±1.93^{a}	***0.032***
Eggshell thickness (mm)	0.431±0.02^{b}	0.461±0.04^{a}	0.437±0.04^{b}	***0.025***
Eggshell strength (kg/cm^2)	3.15±0.518	3.45±0.74	3.19±0.67	0.230
Albumen (%)	63.17±1.61	62.07±1.77	61.91±2.83	0.083
Yolk (%)	23.61±1.66	24.27±1.79	24.87±2.25	0.073
Shell (%)	13.22±1.08	13.65±1.00	13.22±1.5	0.391

P>0.05; A=5% soybean oil (SO); B=2% (SO) + 1.3% rapeseed oil (RO) + 1.3% linseed oil (LO) + 0.5 % microalgae, C=2.0% SO + 1.3% RO + 1.3% LO + 0.5% fish oil (FO)

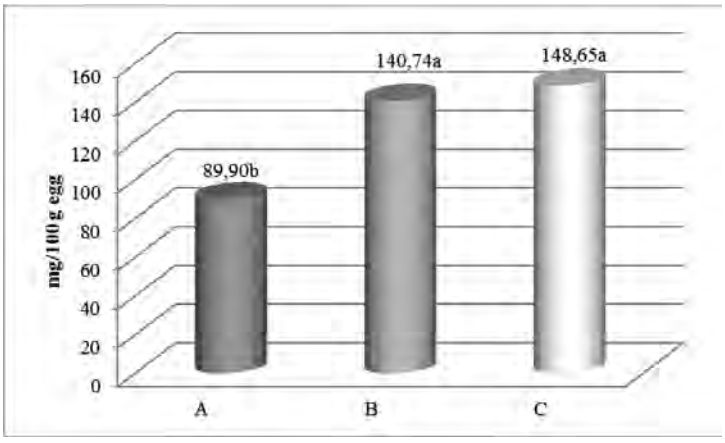
The highest value of the eggshell thickness was found in group B (0.461 mm), which differed statistically significantly (P=0.025) in relation to group A and C (0.431 mm and 0.437 mm). In line with our results, Park et al. (2015) found significantly thicker shell (P=0.003) in eggs of hens which consumed microalgae in feed in relation to the control group (CON=0.367 mm and $MPA_{0.5\%}$=0.379 mm). For other egg quality indicators (eggshell strength and shares of albumen, yolk and shell in egg) no statistical significance was established (P>0.05). Table 3 shows the values of internal quality and freshness of eggs. The results show a statistically significant difference in albumen height (P<0.001) i.e. albumen height was statistically significantly higher in eggs from groups C and A (7.33mm and 6.88mm) compared to group B (5.99 mm). The values of HU ranged in accordance with the values of albumen height, and they were also statistically significantly higher in group C relative to group B (82.48 and 74.36). The results of Saleh 2013. are consistent with our results, as the author states

that there is no significant difference in the HU between the control group and the group with the addition of fish oil to the hens' mixture. There was no statistically significant difference (P>0.05) for yolk color values, which is consistent with the results of Park et al. (2015). Values of albumen and yolk pH were statistically significantly higher in group C compared to groups A and B. The reason for increased pH values can be interpreted with more intense oxidation processes in an edible part of the egg.

Table 3. Indicators of internal quality and freshness of eggs

Indicators	Treatments			P value
	A	B	C	
Albumen height (mm)	6.88±1.14[a]	5.99±1.12[b]	7.33±1.23[a]	***0.001***
HU	79.95±12.93[ab]	74.36±8.49[b]	82.48±9.61[a]	***0.024***
Yolk color	13.20±0.41	13.12±0.43	13.16±0.55	0.835
Albumen pH	8.26±0.12[b]	8.22±0.15[b]	8.37±0.16[a]	***0.002***
Yolk pH	6.05±0.60[ab]	6.04±0.62[b]	6.08±0.07[a]	***0.048***

[a,b] P<0.05; A= 5% soybean oil (SO); B=2% (SO) + 1.3% rapeseed oil (RO) + 1.3% linseed oil (LO) + 0.5 microalgae, C=2.0% SO + 1.3% RO + 1.3% LO + 0.5% fish oil (FO)



Graph 1. DHA content in eggs

[a,b] P<0.05; A= 5% soybean oil (SO); B=2% (SO) + 1.3% rapeseed oil (RO) + 1.3% linseed oil (LO) + 0.5 microalgae, C=2.0% SO + 1.3% RO + 1.3% LO + 0.5% fish oil (FO)

The content of DHA in 100 g of eggs was statistically significantly higher in experimental groups compared to control (A=89.90 mg/100 g egg vs. B=140.74 mg/100 g egg and C=148.65 mg/100 g egg; P <0.05). The reason for this is the addition of microalgae or fish oil to hens' mixtures, which are a rich source of DHA, compared to the soybean oil added to the mixture of the control group of hens. The results of the omega-3 fatty acids content, respectively important docosahexaenoic fatty acid, in the research of different researchers (Park et al., 2015, Becker 2004, Herber et al., 1998) are consistent with our results.

Conclusion

Powder of *Schizochytrium limacinum* strain microalgae is a rich source of omega-3 fatty acids and can replace fish oil in mixtures for laying hens. By adding *Schizochytrium limacinum* microal-

gae to hens' mixture, a similar increase in DHA content in eggs is achieved as in the group in which a fish oil is added to the mixture (B=140.74 mg/100 g egg and C=148.65 mg/100 g egg). This confirms that the microalgae can be an alternative source of DHA in hens' nutrition. It was also found that the use of microalgae or fish oil in mixtures for laying hens has no negative effect on egg quality indicators.

Acknowledgment

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DISINFECTANT AND ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF CAMPYLOBACTER COLI FROM SWINE AND PORK CHOPS

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Abstract: *Campylobacter coli* is a Gram-negative bacteria that is a cause of diarrheal disease worldwide, and *Campylobacter* spp. are among the top 5 important foodborne pathogens in the United States. Disinfectants are used throughout the food chain and hospital facilities to control bacteria; on farms, in processing plants, distribution centers, in homes, in veterinary and human doctors' offices and in hospitals. The disinfectant and antimicrobial susceptibility profiles were determined for 111 *C. coli* strains obtained from market age pigs and retail pork chops for 22 disinfectants and 9 antimicrobials. The prevalence of antimicrobial resistance (AMR) for 99 *C. coli* strains from swine samples was for clindamycin (10.1%), azithromycin (12.1%), erythromycin (12.1%) and tetracycline (55.6%), but the highest prev-

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alence of AMR for *C. coli* was from the pork chop samples 33.3, 41.7, 41.7 and 75%, respectively. The highest observed resistance trait was 'TET'. A high prevalence of disinfectant resistance was found in the 99 *C. coli* strains from swine samples to chlorhexidine (88.9%) and triclosan (100%), and resistance to these disinfectants found in the *C. coli* from retail pork chop samples was 91.7% and 100%, respectively. All strains were susceptible to benzalkonium chloride. The benzy ammonium chlorides (BACs) C12, C14 and C16 were the active constituents in the disinfectant DC&R. Didecyldimethyl ammonium chloride (C10AC) was the most active ammonium chloride (AC). Using molar MICs, C14BAC had similar activity to C10AC, then C12BAC and C8AC, with C16BAC being the least active among the ACs. Pyridinium bromide and chloride and the ammonium bromides had similar disinfectant characteristics as the ACs and BACs. Formaldehyde, providone-iodine, Tek-Trol and tris (hydroxylmethyl) nitromethane (THN) had the highest susceptibilities of all chemicals tested. Continued use of formaldehyde and THN may be questionable because these components are not effective, and inclusion in disinfectants would result in additional unwanted chemicals in the environment.

Keywords: Antibiotics, Disinfectants, *Campylobacter coli*, Resistance, Susceptibility

EFFECT OF DIETARY SUPPLEMENTATION WITH THERMOLYSED BREWER'S YEAST ON GROWTH AND STRESS REDUCTION OF LAMBS

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Abstract: The aim of this research was to determine influence of dietary supplementation with thermolysed Brewer's yeast enriched by dietary nucleotides (Ascogen®) on growth performance, blood parameters and stress reduction in fattening lambs. Biological research was conducted on 32 lambs in fattening during 50 days. Lambs were at the age of 60 days. Thermolysed Brewer's yeast (1.5%/DM) was added to the feed mixture of experimental group of lamb. In the lambs´ blood serum biochemical parameters, enzymes activities, thyroid hormones (triiodothyronine-T3 and thyroxine-T4), and cortisol concentrations were determined. Application of thermolysed Brewer's yeast does not affect the growth of lamb, although better average daily gain of lambs (4.31% higher) in the experimental group was determined. Decreased ($P<0.05$) concentrations of calcium, iron and alanine aminotransferase activity were determined in experimental group of lambs, compared to the control group, as well as significant increase of γ-glutamyl transferase, alkaline phosphatase and thyroid T3 hormone activity. Slightly lower concentrations of cortisol was found in experimental group of lambs compared to the control (40.16:47.57 nmol/L), but differences were not significant ($P=0.33$). Determined minor improvements in growth performances and blood parameters changes of lambs indicate slightly anti-stress effect of dietary supplementation with yeast.

Keywords: blood, biochemical parameters, lamb, thermolysed brewer's yeast

Introduction

Application of yeast in animal diets is well known as the applied method used in the improvement of lambs production traits (Haddad and Goussous, 2005; Mašek et al., 2007; Antunović et al., 2010a). However, the impact of nucleotides utilization alone or in combination with yeast, in the alimentary system of sheep is not yet fully established. Nucleotides are found in all feed animal and vegetable origin as free nucleotides and nucleic acids. They are involved in most cellular processes and have an important role in structural, metabolic, energetic and regulatory functions (Lee et al., 2007). They are important for the organism, especially in time of stress state-intensive growth, disease, limited nutrition, etc. (Rossi et al., 2007). Nutritional supplements of nucleotides can improve the immunological properties of young animals (Uauy et al., 1990; Romano et al, 2007; Martinez-Puig et al. 2007). Stress in lambs at weaning is a serious problem and has a significant effect on their growth and development, and it leads to immune system disorders. The increase

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of stress indicators, such as cortisol, is directly associated with physiological stress (Mellor et al., 2002). Cortisol, despite its variability and short life, is still one of the most used indicators of stress. Other blood constituents related to stress are packed-cell volume, glucose, lactate, insulin, free fatty acids and activity of creatine kinase (Shaw and Tume, 1992, Broom, 2003, Tadich et al., 2009). The aim of this research was to determine influence of dietary supplementation with thermolysed Brewer's yeast enriched by dietary nucleotides on growth, blood composition and stress reduction in fattening lambs.

Material and methods

Biological research involved 32 fattening Merinolandschaf breed lambs divided by sex into two groups. Fattening lasted for 50 days. Lambs were at the age of 60 days. Lambs have been nourished, fed with the meadow hay and forage mixtures of the same composition *ad libitum*, except that in the experimental group, where 1.5% Ascogen was added. Ascogen® (Chemoforma, AG, Switzerland) is nutritional supplement containing thermolysed extract of Brewer's yeast (84%), RNA/nucleotides (3.5%) and specific organic acids (12.5%). Lambs were weighted 1st, 25th and 50th day of the trial. After that, daily gain of lambs was calculated. Blood samples (10 ml) were taken at the end of the experimental fattening from the jugular vein in sterile vacuum tubes Venoject® (Leuven, Belgium). Mineral content, biochemical parameters and enzymes activities in blood serum were determined by Olympus AU640. Activity of total T3 and T4 in blood serum was determined by means of duplicate determinations using commercial kits for clinical use in humans (Abbott Laboratories, USA) by Imx-Abbott immunoanalyser. Methods for determination of T3 and T4 were MEIA (Microparticle Enzyme Immunoassay) and FPAI (Fluorescence Polarization Immunoassay). Sensitivity of the assay was less than 0.4 nmol/L (T3) and 12.8 nmol/L (T4). Mean recovery rates were 98.6%. Cortisol was determined by immunoenzymatic electrochemoluminescene (ECLIA method) on an automatic detector Elecsys Roshe 2011. The lower detection limit was 0.50 nmol/L. Each serum sample was tested two times and the mean value of these two measurings was used as the result. Blood samples were analysed in the Laboratory for small ruminants and non-ruminants of the Faculty of Agrobiotechnical Sciences, J. J. Strossmayer University of *Osijek. The research results were processed according to LSD test by the Statistica (2008).*

Results and Discussion

No significant differences regard the effect of dietary supplementation with thermolysed Brewer's yeast enriched by dietary nucleotides on the growth of lambs (body weight and daily gain of lambs; Table 1) were obtained between experimental and control group. However, increase in daily gain of lambs from 1st to 25th day was visible in the experimental compared to the control group for 4.31%. Messke and Merwe 2006. found similar results in cow.

Table 1. Body weight and daily gain of fattening lambs

Indicators	Control (mean ± s)	Experiment (mean ± s)	Significant (P-values)
Initial body weight, kg	19.3 ± 2.49	19.23 ± 2.75	0.84
Body weight 25th day, kg	27.53 ± 3.61	28.10 ± 2.87	0.64
Final body weight, kg	37.11 ± 3.44	37.67 ± 3.91	0.69
Daily gain from 1st to 25th day, g	340 ± 87,83	354.67 ± 35.02	0.55
Daily gain from 25th to 50th day, g	382.93 ± 100.41	382.67 ± 90.03	0.99
Average daily gain (1st to 50th day), g	361.47 ± 53.64	368.67 ± 51.39	0.71

S - standard deviation

Determination of biochemical parameters which are used in evaluation of certain food additives, is required, particularly with a view to modulate health status, growth and weight monitoring of the certain animal categories. Application of thermolysed Brewer's yeast supplement enriched by nucleotides has led to the decreased (P<0.05) concentrations of Ca, Fe, alanine aminotransferase (ALT) activity, and a significant increase in γ-glutamyl transferase (GGT) and alkaline phosphatase (ALP) activity, as well as the hormone T3 activity (Tables 2 and 4). Nucleotides improve iron absorption. Faelli and Esposito (1970) reported that nucleotides improve the iron status in rats, but the mechanism of action was still unclear. Our results are in compliance with Hernell and Lönnerdal's (2002) report that proved lower level of serum Fe in infants fed with milk formula with nucleotides addition, but higher concentration of serum feritin in relation to the infants on the breast. The determination of serum Ca and P is recommended for detection of disorders in calcium and phosphate metabolism. Total serum calcium is influenced by the concentration of total proteins, particularly albumin. As our data showed, there is no disbalance in albumin level in the experimental group of lambs. Therefore, lower level of total calcium is not necessarily a sign of any health disorder. This lover level of calcium is easily influenced by the different nucleotides actions on calcium activated channel (Reale et al., 1994). Riddle et al. (2007) found, for an example, that extra cellular ATP is a prerequisite for fluid flow induced increases in intracellular calcium concentration. In lambs in which the stress is induced by transport, fall in the concentration of glucose in the blood is also determined, and the differences were significant (Tadich et al., 2009). In investigation of Sowinska et al. (2001) with lambs in weaning stress caused, an increase in glucose concentration in 50-day-old lambs (from 2.79 to 3.11 mmol/L) was determined, but not in 100-day-old lambs (2.39 and 2.34 mmol/L before and after weaning, respectively).

Table 2. Concentration of minerals in blood serum of fattening lamb

Minerals, mmol/L	Control (mean ± s)	Experiment (mean ± s)	Significant (P-values)
Ca	3.38^{a} ± 0.21	3.01^{b} ± 0.11	0.013
P-inorganic	4.04 ± 0.25	4.08 ± 0.26	0.82
K	6.00 ± 0.69	6.10 ± 0.40	0.79
Na	172.50 ± 7.26	165.60 ± 3.58	0.09
Cl	123.00 ± 5.52	120.33 ± 2.27	0.35
Fe, μmol/L	50.05^{a} ± 9.63	36.80^{b} ± 4.67	0.03

a,b- (P<0.05); s-standard deviation

Table 3. Concentration of biochemical indicators in blood of fattening lamb

Indicators, mmol/L	Control ($\overline{X}$ ±s)	Experiment ($\overline{X}$ ±s)	Significant (P-values)
Glucose	5.33 ± 0.30	5.10 ± 0.40	0.34
Urea	10.63 ± 1.70	9.51 ± 0.93	0.31
Creatinin	74.25 ± 6.98	70.00 ± 1.87	0.23
Total proteins, g/L	70.57 ± 5.60	68.70 ± 2.93	0.53
Albumins, g/L	32.60 ± 1.22	32.00 ± 1.48	0.50
Triglycerides	0.35 ± 0.05	0.37 ± 0.09	0.66
Cholesterol	1.33 ± 0.23	1.38 ± 0.57	0.85

s-standard deviation

In the experiment with sheep and lambs Zomborszky-Kovacs et al. (1998) found similar results with the Ascogen addition. They noticed the same concentration of total protein, urea, albumins and decreased ALT activities. The same authors showed that the decrease in urea can be connected with the increase of microbial protein synthesis with regard to the stimulating effect of yeast in feed on growth of rumen microorganisms. To similar results in the research with red deer came Zomborszky et al. (1996) where they found a significant decrease in ALT activity, and a minor decrease in blood glucose levels, total protein, activities of creatine kinase (CK) and lactate dehydrogenase (LDH). The authors show that the application of Ascogen preparation operated antistress. In lambs in which the stress induced transport was also found decreased CK activity in the blood, and the differences were significant (Tadich et al., 2009). Frankič et al. 2006. found decreased ALT and AST activities in the blood of poultry when nucleotides were added. Higher ALP activity indicates increased growth of the experimental lambs, which showed a higher osteoblasts activity and later development of muscle. Chiofalo et al. (2004) determined similar results with goat kids in experiment with the addition of probiotics Bios. Increase in triiodthyronin (T3) content indicates intense metabolic processes and possible faster recovery of the experimental lamb group, which can be connected to somewhat better daily gain, especially during the first fattening period. In the lambs of experimental group that consumed the mixture with the addition of thermolysed Brewer's yeast supplement enriched by nucleotides were found slightly lower concentrations of cortisol, compared to the control group (40.16 and 47.57 nmol/L), but differences were not significant (P=0.33). This indicates a minor anti-stress effect in lambs. In investigation of Sowinska et al. (2009 and 2001) was determined similar blood concentration of cortisol in lambs during weaning. However, authors determined significance lower cortisol concentration in lambs during 100th day of weaning in comparison to 50th day of weaning. Similar content of thyroid hormones (T3 and T4) in blood of fattening lambs determined Antunović et al. (2010b).

Table 4. Enzyme activities and hormones in fattening lamb

Enzymes, U/L	Control ($\overline{X}$ ±s)	Experiment ($\overline{X}$ ±s)	Significant (P-values)
Aspartate aminotransferase – AST	130.85 ± 25.88	108.67 ± 7.22	0.10
Alanine aminotransferase – ALT	19.25^{a} ± 6.42	11.33^{b} ± 1.29	0.03
Alkaline phosphatase – ALP	561.80^{a} ± 45.77	626.33^{b} ± 19.27	0.02
Creatin kinase – CK	271.34 ± 124.28	204.81 ± 77.96	0.15
γ-glutamyl transferase – GGT	91.50^{A} ± 4.92	115.67^{B} ± 3.63	0.001
Lactate dehydrogenase – LDH	425.25 ± 57.69	413.00 ± 40.24	0.71
$T_{3,}$ nmol/L	1.48^{a} ± 0.16	1.89^{b} ± 0.31	0.03
$T_{4,}$ nmol/L	102.35 ± 8.97	95.75 ± 3.62	0.17
Cortisol, nmol/L	47.57 ± 18.49	40.16 ± 20.03	0.33

A,B- (P<0.01); a,b- (P<0.05); s-standard deviation

Conclusion

Application of thermolysed brewer's yeast enriched by dietary nucleotides did not affect the growth performance of lambs. Concentrations of calcium, iron and alanine aminotransferase activity in blood decreased, while γ-glutamyl transferase, alkaline phosphatase and thyroid T3 hormone activity increased in lambs´ blood of experimental group. Mentioned changes indicate slightly moderate anti-stress impact of thermolysed Brewer's yeast supplement enriched by nucleotides in food of fattening lambs.

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QUALITY OF WORK OF ROTARY CUTTING MOWERS WITH OSCILLATOR-PERMITTED REPAIR APPARATUS IN CUTTING THE LIVESTOCK PEAS

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Abstract: Livestock peas is an excellent quality fodder for all kinds and categories of domestic animals, because it is characterized by high content of raw proteins of 20-22%, vitamins and minerals, and can be used as well as a green mass for preparing of hay and silage. For the hay preparation, the best mowing is to be done in the full flowering phase when the lower legumes begin to form. Good results are being achieved with respect to the optimal mowing period. The paper presents results of testing the quality of rotary and mowers with oscillatory-supporting cutting device for mowing peas in the exploitation conditions of Toplica district. The aim of research was to determine the quality of operation of mowers with different cutting devices in the specific operation mode; the influence of the type of the cutting device on the losses and the height of the cut during the cutting of the cattle peas. The obtained results indicate that there is a significant influence of the change in the regime of mowers and the defined parameters, on quality of the work of the examined mowers. The highest cut height was measured when mowing the crop with rotation mower with disc blades type R1 - 9.10 cm, and the lowest with a classic mower with two cutting blades type C2 - 4.93 cm. Total losses after mowing ranged from 1.26 to 4.48%.

Keywords: mowers, cutting device, cut height, losses, livestock peas.

Introduction

In livestock feeding, different leguminous plants are being used. Areas under one-year fodder leguminous plants in Serbia occupy about 30000 ha (Karagić et al., 2011). Livestock feed production is one of the cheapest, best-quality and most cost-effective forms of livestock feed production in arable land in various agro-ecological conditions, especially considering that it belongs to high productive crops because it produces 40-60 t ha-1 of green fodder, and 8- 11 t ha-1 of hay. Thanking to the ability to cultivate in different periods of the year, as well as constant high yields and high content of proteins of good biological value, these plants can successfully replace some other nutrients like perennial leguminoses (Samarappuli et al., 2014; Đorđević et al., 2018). Period of exploitation, yield and quality of the leguminous hay depends on the height and method of mowing during the vegetation, while high mowing results in a higher loss of nutrients and the production of less quality feed (Crasi et al., 2001; Kallenbach et al., 2002 Nagy, 2003; Veronesi et al., 2006). Gottfried (2008) emphasises constructive solution mower from the standpoint of simplicity, ease of maintenance, functionality, reliability which are important for the quality of mowing. Wiersma

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et al.,(2001) recommended mowing of leguminous plants at a height of 5.08 cm in order to reduce losses. The lowest cutting height was measured at mowing with a classic mower on average 6.42 cm, and the highest in rotary mowers, and 7.50 cm with 6 disc-blades mower and 7.47 cm with 14 disks (Potkonjak et al., 2009). Jugović et al., 2013. finds that the lowest cutting height of 5.15 cm is achieved at the aggregate speed of 5.89 km h-1 and the maximum of 6.50 cm, at a speed of 9.29 km h-1. The same authors report that the losses appeared as a result of the cut height of 2.65%, while the losses accumulated due to the crushing mass decreased as the result of increase the speed of movement from 1.06 to 0.82%. Vuković et al., (2015) point out that the highest total losses were measured by mowing with a rotary mower with drums of 3.04%, with losses due to the crushing of the mass of 1.86% and the cutting height of 1.18%. According to the same authors, the lowest overall losses were measured with an oscillating mower of 1.25% with loss due to crushing by 0.40% and a cutting height of 0.85%.

Material and Methods

During the year 2018, in the agro-ecological conditions of the Toplica district 43°14'28.2"N 21°38'18.0"E, the quality of the work of various mowers during the mowing of winter crop was performed. Rotary mower with discs Vicon CM 2400-type R1, rotary mower with drums Celmak RDM 195 - type R2, classical oscillator mower IMT 627.027 - type C1 and oscillator mowers with two sticks FMP 627.794 - type C2. The plot was planted with the winter pea NS Mraz (85-90%) in a mixture with triticale NS Odyssey (10-15%). In the first phase the yield of livestock pea was determined by taking samples from 1 m2 in diagonal of the plot and then calculated to an area of 1 ha. The average yield of green mass was about 45.3 t ha-1, and hay 8.4 t ha-1. In the next phase of the study, the quality of the work of various mowers in the mowing of livestock pea was determined. Work quality, included measuring the cut height, cutting height losses and cutting speed. The height of the cut is determined at the point of determining the losses by measuring the cutting height of the livestock pear on the appropriate rectangular surface. The mowing losses were measured from a surface of one meter of length, in the width of the wor width of the appropriate type of test machine with a rectangular frame, in the same place where the cut height was determined. Total losses are presented as the sum of losses caused by cutting height and losses caused by the crushing of plant mass. All values were considered in five repetitions. The speed of work is determined by chronometry, measuring at 50 m long tracks, as well as the basic time on the total length of the plot of 90 m. For the mower type R1 the operating speeds were: 9.23, 9.61, 10.14, 10.84 and 11.93 km h-1, for type R2: 8.36, 9.18, 9.67, 10.29, and 10.43 km h-1, for type C1: 4.93, 5.48, 5.71 and 5.86 km h-1 and for mower type C2: 5.83, 6.17, 6.94. 7.83 and 8.95 km h-1. Mowers were operated as an aggregate with tractors of 30, 43 and 47 kW.

Results and Discussion

The cutting height of stems is of high importance to the outcome of the production process, as it essentially affects its efficiency, or loss of crop mowing. Charts 1 and 2, show the influence of changes of the operating speed regime, on the cutting height of the stem of winter livestock peas, at different working speeds of the examined mowers.

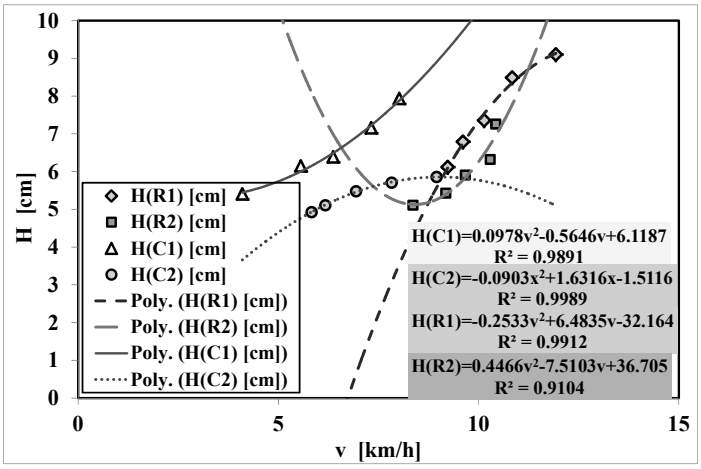

Figure 1. The dependence of the height of the mowing of the livestock peas stem on the speed of movement of the examined mowers of the described with second-line parabolas

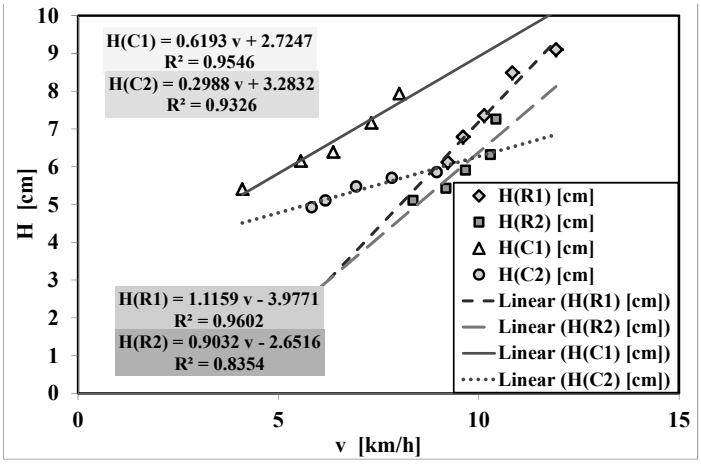

Figure 2. Dependence of the cut height of the livestock peas stems, on related to the speed of movement expressed by linear functions

On the basis of obtained results, it is obvious that in all the examined mowers the cutting height increased with the increase of the operating speed. The lowest cutting height was measured with a conventional oscillatory moqwer with two cutting blades Type C2- 4.93 cm, with operating speed of 5.83 km h-1, and the highest in rotary mowers with a disc blades Type R1- 9.10 cm at a working speed of 11.93 km h-1.

Chart 1 shows that these interdependencies are precisely described by parabolic functions of the second line. Correlation indices in all cases high values, over 0.9.

The small values of the coefficients with the square points in the model functions indicate the possible application of linear functions for describing the connections, shown in Figure 2. In this case as well, in addition to the mowers type R2 (coefficient of correlation 0.84), the correlation coefficients in all cases gains high values, over 0.9 (Fig. 2).

Charts 3, 4 and 5 show the values of the loss at mowing of winter livestock peas with the examined mowers.

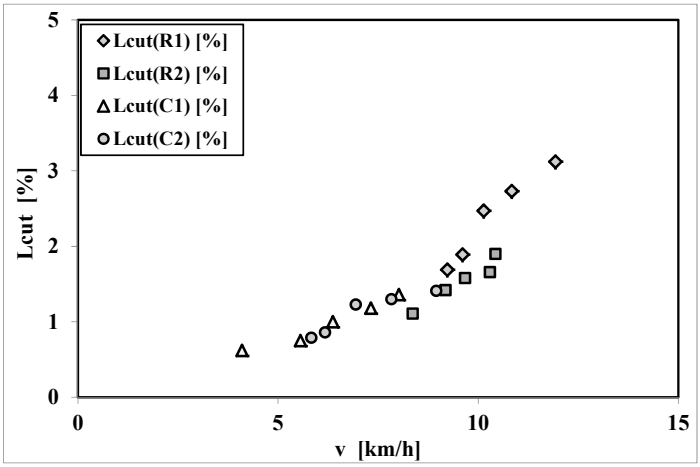

Figure 3. Interdependence of losses caused by the cutting height of livestock peas and the speed of movement of the tested mowers

Based on the results in Figure 3, it is noted that the minimum loss values due to the cutting height were measured with classic mowers Type-C1, within the values of 0.62-1.36% of the yield of winter peas. Working speed was 4.10 and 8.02 km h-1. The highest values of losses due to the cutting height in relation to all examined mowers were measured in rotary mowers with disc blades Type R1. These were in the range of 1.69-3.12%, at operating speeds of 9.23 and 11.93 km h-1 (Figure 3).

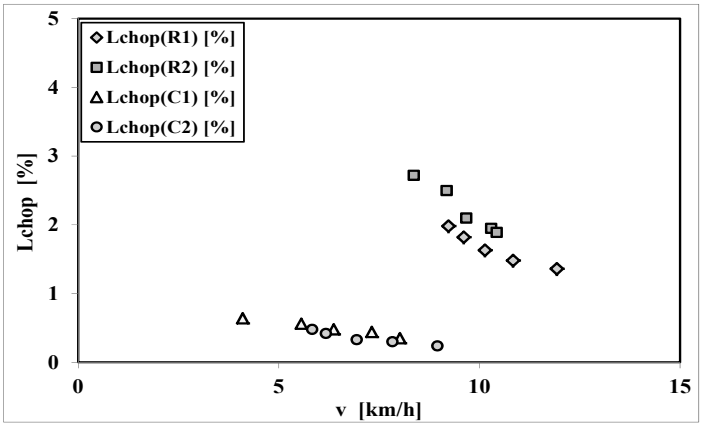

Figure 4. The interdependence of losses caused by crushing the pea stalks and the speed of the movement of the tested mowers

Considering mowing losses of winter grain produced by crushing, it can be noticed that the lowest values were measured when using an oscillator mower with two cutting blades (Type C2) and ranged from 0.24-0.48% of the livestock peas, in speed of 8.95, or 5.83 km h-1). The highest loss

values due to the crushing of the mowing mass with the examined mowers were measured at rotary mowers with drums (Type R2), so they ranged from 1.89 to 2.72% at operating speeds of 10.43 and 8.36 km h-1 (Figures 4).

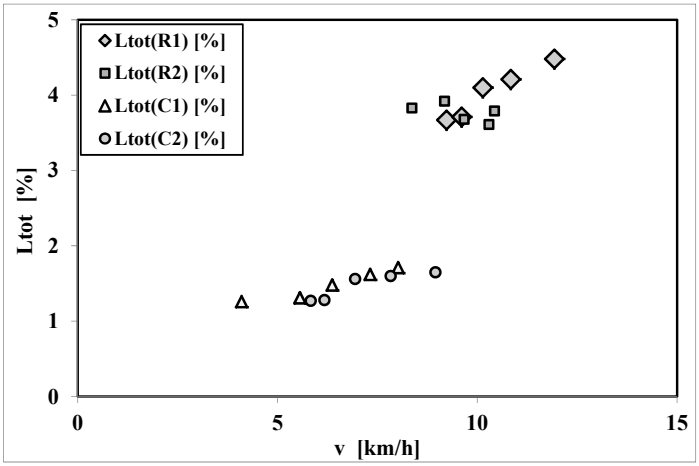

Figure 5: Interdependence of total livestock losses and speed of movement of the tested mowers

Total mowing losses are an important indicator of the quality of work, and represent the sum of the losses caused by cutting height and losses due to the crushing of the mown masses after cutting. The lowest value of the total loss of mowing of winter livestock peas was carried out during the operation of the classical oscillator mower - Type C1 and amounted up to 1.26% of the yield of winter livestock peas (operating speed of 4.10 km h-1). The highest values of total losses were measured during mowing with rotary drum mower Type R2 - 4.48%, operating speed of 11.93 km h-1 (Figure 5).

The quality of mowing was also analyzed by other authors who, in their studies, give similar results (Wiersma et al., 2001, Kallenbach et al., 2002, Nagy, 2003, Veronesi et al., 2006; Potkonjak et al., 2009; Jugović et al., 2013, Vuković et al., 2015).

Conclusion

Based on the obtained results, it can be concluded that there is a significant influence of the change of the defined parameters on the quality of the work of the examined mowers. With all the examined mowers the cutting height of mowing was increased with the increase in operating speed. The lowest cutting height was measured with a conventional oscillatory mower with two cutting blades Type C2- 4.93 cm, and the highest in rotary mowers with a disc Type R1- 9.10 cm. The small values of the coefficients with the square points in the model functions indicate the possible application of linear functions for describing the links, and the coefficients of correlation besides mower R2 (coefficient of correlation 0.84), in all cases reach high values over 0.9. The lowest values of losses due to cutting height compared to all examined mowers were measured in the type Class C1 of oscillatory mower, amounting to 0.62% , and the highest in rotary mowers with type R1, which is 3.12%. The lowest values of total losses in amount of 1.26% were measured in the operation of

the classic oscillator mower - Type C1, while the highest values of total losses were measured with mowing with rotary mowers with drums Type R2 - 4.48%. Bearing in mind that the upper limit of total losses is 5%, the general conclusion is that the examined mowers can be successfully used for mowing the winter livestock peas in the observed agro-ecological area.

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THE IMPORTANCE OF ANTIOXIDANTS IN THE HEALTH PROTECTION, PRODUCTIVE AND REPRODUCTIVE TRAITS OF FARM ANIMALS

Davidović V.*[1], Stojanović B.[1], Božičković I.[1], Savić Radovanović R.[2], Bojanić Rašović M.[3], Jovetić B.[4]

Abstract: The exposure of cells to reactive oxygen species leads to damage of membranous structures, lipid peroxidation, decreased activity of enzymes and other proteins, and genetic mutations. Dependence between the consumption of oxygen and production of reactive oxygen species depends on the physiological state of the individuals. Enhancement of the metabolic processes in lactation, increased physical activity and thermal stress lead to increased production of these metabolites. Antioxidants are substances which can postpone, prevent or decrease oxidative damage of target molecules and they act via enzyme and non-enzyme mechanisms. Imbalance between the velocity of production of reactive oxidative metabolites and their neutralisation by antioxidants leads organism into a state of oxidative stress which contributes to the development of postpartum diseases and can cause metabolic disorders.

Keywords: antioxidants, health, productive and reproductive traits

Introduction

Free radicals include reactive oxygen species (ROS), reactive nitrogen species (RNS) and non-reactive radical agents, the free radicals originating from the oxygen of the most important reactive species which are being generated in the organism. Reactive oxygen species are the products of natural metabolism of oxygen which are being continually formed in the cells as a result of oxidative biochemical reactions. Primarily they are formed as the end products of mitochondrial respiratory chain or are being catalised by activated NADPH oxidase after intake of pathogenic agents. The most important forms of ROS are: superoxid anion ($O_2^{\cdot}$), hydrogen peroxid (H_2O_2), hypochlorous acid (HClO) and the most reactive hydroxyl radical ($OH^{\cdot}$) (Sordillo and Aitken, 2009). Superoxid radical is being produced mostly in mitochondria, it goes through their membrane and reacts with DNA molecules damaging purine and pyrimidine bases and deoxyribose. Such a prolonged oxidative damages cn result in mutagenesis and carcinogenesis (Valko et al., 2004). Hydrogen peroxid is produced in peroxizomes but these organelles contain enzyme which can neutralise it so that they maintain balance between its production and neutralisation. However, when peroxizomes are damaged this free radical can enter cytoplasm and cause different pathological conditions.

At low concentrations, reactive oxygen species exhibit useful effect in response to agents of infective and non-infective nature, while high concentrations are harmful to biological molecules what is called an oxidative stress (Ronchi et al., 2000). Uncontrolled oxidation can cause damages

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of cell structures and tissues, degenerative processes associated with aging and many diseases (atherosclerosis, diabetes, rheumatoid arthritis), and ROS are also potential cancerogenes because they can facilitate mutagenesis, development and progressing of tumour.

In physiological and stressful conditions free radicals have important functions in the activity of the effectors of cell defence, cell signalisation, apoptosis and mobilisation of ions transport system. During the action of stress factors (UV radiation, exposure to heat, radioactive substances, cyclic xenobiotics, drugs) or in pathological and degenerative conditions, cellular level of ROS/RNS significantly increases. Increased production of ROS which have a role in destruction of bacteria during phagocytosis is generated during inflammatory process due to action of neutrophiles and synthesis of prostaglandines. Polymorphonuclear neutrophiles (PMN) get activated after internalisation of pathogens wherein the oxygen, under the effect of nicotinamide dinucleotide phosphate (NADPH) becomes superoxid radical. This functional response, called oxidative or respiratory burst, together with cells migration and degranulation contributes to host defence but can also lead to colateral inflammatory tissue injury (Chen and Junger, 2012). There are several congenital disorders (for example chronic granulomatosis disease), in which the creation of oxidative burst is prevented due to inability of neutrophile granulocites to properly gather multiprotein NADPH oxidasis complex on cellular membranes during activation (Hager et al., 2010).

Oxidative stress – postpartum diseases

Peripartum period in dairy cows is the most important stage during lactation cycle because numerous physiological, metabolic and nutritive changes occur at that time by action of a number of physiological and external factors during which the function of immune system decreases. Metabolic disorders and change in the level of some hormones (glucocorticosteroid, estradiol and progesterone) most likely affect the decrease of immune function (Waldron and Revelo, 2008). The way in which changes happen and develop has a great effect on lactation performances, subclinical and clinical postpartum diseases, reproductive disorders and therefore on the efficiency of an individual (Joksimović and Davidović, 2012, 2013a). The cause of peripartum immunosupression is not known but it is assumed that immune disfunction is caused by metabolic and endocrine factors (negative energy balance, unsaturated fatty acids, ketone bodies and hypocalcaemia) (Kimura et al., 2006; Waldron, 2010). Ketosis can increase the possibility of the occurrence of mastitis in cows with immunosupression because immune cells suffer negative effects and immune mechanism of mammary gland are weakened. In cows with ketosis blastogenesis of lymphocites is supremated, the activity of respiratory burst decreases and chaemotaxic ability of neutrophiles and the titre of γ-interferon and TNF-α in endothelial aorta cell in cattle is reduced (Suriyasathaporn et al., 2000a).

The occurrence of oxidative stress is associated with disorders of health state in high yielding dairy cows such as mastitis, retention of placenta and metabolic disorders (Ronchi et al., 2000). Physiological stress during transitional period is associated with rapid differentiation of secretory parenchyma and the growth of mammary gland in the beginning of copious synthesis and milk secretion what implies very high energy needs and increased demand for oxygen. These increased requirements for oxygen lead to increased production of ROS while oxygen, although indispensable, is, at the same time, very toxic, what can be marked as "oxygen paradox" (Sharma et al., 2011). Research by Lohre et al. (2005) showed that cows with higher milk production also have higher concentration of hydroperoxide in serum compared to individuals with lower production. In

cows with higher body mass at calving and substantial loss of weight after calving the production of ROS is increased (Bernabucci et al., 2005). Balance between the production of reactive oxidative metabolites and antioxidative protective system is shifted to the advantage of oxidative status what results in oxidative stress.

Both forms of mastitis, subclinical and clinical can provoke increased production of free radicals and decrease in antioxidative capacity, leading to oxidative stress, particularly in the beginning of lactation (Atakisi et al., 2010). In the course of inflammatory process, epithelial cells of mammary gland and mononuclear phagocyte stimulated by lipopolisaccharides and cytokines produce superoxid anion and nitric oxide ($NO^{\cdot}$) and in that way they intensify oxidative reaction (Boulanger et al., 2001). By the action of reactive oxygen species there occur injuries of protein and polyunsaturated fatty acid along with the death of cell by apoptosis or necrosis and structural damages of metabolically active tissues (De Nigris et al., 2001; Lykkesfeldt and Svendsen, 2007). Proteins are liable to oxidative modification and as a final product of lipid peroxidation there occurs cytotoxical malonildialdehyde (MDA) which has a mutagenic and cancerigenic effect and can be alternatively used as a marker of oxidative stress in mammary gland. Adela et al. 2006. determined the highest level of MDA in serum of dairy cows in the first week of lactation (62 μmol/l), and the lowest in the sixth week (35 μmol/l) what confirms that the most intensive lipolysis occurs postpartum because in that period the activity of antioxidative enzymes of superoxid dismutases (SOD), glutathione peroxidasis (GSH-Px) and catalase (CAT) is the lowest.

In more severe forms of mastitis a huge quantity of peroxynitrite ($ONOO^{\cdot}$) which is produced by the oxidation of NO is being released (Beckman et al., 1990). Abrupt releasing of this reactive nitrogen metabolite can have as a consequence disturbance in the balance of antioxidants (Chaiyotwittayakun et al., 2002) and oxidative violation of secretion of mamary gland (Atakisi et al., 2010). Somatic cell count (SCC) is an indicator of health status of mamary gland and one of parameters for estimating the milk quality (Suriyasathaporn et al., 2000b). A positive correlation between SCC and production of nitric oxide (Atakisi et al., 2010) has been determined as well as the increase in somatic cell count in milk what is associated with increase in MDA level (Adela et al., 2006). Excessive oxidative reactions can cause degenerative changes in mammary gland tissue and decreased quantity of milk in the case of increased somatic cell count what can be a consequence of increased level of oxidative stress (Suriyasathaporn et al., 2009; Joksimović and Davidović, 2007a). By intensifying the inflammatory processes and development of disease into a chronic state, greater quantity of free radicals are being released what is followed by increased oxidative damage of tissue, peroxidation of lipids and oxidative stress (Savić Radovanović et al., 2017).

Antioxidative status is one of the factors which affect reproductive functions of dairy cows (Davidović et al., 2016). Antioxidants deficit leads to reproductive disorders: placenta retention, ovarian cysts, metritis, decreased rate of conception and prolongation of service period, abortion, delivery of non-viable and still born calves (Formigoni et al., 2011). Placenta retention is a postpartum disease in dairy cows, which has harmful a impact on reproduction, milk yield and health of mammary gland. All this leads to shortening of production period in dairy cows and need for extra expenditures. The incidence of placenta retention in postpartum cows accounts for 4-18% (Han and Kim, 2005), while absence of leukocytes leads to placenta retention in 100% dairy cows (Putnam and Comben, 1987). The aetilogy implies both mechanical and nutritive factors, inadequate management (uncomfortable conditions) and infective diseases and oxidative stres which can also contribute to placenta retention (De Rensis and Scaramuzzi, 2003; Grummer et al., 2004; Gunay et

al., 2011). By addition of antioxidants in food the occurrence of placenta retention is reduced if it is not caused by mechanical and pathological factors this suggests that oxidative stress is one of the factors responsible for development of this disease (Julien and Conrad, 1976). An increased content of malonildialdehide in erythrocytes and increased concentration of cortisole are considered as major indicators of developing placenta retention. Decreased activity of mieloperoxidases in cotyledon indicates a decreased function of neutrophiles and high activity of lysosome and acid phosphatase in placenta retention indicates acute inflammatory reaction of fetomaternal connection (Gupta et al., 2005).

Antioxidative immune system – antioxidants

Antioxidative protection can be realised at several levels – by inhibition of production and releasing of ROS or removing of oxygen, catalyst ions of metal or ROS (Halliwell and Gutteridege, 2007; Apetrei et al., 2011). Antioxidants donate an electron to oxidants preventing in that way their reactivity and the damage of biological macromolecules. During this process the antioxidants become radicals but they are stable and cause no damage to cells and in time they move into an active reduced state (Lykkesfelat et al., 2003). If antioxidants are defficient the causative agents of oxidative stress can affect metabolic and physiological processes, damage cellular lypids, proteins or DNA, and in that way endanger functions of cells, health state and production and reproductive abilities of an individual. Damage of DNA leads to mutations, translatory errors, inhibition of protein synthesis while injured proteins can cause modifications in ions transport and changes in enzyme activity (Spears and Weiss, 2008).

Preventive antioxidative immune system acts through enzyme (superoxide dismutase, glutathione peroxidase, catalase, lactoperoxidase, thioredoxin reductase and peroxiredoxin) and non-enzyme mechanisms (vitamin E, selenium, ascorbic acid, glutathione, heat shock proteins, ubihinon, ß-carotene, flavonoids). The most important antioxidant in cytoplasm is tripeptid glutathione (GSH) which directly donates hydrogen to reactive oxygen species and reduces hydrogen peroxid and other peroxides in the water (Arthur, 2001). Adela et al. 2006. determined that in cows in the first week postpartum there occurred a significant fall in the acitivity of enzyme superoxid dismutases, glutathione peroxidase and catalase while in the second week of lactation there occurred a significant increase what can be explained as an organism reaction to high levels of ROS. Membranous antioxidants are vitamin E, beta carotene and coenzyme Q. Metals (copper, zinc and manganese) are not strict antioxidants but their concentration and utilisation can affect redox status (Sharma et al., 2011). Zinc has an important role in DNA and RNA synthesis by increasing replication and cell proliferation, as well as catalytic, structural and regulatory function (Spears and Weiss, 2008). It affects reproductive functions, secretion of gonadotropine, androgens, prostoglandin and prolactin, and also that of antioxidants (Arthur, 2001).

The use of antioxidants in dairy cows promotes the health of mammary gland, decrease oxidative damages of mammary tissue, enhances the resistance to mastitis, has a favourable effect on chemotactic activity and phagocytic capacity of neutrophiles (Spears and Weiss, 2008; Davidović et al., 2017). Precise mechanisms into the improvement of health of mammary gland are not completely known but they are associated with their antioxidative functions (Joksimović Todorović and Davidović, 2007b; Joksimović Todorović et al., 2012). Kellogg et al. (2004) report that zinc-methionine can significantly increase lactation performance and improve health of mammary gland since

it reduces the somatic cell count by 33.3%. The results obtained in study Davidović et al. (2014, 2015) indicate that addition of different forms of zinc or selenium in dairy cows rations, starting from the twentieth day before calving up to the sixtieth day of lactation, can lead to the increase of this nutrients content in blood and milk. Addition of antioxidants into food reduces the frequency of the incidence of uterus infection and placenta retention in dairy cows since the number of leukocytes in uterus and placenta increases, prompts leukocyte chemotaxis, helps to weaken the links between fetomaternal relationship and placental expulsion. Many studies indicate that adequate levels of selenium, copper, zins and certain vitamins, which play a role of antioxidants, can decrease the percent of individuals with retained placenta disease (Tillard et al., 2008, Sharma et al., 2011, Joksimović Todorović and Davidović, 2007a, 2013a,b; Joksimović Todorović et al., 2016). Fifty years ago Trinder et al. (1969) determined an increased number of cases of placenta retention in dairy cows with nutritive muscular dystrophy and reduced the number of diseased animals by adding the selenium and vitamin E in the food. These authors reported that selenium and vitamin E feeds supplements solved the incidence of muscular dystrophy and placenta retention. Julien et al. (1976) prevented the incidence of placenta retention in dairy cows by injecting 50 mg Na_2SeO_3 and 680 IU vitamin E) about three weeks prepartum or by administering 0.92 mg Se/kg in the form of Na_2SeO_3, daily, 60 days prepartum. Michal et al. (1994) determined that supplementing 300-600 mg/day vitamin A in the food can decrease the frequency of the incidence of metritis by about 10%. The adequate levels of copper in food are indispensable for optimization of immune system, since copper reduces the occurrence of development of metabolic and oxidative stress in dairy cows (Cortinhas et al., 2010).

Key food ingredients which function as oxidative protection are selenium and vitamin E. This vitamin is highly reduction substance, it is the major antioxidans in plasma membranes and represents the first line of cell defence against free radicals (Wang and Quinn, 2000). The supplementation of these antioxidants increases the level of vitamin E in erythrocytes, neutrophiles and plasma along with the activity of enzyme of glutathione peroxidase (Joksimović and Davidović, 2007b, 2013b). Selenium plays its biological role in the organism through enzyme glutathione peroxidase (GSH–Px) which is localised in cytosol and uses glutathione reduction potential (Arthur, 2001). The activity of the enzyme glutathione peroxidases increases in plasma with the increase of its level in the food or water what can be used as a reliable indicator of biological adoption of selenium and antioxidative status of the organism (Joksimović Todorović and Davidović, 2005). During the increase of the selenium levels above needed ones the activity of GSH–Px shows the plateau effect so that levels above 5 mgSe/day do not lead to further increase in the activity of this enzyme (Weiss et al., 1990; Joksimović Todorović and Davidović, 2005). The concentration of Se in tissue is in high correlation with the activity of GSH-Px and directly depends on selenium intake.

Besides the effect on production and reproductive characteristics selenium and vitamin E have an important role in immunological reactivity of organism. These nutrients increase migration of neutrophile granulocytes into infected parts of udders where they phagocyte and destroy present bacteria (Hogan et al., 1993). Allison and Laven 2000. determined that vitamin E, but not Na-selenit, enhances phagocytosis opsonized bacteria *Staphyloccocus aureus.* In the trial of Weiss et al. (1997) it was determined that doses of vitamin E 1000 IU/day for the first 46 days of dry period, 4000 IU/day during the last 14 days of dry period and 2000 IU/day during lactation can decrease the incidence of novel udder infections. Biological availability of selenium represents a quantitative expression of biological utilization of this nutrient from different sources. Organic forms of selenium

(selenised yeast) have an advantage over inorganic forms (selenite and selenate), due to better absorption, faster storage and retention in tissues, lower toxicity. Selenocysteine has somewhat higher and selenomethionine 3 to 4 times higher biological utilization than sodium selenite (Joksimović Todorović et al., 2007a,b). It is thought that levels of 0.2 or 0.3 mgSe/kg food administered 60 days before calving can successfully prevent the incidence of subclinical mastitis and promote its healing (Weiss and Hogan, 2005). The results of the research of these authors indicate that concentration of selenium in the serum of cows at calving and at 28th day in milk was 1.4 times higher in cows fed 0.3 mgSe/kg food in the form of organic selenium (selenised yeast) compared to cows which received the same quantity of inorganic selenium (Na-selenite). Addition of 0.2 mgSe/kg food in the form of selenized yeast daily during 8 weeks in dairy cows has led to significant increase in the activity of GSH-Px compared to control while the infected udders quarters were free from the presence of pathogenic bacteria (Malbe et al., 2003). These authors reported that level of GSH-Px of 3.3 μkat/g haemoglobin in cows keeps the mammary gland healthy and that animals with lower activity of this selenoenzyme are more susceptible to infection and frequent ailments caused by different agents. Jukola et al. (1996) determined that concentration of selenium in blood of 200 pg/L is an adequate level which provides healthy mammary gland.

A low level of glutathione peroxidases in blood can provoke incidence of anestrus or subestrus as well as ovarian cysts (Jukola et al., 1996). Administration of selenium by injection in dry period or vitamin E in the last third of gravidity can prevent the occurrence of ovarian cysts and metritis in dairy cows. Ovarian cysts were diagnosed in 19% cows treated by selenium compared to 47% animals untreated with selenium (Harrison et al., 1984). Erskine et al. (1997) also determined that administration of vitamin E by injection 8-14 days prepartum reduces frequency of occurrence of metritis from 9 to 4%. However, contrary to these studies, Le Blanc et al. (2002) and Bourne et al. (2008) did not determine that once and/or twice administered injections of vitamin E and selenium prepartus may prevent metritis and endometritis.

Conclusion

The absence of adequate control of production of free radicals in metabolically active tissues can result in development of oxidative stress and health disorders in dairy cows. Natural (polyphenols, flavonoids, vitamins, carotenoids and other bioactive plant components) and synthetic compounds which have the ability to decrease generating and releasing and/or to bind, inhibit or remove reactive oxygen species, can have a therapeutic potential in reducing oxidative damages created by excessive ROS generating. For the purpose of preventing the occurrence of oxidative stress and maintaining the oxidative balance it is necessary to enhance immune status in dairy cows via properly balanced nutrition and supplementation of indispensable nutritive components of approved quality, maximum protection against pathogens, hygiene and preserving the tissue integrity by optimal functioning of antioxidative system.

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EFFECT OF BEDDING MATERIAL ON OCCURRENCE OF FOOTPAD DERMATITIS IN BROILER CHICKENS

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Abstract: The study was conducted to evaluate the impact of two types of litter (chopped straw or pelleted straw) on occurrence and severity of footpad dermatitis (FPD) in broilers. A total of 296 Ross 308 broilers were randomly allocated to two treatments with four replicates. Each replicate consisted of 37 as hatched birds per pen. The litter quality (moisture content, pH, litter score) and incidence of footpad dermatitis were evaluated at the age of 4 and 6-weeks. The litter quality was evaluated by scoring method on the scale from 1 to 5 (1-dry and friable litter; 2- friable but slightly wet; 3- friable by some cake formation; 4-fully caked but friable litter beneath; 5-totally caked and wet). The incidence and severity of FPD were measured by the method described by Eichner (2007).

There were no significant differences in litter scores between bedding materials. The moisture content was higher in chopped straw compared to pelleted straw (55.17 vs 52.18 at 4 weeks and 53.08 vs. 50.78 at 6 weeks). The pH of the litter was significantly higher ($P<0.05$) in chopped straw at 4 weeks of age (8.14 vs. 7.38). The FPD score was significantly lower ($P<0.01$) in groups with pelleted straw. At 4 weeks of age average FPD score was 0.59 in pelleted straw group and 0.96 in chopped straw group. At 6 weeks of age the average score was 1.81 in pelleted straw and 2.34 in chopped straw.

In conclusion, the results showed that pelleted straw significantly lowered the incidence of footpad dermatitis in broilers.

Keywords: broilers, FPD, litter, moisture, pH

Introduction

Litter quality is one of the most important factors in poultry production. It plays an important role in moisture absorption and thermal insulation and has a significant influence on health status, productive parameters, carcass quality, and welfare of broilers (Grimes et al., 2002; Bilgili et al., 2009; Garcês et al., 2013). A wide range of materials could be used as litter for broilers but in Central and Eastern Europe the most commonly used material is wheat straw despite of its rather poor characteristics (Meluzzi et al., 2008; Shepherd and Fairchild, 2010, Bjedov et al., 2015). However, there are some treatments that could contribute to improvement of its characteristics like chopping or pelleting (Bjedov et al., 2015, Đukić Stojčić et al., 2016).

Litter moisture and pH are very important factors because bacteria-generated NH3 dissolves at a high moisture level (Meluzzi et al., 2008; Allain et al., 2009; Bjedov et al., 2013) and forms an

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alkaline solution that acts as an irritant to the footpads. The footpad condition is a significant aspect of poultry welfare that in severe cases can cause pain (Berg, 2004), reduced weight gain and changes in behavior of birds (Žikić et al., 2017). According to Grimes et al., (2002) the different particle size of litter material seems to be an important contributing factor in the development of footpad dermatitis (FPD), but further evidence is needed to confirm if the pelleting of the straw could positively influence the paw quality.

The aim of this study was to evaluate the properties of different litter types (pH, moisture content, litter score) and to examine the influence of litter types on occurrence and severity of footpad dermatitis (FPD) in broilers.

Material and Methods

The experiment was conducted at the Experimental farm of the University of Novi Sad, Faculty of Agriculture. A total of 296 Ross 308 broilers were randomly allocated to two treatments with four replicates. Each replicate consisted of 37 as hatched birds per pen with stocking density of 16 birds/m2. The bedding material in the first group was chopped straw and in the second group pelleted straw. Both bedding materials were added in an amount of 4 kg/m2.

The basic environmental parameters were in accordance with the demands of the hybrid used (Aviagen, 2014) with a lighting program of 18 h light and 6 h dark. Feed and water were available *ad libitum*. Broilers were reared to 42 days of age on a 3-phase commercial feeding program consisting of starter (1-21 days), grower (22-35 d) and finisher (36-42 d) diets. The composition of the basal diets is shown in Table 1.

The litter quality parameters and incidence of footpad dermatitis were evaluated at the age of 4 and 6-weeks. The litter quality was evaluated by scoring method on the scale from 1 to 5 (1-dry and friable litter; 2- friable but slightly wet; 3- friable by some cake formation; 4-fully caked but friable litter beneath; 5-totally caked and wet). The litter moisture was measured on aggregate samples taken from the five different sites per pen. After that, the pooled litter was weighed and dried at 105o C for 24 h and weighed again. For determining the pH, 10 g of litter samples were taken and immersed in 100mL of distilled water. A weighted sample mat was then stirred for 15 min in a magnetic stirrer; thereafter, the sample was filtered through filter paper. After completion of filtration, the pH in the filtered liquid was measured using a pH meter (Inolab 720, WTW, Germany).

The incidence and severity of FPD were measured by the method described by Eichner (2007): no lesions (0), lesions cover less than 25% of the footpad (1), lesions in wide areas, covering between 25% and 50% of the footpad (2), more than 50% lesion on the footpads (3).

Table 1. Composition of experimental diets

	Starter (0-12 days)	Grower (13-33 days)	Finisher (34-42 days)
Diet composition (%)			
Corn	39.15	43.85	49.65
Full fat soybean	10.00	10.00	10.00
Soybean meal (44% CP)	32.70	27.60	22.00
Wheat	10.00	10.00	10.00
Sunflower oil	2.60	3.70	3.60
Monocalcium phosphate	1.60	1.40	1.30
Limestone	1.60	1.40	1.40
Salt	0.25	0.25	0.25
Sodium bicarbonate	0.30	0.30	0.30
L-Treonine	0.10	0.10	0.10
L-Lysine HCl	0.20	0.10	0.10
DL-methionine	0.40	0.30	0.30
Valine	0.10	-	-
Vitamin and mineral premix	1.00	1.00	1.00
Calculated nutrient composition			
AME (MJ/kg)*	12.89	13.20	13.40
Crude protein (g/kg)	22.89	20.89	19.00
Lysine (g/kg)	1.42	1.21	1.06
Methionine (g/kg)	0.74	0.61	0.60
Ca (g/kg)	1.05	0.98	0.85
P total (g/kg)	0.76	0.70	0.68
P available (g/kg)	0.50	0.44	0.43

*AME – apparent metabolizable energy

Results and Discussion

The results of the basic parameters of litter quality are presented in Table 2. The moisture content was higher in chopped straw compared to pelleted straw in both terms, but the differences were not significant (P>0.05). Also, there were no significant differences in litter scores between two litter types. However, it can be noticed that litter score of pelleted straw had the same value in 4th and 6th weeks of fattening, while the score of the chopped straw increased. That could indicate that chopped straw was not able to retain its quality over time. The pH of the litter was lower in pelleted straw at both terms, but the difference was significant only at 4 weeks of age (P<0.05).

Several authors (Nagaraj et al., 2007; Eichner et al., 2007; Đuki Stojčić et al. (2016). reported that reduction of litter moisture could be done by chemical treatments, dietary manipulations, microbiological products or changing the physical forms of the litter (chopping, pelleting). However, this effect was not established in this trial.

Table 2. Parameters of litter quality at 4th and 6th weeks of fattening (x ± SD)

Parameter	Age of birds	Litter type	
		Chopped straw	Pelleted straw
Moisture content, %	4 weeks	55.17 ± 3.28	52.18 ± 2.56
	6 weeks	52.32 ± 6.18	50.78 ± 4.42
pH	4 weeks	8.14 ± 0.19[a]	7.38 ± 0.18[b]
	6 weeks	7.85 ± 0.43	7.14 ± 0.66
Litter score	4 weeks	3.75 ± 0.50	4.00 ± 0.82
	6 weeks	4.25 ± 0.50	4.00 ± 0.00

a-bMeans within the same rows with different superscript are significantly different ($P<0.05$)

In contrast to our results, Đukić Stojčić et al. (2016) reported that size of the straw did not influence the pH value of the litter. Lowering the pH could lead to the lowering of ammonia concentration in the litter which is important because ammonia in litter can contribute to the development of FPD (Bilgili et al., 2009).

Table 3. Effect of litter type on average FPD score at 4 and 6 weeks of age (x ± SD)

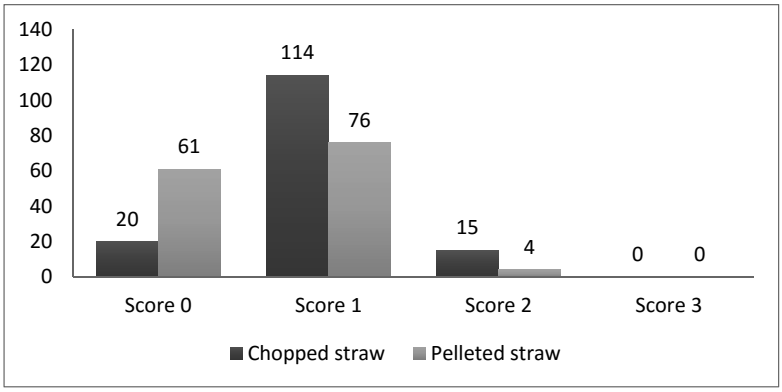
Age of birds	Litter type	
	Chopped straw	Pelleted straw
4 weeks	0.96 ± 0.08^{A}	0.59 ± 0.17^{B}
6 weeks	2.34 ± 0.37	1.81 ± 0.47

A-B Means within the same rows with different superscript are significantly different ($P<0.05$)

Physical form of the straw had a highly significant effect on incidence of FPD ($P<0.01$). The average FPD score was lower in groups with pelleted straw in both terms, but the difference was significant ($P<0.01$) only at 4 weeks of age.

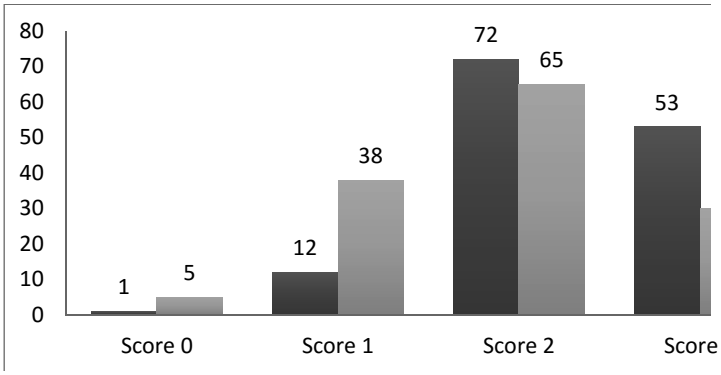
The same effect was reported by Žikić et al. (2017) who stated that reducing the size of the straw on approximately 2 cm significantly decreased occurrence of FPD in broilers. Bilgili et al. (2009) and Shepherd and Fairchild (2010) confirmed that particle size of litter material has an important role in development of FPD probably because of the better condition of the litter and lower ammonia emission.

Analysis of the severity of foot pad lesions showed that highest number of birds with no lesions (score 0) was found in the group with pelleted straw both at 4 and 6 weeks of age. At 4 weeks of age there were no birds with the score 3 (Graph. 1).



Graph 1. No. of birds with different FPD scores at 4 weeks of age

At 6 weeks of age the number of birds with the score representing the deep epithelial lesions of foot pad (score 3) was higher in group with chopped straw compared to pelleted straw (53 vs. 30). That confirms the positive effect of pelleted straw on the incidence and severity of footpad dermatitis in broilers.



Graph 2. No. of birds with different FPD scores at 6 weeks of age

Similar results were reported by **Žikić** et al (2017) who found that the shorter fibers of straw significantly lowered the incidence of FPD in broilers probably because of the better condition of the litter and lower ammonia emission. This is in line with findings of Slobodzian-Ksenicz et al. (2002) who found that long straw had higher ammonia emission compared to chopped straw.

Conclusion

The results of the trial showed a positive effect of pelleted straw on the litter quality by lowering the pH value. Also a pelleted straw significantly lowered the incidence of footpad dermatitis in broilers. It can be concluded from the results of trial that pelleted straw is more suitable for the bedding for broiler chickens compared to chopped straw.

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THE MEDICINAL EFFECTS OF PLANTS ON DOMESTIC ANIMALS

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Abstract: Phytotherapy (herbal medicine) is becoming increasingly popular, it is a medical discipline that uses herbs as a source of biologically active substances. Phytoncides are substances with the bactericidal effect, secreted by certain plants. They are plant or natural antibiotics. A large number of plants contain phytoncides, mostly those that are of a medicinal, spice and aromatic nature. Phytogenic additives – in addition to other additives: probiotics, prebiotics, enzymes, antioxidants, acidifiers – phytogenic additives are one of the most recent alternative growth stimulators. Ethnoveterinary medicine (EVM) or traditional veterinary medicine is the application of folk knowledge and skills in animal health care, production and breeding. This means the application of diagnostic, preventive, therapeutic skills and treatments in order to improve the health of animals. EVM has great significance in organic agriculture and environmental protection. Advances in pharmacology and phytomedicine lead to greater knowledge of the chemical composition of different plants, including their antibiotic substances. We strive to apply antibiotic and defense stimulating plants, thus replacing synthetic antibiotics. Plants as medications can be used thanks to the content of active substances, which can be utilized in veterinary medicine. They are used in the treatment or prevention of disorders and diseases, which include not only large animals, but also dogs, fowl (poultry) and rabbits. Natural herbal products are often used as antibacterial agents, antimycotics, antiparasitics, disinfectants and immunologic adjuvants. In this study (paper), several plant species and their influence (positive or negative) on some domestic animals are described.

Keywords: phytotherapy, veterinary medicine, domestic animals, plants

Introduction

Pursuant to the Article 48 of the Ordinance on the Methods of Organic Livestock Production (2002), in animal healthcare in organic production, phytotherapeutic (plant extracts, essences, etc.) and homeopathic products are used under the condition that the treatment effect is positive in animals for which it is intended.

Phytogenic additives are supplements obtained by processing medicinal and aromatic herbs. Fertilizers, pesticides, growth regulators, antibiotics, food additives, and genetically modified organisms are completely prohibited or strictly controlled in organic agriculture. Organic agriculture methods are governed by national and international laws, which are being implemented in many countries, and which are largely based on the standards of the International Federation of Organic Agriculture Movements (IFOAM) (European Commission, 2012).

Phytogenic additives are composed of extracts and essential oils derived from plants: oregano, mint, garlic, citruses, etc. that have an antimicrobial, antiviral, antifungal and antioxidant effect.

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Phytogenic additives fulfill their function through the gastrointestinal tract by stimulating the secretion of enzymes, improving digestion, reducing the number of pathogenic organisms, and boosting the immune system (Table 1). Essential oils can stimulate the growth and functioning of the body, which improves health in chickens and their product quality (Adaszyńska-Skwirzyńska and Szczerbińska, 2016). The components of the antibacterial activity of essential oils are: linalyl acetate, menthol, a-pinene, b-pinene, camphor, carvacrol, 1,8-cineole, linalool, limonene and thymol, they have been researched in human pathogenic organisms (Soković et al., 2010).

Jaiswal et al. (2017) points out to the determination of potential and possible mechanisms that are alternative to Antibiotic Growth Promoters (AGP), in the improvement of the intestinal microbial ecosystem, the immune system, and also the growth performance in poultry.

An experiment that used sunflower meals in the feed, Grčak et al. (2015) has shown a significant influence of sunflowers on the stability of the intestinal microflora in weaned piglets. The influence of premixes on the quality of chicken eggs for consumption has shown positive characteristics (Grčak, 2015). The use of probiotics in weaned piglets has shown positive characteristics in the production ability of piglets (Grčak, 2003).

The *in vivo* experiment examined the effect of spores of probiotic *Bacillus coagulans*, with or without the prebiotics, inulin, on the gastrointestinal (GI) tract of healthy mice and their ability to survive within the GI tract. The presented results have shown that probiotic *Bacillus coagulans* is efficient. Daily consumption of probiotics is essential for a long-term effect (Abhari et al., 2015). Laudato and Capasso 2013. point out to the effect of various plant species to certain organs (skin) and systems-apparatuses (cardiovascular system, digestive and respiratory apparatus) in the body of animals, and to certain parasites (helminths).

The importance of ethnoveterinary medicine is becoming more topical and lies in applying its knowledge in various forms of animal breeding, especially in organic growing, environmental protection (Adedeji et al., 2013; Šubarević et al., 2015).

Phytoncides from plants are used for the treatment of humans and animals, and in plant production, they serve as catch crops, pre-sowing plants, trap crops. Planting the following Phytoncide woody plants is recommended: white poplar, birch, lemon, walnut, laurel, etc.

The specificity of feeding poultry, compared to other domestic animals (sheep, goats, pigs, etc.), regarding the gastrointestinal tract, is the fact that the gastrointestinal tract of poultry is much shorter in relation to the body length, when compared to the length in other domestic animals, e.g. the intestines of cattle are about 20 times longer than their body length. The ratio of poultry intestines to their body length is about 1:7. They do not have teeth to chew feed, so easily digestible feed should be given to poultry. All poultry breeders know very well that poultry have an irresistible need to "graze." Green feed is very important in feeding poultry, because it serves as prevention of diseases, utilizing a variety of plant species (Lagu and Kayanja, 2010).

Some of the plants that are used for feeding domestic animals

Hellebores (*Helleborus* L.) are plants whose organs are used in traditional (folk) medicine. They were used as a human remedy against deafness, scabies, leprosy, delirium and insanity. Hellebores have a very diuretic, emetic (induces vomiting), stimulant and narcotic effect. The said effects come from hellebrin glycoside and helleborinsaponoside. Kusan (1956) indicates that fresh parts of hellebores must not be used, due to their effect on the central and peripheral nervous system, which

can cause insomnia, vomiting, diarrhea, convulsions and stiffness, and even death in humans and animals.

Purple hellebores (*H. purpurascens* W. et K.) were used for watering cattle and sheep. Purple hellebores were put in water, so that they would destroy parasitic larvae in the liver and also as a remedy in situations when animals refuse to eat.

In cattle, hellebores were used for reducing inflammation, for example, mastitis in sheep and for reducing inflammation (edema) in cattle's extremities. They are used in several ways: a little piece of sharpened rhizome is slipped through a pierced ear or tail (pig, sheep), or the neck (cattle) and the breast skin in horses, and left like that for 1-2 days. After removing the rhizome, transient swelling appears at the implantation spot. In this way, the animal's immune system is being strengthened, creating a greater number of leukocytes. This procedure is also performed when there is an insufficient development of animals, the loss of appetite, cough, lung and intestine diseases, erysipelas and against acute diseases.

Hellebores can be applied externally on swollen joints, or they can be dried, and triturated rhizomes with roots are given to animals in feed. As a means of lowering the body temperature, the hellebore's underground organ is used in pigs, cattle and sheep. In the Italian folk medicine, hellebores were used by grinding their underground organs in the form of decoctions (boiled juice) and bitter tonics as cardiac agents and digestion improvement remedies (Davidović, 2013).

Oregano (lat. *Origanum vulgare*) – its essential oil, which the plant contains from 2 - 4%, is important, since it contains about 80% of the aromatic carvacrol and about 50% of thymol. These substances have an effect on the causes of many diseases. In a concentration of 0.007%, thymol kills certain bacteria (staphylococcus, salmonella and listeria). At higher concentrations, it also kills fungi (*Aspergillus flavus* and *A. versicolor*).

Bacteria cannot defend themselves from natural antibiotics. This is because antibiotic substances are not the only ones that fight bacteria, but other substances, whose concentration changes depending on the plant, also fight bacteria (related to natural substances, saponins and bitter substances). Not only does oregano oil fights against bacteria and fungi, but it also kills coccidia.

Oregano can be given to poultry as a supplement to green feed. Oregano essential oil serves as an alternative to promote the growth stimulator in piglets, chicks and turkeys. Basil (*Ocimumbasilicum*) has a similar effect (Anon., 2018). Fonseca-García et al. (2017) conclude in their paper that oregano oil in the feed of broilers has a positive effect on the production parameter and the length of intestinal villi and also has an antioxidant function.

St. John's wort (*Hypericum perforatum*) has an antibacterial, antiviral, and antihelminthic effect, is an antidepressant, prevents and relieves inflammation, prevents bleeding, protects blood vessels, induces secretion, prevents the occurrence of deformed cells in the body (cancer cells), and protects cell membranes. St. John's Wort is a natural medicine, whose composition has a holistic effect, by suppressing pathogens and inducing self-healing. Poultry should be given the ground part of the plant at the time of flowering. Leaves and flowers should be separated from their stems and mixed into soft feed; it will not be eaten if we do not offer it fresh and clean. St. John's Wort can be picked, tied in bundles and dried in a cool, shady place, and given to poultry in winter (Stojanović,

2012).

Marigolds (*Calendula officinalis*) – the well-known components of marigolds are essential oils, saponins, flavonoids, carotenoids, xanthophylls, resin, triterpenoids, mucilage, proteins, sugar, enzymes and organic acids. The cooperation of all the listed components makes the plant effective. Marigold essential oils are used to prevent the growth of various bacteria and fungi. It has an antibiotic effect thanks to flavonoids and saponins. An extremely high content of saponins in marigolds has a strong effect against the harmful fungi in the intestines, preventing the creation of their membranes. Flavonoids act, e.g. through vitamin K1, and regulate blood, thus preventing inflammation and vascular occlusion.

The entire above-ground part of marigolds is used in feed. Aphids frequently attack marigolds, so, poultry is provided with high-value animal proteins when they are given feed with marigolds (Anon., 2018; Modrić, 2016).

Common yarrow (*Achillea millefolium*) contains a bitter substance achilein, essential oil, tannins, resins, asparagine, and various acids and minerals. It has an antibacterial and anti-inflammatory effect. In phytomedicine, it cures stomach ailments, stimulates appetite, cleanses blood, is an anthelmintic, is used for treating wounds, malaria, inflammation and liver diseases, and its richness in potassium supports the kidney function. Young leaves, which appear from late February to late October, are given to poultry chopped in order to boost their immunity (Anon., 2018; Modrić, 2016).

Sage (*Salvia officinalis*), whose main components are thujone and camphor, is given to poultry in feed in order to improve their fitness and health. Chopped leaves are mixed with other green or soft feed. Dried leaves can be used during winter. Middle leaves contain thujone, and large leaves have more camphor. Sage helps the healing of wounds, has an antibiotic effect (thujone), cures intestinal infections and prevents inflammation (camphor). Cineol kills germs in wounds (antiseptic). An excessive use of sage can cause improper functioning of the kidneys and liver problems. Sage essential oil contains thujone, whose excessively large amount influences the central nervous system. Therefore, it is proposed that sage feed is paused for about ten days, and replaced with different green feed (Anon., 2018; Stojanović, 2014).

Nasturtium (*Tropaeolum majus*) should be given to poultry periodically, as it causes irritations of the digestive tract. Only fresh leaves, flower buds and flowers are used. It has an antibiotic, antiseptic and a fungicide effect. All above-ground parts are rich in sulfuric heteroside glucotropaeolin (Anon., 2018). When *Tropaeolum leaves* are consumed, their antimicrobial activity is exhibited (Kleinwächter et al., 2008).

The onion (*Allium cepa*) is a unique plant with a large number of medicinal properties (about fifteen), which is used for treatments of twice as many diseases. It boosts metabolism throughout the entire digestive system. It strengthens the immune system with its antibiotic effect, stimulates the production of blood and pink structures in poultry (nails, feathers, beaks, scales on legs). It also contains a high level of vitamin C (Aleksić, 2015).

Table 1. The above mentioned plants in the service of veterinary phytotherapy

Scientific name	Used parts	It is used as (against)
Helleborus L.	Underground parts, rhizome3	It boosts (improves) immunity, metabolism, the excretion of harmful substances from the body3.
Origanum vulgare	Ground part4	Antibacterial effect, antifungal4
Hypericum perforatum	Flowers1	Wounds1
Calendula officinalis	Leaves, flowers1	Wounds, gingivitis1 Antibiotic, antifungal4
Achillea millefolium	Leaves4	Antibacterial effect, anti inflammation 4
Salvia officinalis	Leaves, flowers1	Endoparasites, dehydration1 Antiseptic4
Tropaeolummajus	Fresh leaves, flower buds and flowers4	Antibiotic, antiseptic, antifungal4
Allium cepa	Bulb2	Bacteria, viruses, fungi, and many other parasites2

1Laudato and Capasso2013.; 2Aleksić(2015); 3Davidović et al. (2010); 4Anon. (2018).

In addition to the said herbaceous plants that show healing effects in animals, pits of stone fruits can be included in animal feed. In Serbia, the dominant type of stone fruit is plum (Grčak, 2017). Pits of plums are rich in vitamin B17. Pits of plums also contain a bioactive substance amygdalin, which helps in the treatment of cancer. The amygdalin molecule belongs to the group of tumor-selective agents, which destroy only tumor cells, leaving healthy cells intact (Savić et al., 2016). Pigs are domestic animals that like to eat pits.

Conclusions

Herbal remedies are the oldest forms of treatment for various diseases. Phytotherapy is not alternative medicine, but part of it provides (offers) the possibility for an adequate (more holistic) treatment. The application of phytotherapy has been intensified along with organic livestock production. In recent years, a number of studies have been carried out, proving that certain plants, with their active substances, influence certain organisms or certain physiological processes in domestic animals. In order to prove scientifically the influence of plants on animal health, it is necessary to clinically validate their use, and to determine the risks of their use, if any. The plants that we have described showed their specific effects on domestic animals, such as the effect on: wounds, gingivitis, as antibiotics, antifungal activity, antibacterial, anti-inflammation, antiparasitic, antiseptic activity.

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THE OCCURRENCE OF AFLATOXIN M1 IN MILK SAMPLES ON SOME HOUSEHOLDS AND DAIRY PLANTS IN SERBIA

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Abstract: A total of 63 raw milk samples were analyzed on aflatoxin M1 (AFM1) content using HPLC-FLD method after immunoaffinity column cleanup. Samples were collected from households (23 samples) and from dairy plants (40 samples) in Serbia during the period of October 2017 – June 2018. The average AFM1 content in 87.3% positive samples was 0.137 μg/kg, and it was ranged from 0.011 to 1.218 μg/kg. The occurrence of AFM1 was extremely high, although higher in samples from households (100.0%), compared to samples collected on dairy plants (80.0%). Likewise, AFM1 levels were higher in samples originating from households (0.025-1.218 μg/kg) in comparison with the samples from dairy plants (0.011-0.295 μg/kg). This was expected since all samples from dairy plants were bulked milk from a number of farms. In this manner, regulation compliance was also very different between households and dairy plants samples. On households, 87.0% of raw milk samples were above EU maximum limit (ML) and 26.1% above Serbian ML. On the other hand, significantly lower number of samples (28.1%) was above EU MLs and only one sample above Serbian MLs, considering samples collected on dairy plants.

Keywords: aflatoxin M1, HPLC-FLD, households, dairy plants, Serbia

Introduction

Aflatoxins (AFs) as food safety hazards, were the center of public attention in the last few years in Serbia. The reasons for this were socio-economic consequences of severe milk contamination with carcinogenic aflatoxin M1 (AFM1). According to the World Bank (2018), Serbia belongs to a group of developing countries. Williams et al. (2004) stated that since security blankets in crops at pre-harvest and post-harvest level are not as strict as in developed countries, populations of developing countries are the most susceptible to aflatoxicosis illness. Furthermore, Lizárraga-Paulín et al. (2011) concluded that the same problem occurs with milk derivates in developing countries for the reason that they have not accepted and assumed amenities as quick as developed countries. Unquestionably, this is not entirely true for Serbia, since the country is an EU candidate and therefore is harmonizing the legislation with the EU.

Aflatoxins M1 (AFM1) and M2 (AFM2) are thermo-resistant hydroxylated metabolites produced by lactating animals consuming aflatoxin contaminated feeds (Lizárraga-Paulín et al., 2011). The conversion rate of aflatoxin B1 (AFB1) into AFM1 was found to be 1–3% (Herzallah, 2009). In milk of dairy cows AFM1 was found within 12-24 hours of AFB1 contaminated feed ingestion, while the highest levels were reached after a few days (Ayar, 2007). On the other hand, when con-

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taminated feed is excluded from the diets, the AFM1 levels in the milk were decreased to an undetectable level after 72 hours (Van Egmond, 1989; Gimeno, 2004; Özdemir, 2007). However, AFs carry-over from feed into milk is exponentially increased (Britz et al., 2013). The aflatoxin carry-over ranges from 0.6 to 6% (Masoero et al., 2007) and can be up to 11.4% in case of high yielding cows (Britzi et al., 2013). Britzi et al. 2013. also suggested that in the case of high yielding cows with the average milk production of 45 kg and daily intake of 25 kg dry matter (DM), AFB1 needed to be below 1.4 μg/kg in cows' diet to ensure milk production with AFM1 levels lower than 0.05 μg/kg (EU legal limit).

To reduce the risk of exposure, many countries have regulated the maximum level (ML) of AFB1 in feed (and have set or proposed ML of AFM1 in milk). Currently, the legal limits of AFB1 in feedstuffs are highly variable from the European Union (EU) countries to other countries (the EU has a limit of 5 μg/kg for dairy feed) (European Commission, 2003). In Serbia, proposed ML of AFB1 is harmonized with EU since the April 2014 (Serbian Regulation, 2014). Regarding the regulation of AFM1, European Union has established ML in raw milk of 0.05 μg/kg (European Commission, 2006). This level was also set in Serbia (Serbian Regulation, 2011) but since then, it has been changed several times. At this moment, the ML in Serbia is set to 0.25 μg/kg (Serbian Regulation, 2017).

The aim of this paper was to investigate and compare the occurrence of AFM1 in raw milk samples on households and dairy plants in Serbia during the period of October 2017 – June 2018.

Material and Methods

Samples. Raw milk samples were randomly collected from households with less than 20 cows (23 samples) and from dairy plants with milk bulked form household and large dairy farms (40 samples) in Serbia during the period of October 2017 – June 2018. The samples from households were collected directly from cooling tanks after morning milking, while samples from dairy plants were collected from bulk tanks. All samples originated from dairy cows fed maize and/or maize silage.

Reagents. Acetonitrile was purchased from Sigma Aldrich (Buchs, Switzerland) while n-hexane was obtained from Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA) was purchased from Thermo Fisher Scientific (Cheshire, United Kingdom). Sample clean-up for high-performance liquid chromatography (HPLC) analysis was done on AflaStar™ M1 R-Immunoaffinity Columns (IAC) (Romer Labs Inc., Union, MO, USA). AFM1 standard (c=10 μg/ml) was purchased from Sigma Aldrich (Buchs, Switzerland).

Sample preparation. Twenty-five ml of milk was filtered through a quantitative filter paper (Filtros Anoia, Barcelona, Spain) and applied to the IAC. Flow rate of milk was approximately 1-3 ml/min. After the milk completely passed, IAC was rinsed with 20 ml of deionized water. The AFM1 was eluted with 4 ml of acetonitrile. Eluate was collected and evaporated just to dryness at 50 °C using gentle stream of nitrogen. AFM1 was derivatized by adding 200 μl of TFA and the same volume of n-hexane to the residue and to the AFM1 standards, vortexed for 30 s, and kept in the dark for 10 min at 40 °C. After evaporation, 300 μl of water:acetonitrile (75:25, v/v) mixture was added to the vials and vortexed for 30 s.

HPLC determination. The HPLC instrument was an Agilent 1260 (Agilent Technologies Inc., USA) system with fluorescence detector and Agilent Hypersil ODS C18, 4.6 x 100 mm, 5 μm col-

umn. The mobile phase consisted of an isocratic mixture of water:acetonitrile (75:25, v/v) at flow rate of 1.0 ml/min. Twenty microliters of standards and samples were injected into the HPLC column. The fluorescence detector was set to an excitation and emission wavelengths of 360 and 423 nm, respectively. The retention time was around 2.1 min. Analytical quality control was implemented according to the Commission Regulation (European Commission, 2002). The linearity of the method was assessed by standard ranging from 2.5–50 ng/ml. The limit of quantification (LOQ) was 0.25 ng/ml of AFM1, which is equivalent to 0.010 μg/kg of AFM1 in sample.

Statistical analysis. Statistical analysis was done using Statistica software (Version 13.3, TIBCO Software, Palo Alto, CA, USA).

Results and Discussion

A total of 63 raw milk samples were analyzed on AFM1 content. Samples were collected from households (23 samples) and from dairy plants (40 samples) in Serbia during the period of October 2017 – June 2018. Monthly ratio between number of samples collected on households and dairy plants was presented in Figure 1, while the obtained results were summarized in Table 1.

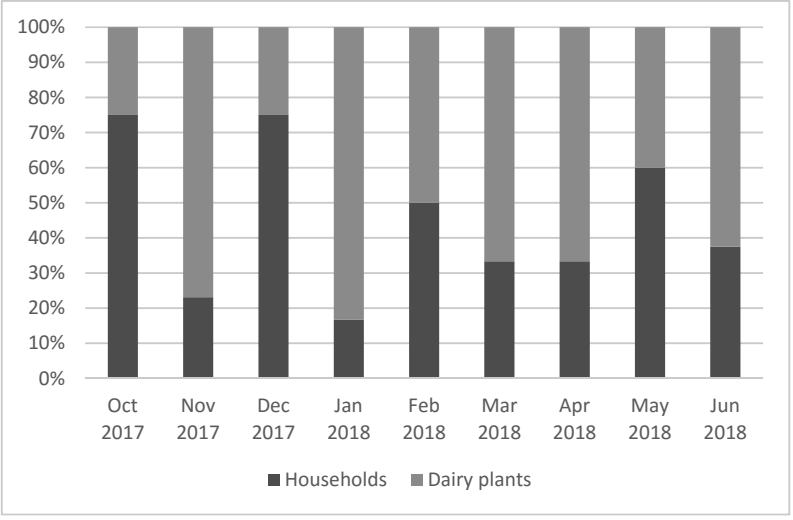

Figure 1. Monthly ratio between the number of samples collected on households and dairy plants.

The average AFM1 content in 87.3 positive samples was 0.137 μg/kg, and it was ranged from 0.011 to 1.218 μg/kg. The occurrence of AFM1 was extremely high, although higher in samples from households (100.0%), compared to samples collected on dairy plants (80.0%). Likewise, AFM1 levels were higher in samples originating from households (0.025-1.218 μg/kg) in comparison with the samples from dairy plants (0.011-0.295 μg/kg). This was expected since all samples from dairy plants were bulked milk from a number of farms. In this manner, regulation compliance was also very different between households and dairy plants samples. On households, 87.0% of raw milk samples were above EU maximum limit (ML) and 26.1% above Serbian ML. On the other hand, significantly lower number of samples (28.1%) was above EU MLs (European Commission, 2006) and only one sample above Serbian MLs (Serbian Regulation, 2017), considering samples collected on dairy plants.

Table 1. AFM1 in raw milk samples on households and dairy plants in Serbia (October 2017 – June 2018)

	Households	Dairy plants	Total
N	23	40	63
N (>LOQ)	23 (100.0%)	32 (80.0%)	55 (87.3%)
Average±SD (μg/kg)	0.250±0.306	0.056±0.064	0.137±0.223
RSD (%)	122.6	113.6	163.0
Range (μg/kg)	0.025-1.218	0.011-0.295	0.011-1.218
N (> EU regulation)	20 (87.0%)	9 (28.1%)	29 (12.7%)
N (> SRB regulation)	6 (26.1%)	1 (3.1%)	7 (12.7%)

N – number of samples, N (>LOQ) – number of samples above the limit of quantification, SD – standard deviation, RSD – relative standard deviation, N (> EU regulation) – number of samples above EU regulation, N (> SRB regulation) – number of samples above Serbian regulation

Since the presence of AFM1 in milk depends primarily on the presence of AFB1 in feed, hot and dry weather conditions during maize growing season of 2017 could be considered as favorable for *Aspergillus* molds growth and AFs productions. Namely, reports of Serbian hydrometeorological service described that vegetation period of 2017 (April-September) was warmer and drier when compared to the multiannual average, with 20% less rainfall. On the other hand, standardized precipitation index (SPI-6) showed normal humidity conditions on most of the territory of Serbia. During the summer (June-August), the precipitation was uneven both, in terms of quantity and geographical distribution. The rainfall did not meet the requirements of the plants for moisture, especially in periods of hot waves when the maximum temperatures were above 35 °C for several days, and some values measured at about 40 °C. Such unfavorable temperature and humidity conditions were recorded on most of the territory of Serbia, which in some parts of the country resulted in a significant reduction in yields, especially maize (30% lower yields compared to the average). Fortunately, in some regions like Bačka, the excellent yields of this culture were recorded.

By comparing weather conditions with our results, it can be said that weather conditions might be coincided with relatively high AFM1 levels in milk, even there were no reports on AFB1 contamination of maize from 2017 harvest, to the best of our knowledge at the moment. On the other hand, Glamočić et al. (2018) reported aflatoxin contamination of maize silage collected from households in Vojvodina during the period November 2017 –January 2018. The authors have found aflatoxins at levels up to 24.64 μg/kg.

It is also important to point out that considerable differences between samples collected on household and dairy plants (Figure 2) could be an outcome of a fact that all samples from dairy plants were bulked milk from a number of farms including large farms with better feed quality control systems on larger dairy plants, whereas on households those are mostly hasty or does not even exist.

Presence of AFM1 in milk in an earlier period in Serbia was published in few reports (Janković et al., 2009; Polovinski-Horvatović et al., 2010). The studies showed lower occurrence of AFM1 and could be explained by the absence of AFs in maize and other feed material (Kokić et al., 2009; Jakšić et al., 2011). In 2013, the situation was completely different with both, high contamination of milk (Škrbić et al., 2014; Vuković et al., 2013; Kos et al., 2014; Torović, 2015) and high contamination of maize (Kos et al., 2013). Similar situation was in the neighboring Croatia (Bilandžić et al., 2014). Re-

sults obtained in our research were well above those published in 2009-2010 period. Unfortunately, in samples originating from households the results were not much below ones from 2013-2014. On the other hand, the situation on dairy plants in considerably better and mostly under control.

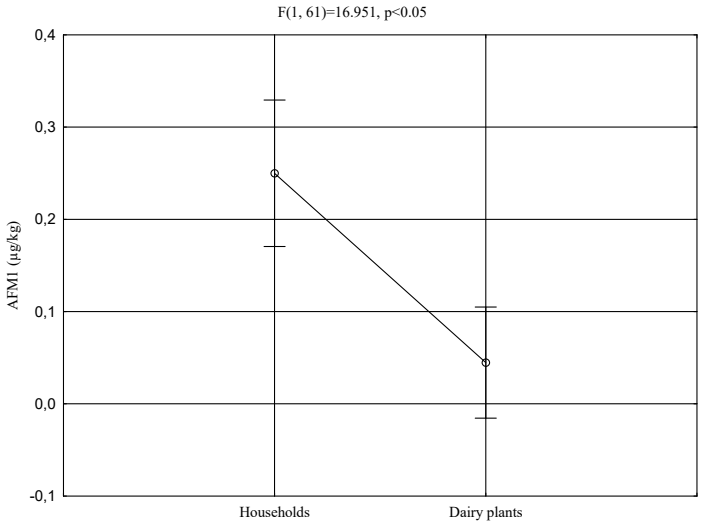

Figure 2. Differences in aflatoxin M1 content in raw milk between households and dairy plants in Serbia (ANOVA).

Conclusion

This paper confirms that the problem of milk contamination with AFM1 still exists and should be considered as high priority when developing national strategies in terms of food safety. Our results may reveal, that the control of aflatoxins in milk is still quite problematic on household in Serbia, although a larger study is needed. It is imperative that small primary producers are well educated regarding the importance of feed quality control procedures and its frequencies.

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