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Preface

Institute for Zootechnics of the Faculty of Agriculture University of Belgrade and Department of Animal Sciences of the Faculty of Agriculture University of Novi Sad both have a long tradition in organizing scientific events. Institute for Zootechnics has been organizer of nineteen national conferences "Innovations in animal science" that 2012 evolved into "The first international symposium on animal science". At the same time, Department of Animal Sciences twenty three times was successful organizer of the International Symposium "New Technologies in Contemporary Animal Production". This year, these two institutions have decided to continue the tradition by joint forces, and the "International Symposium on animal science 2016" is the result of that cooperation.

The main goal of this Symposium is to establish better collaboration between animal science researches and specialists from industry, trade and other related fields, and producers from Serbia, Western Balkans, EU and other parts of the World in their common fields of interests in science, education and good livestock production practice.

The Symposium covers the most important topics in animal sciences: 1. Breeding, Selection and Reproduction of Domestic Animals, 2. Nutrition of Domestic Animals, 3. Animal Welfare, Health and Farm Biosecurity, 4. Game Animals Production, Fishery and Beekeeping, 5. Organic and Sustainable Livestock Production, 6. Animal Products Technology and Quality, 7. Environment Protection in Livestock Production and 8. Transfer of knowledge and innovations in Animal Science.

We are grateful to the all institutions who responded to our invitation to help us in organization of this event. We thank the Symposium co-organizers: Kmetijski Inštitut Slovenije, (Slovenia), University Of Zagreb, Faculty of Agriculture, (Croatia), Josip Juraj Strossmayer University Of Osijek, Faculty of Agriculture, (Croatia), Faculty of Agriculture-Josip Juraj Strossmayer University of Osijek (Croatia), Banat University Of Agricultural Sciences And Veterinary Medicine Timisoara, Faculty of animal science and biotechnology, (Romania) , National Agricultural And Food Centre, Research Institute for Animal Production in Nitra, (Slovakia), Slovak University Of Agriculture In Nitra, Faculty of biotechnology and food sciences, (Slovakia) and Ministry of Education, Science and Technological Development of Serbia (Serbia). Also, we express a special thanks to European Society of Agricultural Engineers (EurAgEng), as well as all other institutions, sponsors and donators for support. We thank all authors and co-authors for their contribution in achieving the high scientific level of all topics, as well as invited speakers and members of the International Scientific and the Organizing Committees. Finally, we thank all participants, contributors, chairpersons, organizational and technical assistants for the considerable efforts they made in realization of this event.

Sincerely,

Prof. Dr Zoran Popović

Editor in Chief and Chairman of Symposium

Organising and Scientific Committees

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POSSIBILITIES FOR IMPROVING REPRODUCTIVE TRAITS
IN COW-CALF SYSTEM

Perišić P.[1]*, Maletić M.[2], Mekić C.[1]

Abstract

This paper describes production and reproductive parameters determined in some previous studies which were conducted in cow-calf system breeding stocks. The importance of meat production in cow-calf system is highlighted, particularly in the context of current tendencies in cattle production both in EU countries and in our own country.

A success in cow-calf production system depends mostly on the fertility achieved in a breeding stock. An ideal fertility in a cow-calf system is one calving a year, i.e. the length of a calving interval of about 365 days. The most important factors which impact bovine female fertility are indicated in this paper as well as a possibility for improving fertility traits in cows raised in cow-calf system.

Besides application of certain zootechnical procedures which could improve fertility, the application of hormones for the purpose of induction and synchronization of estrus in cows is essential. The protocols which are most often used for induction and estrus synchronization in cows are presented.

Keywords: cow-calf system, fertility, induction and estrus synchronization, meat production

Introduction

Cow-calf system is a major system used in beef production systems in many countries. This production is organized in different variants and levels of extensive production systems. The pastures are most commonly used since they provide for the most profitable cattle fattening. In regions where seasonal changes are more distinctive, cattle are kept on pastures in the summer season, while in the winter they are kept in stables with a free range system and nutrition based on forage feeds and by-products of feed industry.

The most important factor which the success of production in cow-calf system depends on is a good fertility. The ideal fertility in beef breeds, raised in cow-calf system means one calving a year, i.e. the interval between calvings being about 365 days. However, in real production the situation is often far from optimal and regular cow fertility is primarily impaired what can directly influence the production success.

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In order to improve beef production various activities are being applied (improved nutrition, suckling restrictions, hormonal treatments in breeding females) directed mostly to improving bovine female fertility. Thus the induction and estrus synchronization is being often applied in cows for the purpose of obtaining shorter possible duration of service-period as well as of performing synchronized cow calving what seems to be important from the organizational aspect.

Trends in beef production in EU countries

In EU countries there is a trend to reduce the number of households which raise the breeding stocks of dairy cows but at the same time there is an increased number of cows per households engaged in dairy production. Besides enlarging the households engaged in dairy production a trend of reducing a total number of cows is observed as well. Additional reasons which can impact reducing the number of cows can be a still present trend to increase dairy production per cow and current milk reserves (Popović, 2008; Perišić et al. 2012).

In a meat production sector a decrease in totally produced quantities of beef meat is observed as a consequence of reduction of total number of cattle primarily due to reduced number in dairy cattle. There appears a negative net balance (between 200 000 and 400 000 tons of beef meat annually), on the market what gives space for further increase in the volume of meat production in the EU member countries.

A decrease in the number of cattle per average rate of 2-3 % annually is observed in Serbia in the last decade followed accordingly by all negative tendencies in meat production. Having in mind free markets for beef meat (in EU countries, and in other countries outside EU) Serbia should begin to strategically organize the production of beef meat meant for export (Perišić et al. 2012).

Production results in cow-calf system

In cow-calf production system different breeds and their cross-breds can be used. Thus in eastern Germany, Deblitz et al. (1992) studied the effects of extensive keeping of 221 herds of purebred nursing cows and cross-breds of Galloway, Montafon, Limousine, German Red Pied, German Angus, Aberdin-Angus, Charolais and Simmental nursing cows on reproductive results and production of calves. The animals were kept outside during the winter in 40% herds. Number of calves weaned per cow ranged from 0.87 for the breeds with more robust constitution and up to 0.91 for large breeds (Charolais and Simmental) but there were no significant differences between the breeds in body mass of calves produced at 100 kg of cow body mass. Nutrition costs were by 15% higher for the cows which spent the winter outside in comparison with the cows kept in stable. It was concluded that, under the conditions which prevail in Germany, extensive keeping of smaller format cattle breeds has certain advantages.

Cundiff and Gregory (1999) reported results of the trial conducted by the U.S. Meat Animal Research Center (Nebraska, SAD) with the aim of determining the   differences

between breeds in growth rate, reproductive results, characteristics of carcass and meat for possible use in production of composite herds. Continental breeds (Chianina, Charolais, Limousine, Salers, Maine-Anjou, Gelbvieh, Holstein, Simmental and Dark-Brown) had larger body mass in comparison with British breeds (Hereford, Shorthorn, South Devon, Devon, Aberdin-Angus) which had high levels of marbling, but also excess fat. The 50:50 ratio between British and continental heritage gave optimal body mass, dressing percentage and level of marbling. Belgian Blue and Piedmontese cattle had high slaughter dressing percentages but low evaluation of carcass. The descendants of Tuli bulls had low average daily gains. Cattle with Tuli or Boran heritage matured earlier than cattle with Brahman heritage. Offspring body masses at weaning were larger in cattle with Nellore or Brahman heritage than in those with Boran or Tuli heritage.

Mazurovsky and Litovchenko (2000) presented the results on creating a new meat type of Simmental cattle in the region of Orenburg (Russia). During creating a Simmental purebred meat type the best performance during the first phase of creating had animals produced by the bulls of German and Canadian selection. During introductory cross-breeding the utilization of Limousin breed produced some good effects. During the first phase an average gain in fattening was 1300-1400 g per day.

In Poland Choroszy B. and Choroszy Z. (2001) conducted a trial for the purpose of using the ecological pastures for fattening the Simmental, Red and White cattle and cross-breds of fattening heifers for slaughter. A period of five-month pasture had a positive effect on daily gains which ranged from 601 to 734 g. The analysis of slaughter traits showed that the highest dressing percentage and best composition of carcass tissues were realized by cross-breds fattening heifers. Heifer carcasses were well covered by muscles (76.79%) and moderately covered by fat (5.42%) regardless the breed. Quality of heifers meat corresponded to the requirements for the quality of young beef meat (a large part under muscles, light color and required softness). The results obtained confirmed the possibility of using ecological pastures for fattening the young cattle for slaughter.

Tschumperlin et al. (2001) compared production efficiency of dual-purpose medium size Simmental cows and Aberdin-Angus bovine female breed. A whole herd was mated with Angus bulls. Bullcalves cross-breds of Simmental x Aberdin-Angus had higher live weight gains and net mass gain compared to purebred Angus bull calves. Simmental x Angus bull calves had higher yield and better quality of meat compared to Angus bull calves (difference of 0.6% and 1.0%, respectively). Superiority of dual-purpose type of cows in relation to specialized meat type in this system of meat production was caused mostly by higher milk yield produced by Simmental cows.

As a maternal base Simmental breed is often used in specialized systems of meat production ("cow-calf" system), where crossbreeding with terminal fattening breeds (Charolais, Limousine, Belgian Blond-White) is often organized. Research on production traits (fattening and slaughter) of Simmental breed and its cross-breds with beef breeds was done by a number of researchers in a previous period. Thus Čobić et al. (1990) studied fattening abilities of male F_1 generation cross-breds of Charolais and Limousine breed with Simmental. Main conclusions drawn suggested that the gain of cross-breds with Charolais was significantly higher than the gain of crossbreds of Limousine and Simmental breeds. Consumption of net energy per kilogram of gain was by 16.79% lower in crossbreds with Charolais in comparison with Simmental breed, digestible crude

protein by 14.52 %, while crossbreds with Limousine breed had slightly lower consumption of net energy (2.04%) and digestible crude protein (9.91%) in relation to Simmental breed. Bullcalves of Simmental breed had relatively heavier head, skin and some internal organs, what affected carcass lower dressing percentage in relation to cross-breds while differences in dressing percentages between cross-breds were insignificant.

Medić et al. (1991) studied fattening and slaughter qualities of cross-breds obtained by crossbreeding of combined and dairy production cows with bulls of beef breeds. They reported that the largest mass at birth was obtained in crossbreds with Charolais and Limousine breeds and on the basis of this knowledge by the use of crossbreeding between these two breeds in larger production systems higher frequency of difficult calvings and loss of calves and cows may be expected. Bulls of Blonde d`Aquitaine breed had no effect on increasing the mass of calves at birth. The best fattening results were obtained by crossbreds with Charolais and Blonde d`Aquitaine breeds (1288 g/day, 1263 g/day). As for slaughter traits the advantage was given to Limosine and Blonde d`Aquitaine breeds due to increased slaughter dressing percentage (63.7% and 65.4%), as well as larger yield of the best quality meat parts (ham and back).

Čepon et al. (2001) studied carcass traits of the bull calves meat of Simmental, Brown and Holstein-Friesian breeds and crossbreds between Brown and Belgian Blue, Holstein and Belgian Blue and Holstein and Piedmontese. Among these 3 pure breeds Simmental breed showed best carcass quality followed by Brown and Holstein breeds. As for meat properties there was no significant differences between these 3 breeds. Crossbreds with Belgian Blue had higher dressing percentage, higher content of lean meat and lower content of carcass fat. In meat properties differences were less pronounced. Crossbred bulls had lower intramuscular fat compared to purebred bulls and better subjectively evaluated softness of meat in comparison with Simmental bulls. Crossbreds between Holstein and Piedmontese had better dressing percentage, conformation and carcass composition and better softness of meat than Holstein bulls.

Perišić (2007) states the results which justify the use of crossbreeding between Simmental breed and terminal beef breeds (Charolais and Limousine) when meat production is organized in cow-calf system.

Reproductive parameters in cow-calf system

In free range keeping of cattle in cow-calf system the contact between the breeder and the animals is done at longer distance so it is harder for farmer to observe all changes in animals regarding health state, occurrence of estrus and alike. For these reasons parameters of fertility in cows and therefore production in general in indirect way, depend strongly on breeders, particularly if they apply insemination in cow herds instead of mating.

Reproductive parameters for Simmental breed, cross-breds of beef breeds with Simmental and beef breeds and cross-breds in majority of studies differ from the optimum expected for "cow-calf" production system. For a number of European countries and also for Serbia, Simmental breed has a great importance. This breed can be equally well directed towards combined production (milk-meat) and towards specialized   meat

production (cow-calf system) according to the reports of Perišić et al. (2008) and Perišić et al. (2009).

Fiss and Wilton (1989) studied the effect of system of keeping Hereford and Simmental breed, as their crossbreds obtained in rotation crossbreeding and which were of different format and production purpose (fattening, dual-purpose). Simmental heifers were of the oldest age at first calving and had longest duration of first service-period, while the best reproductive parameters had Hereford breeding females and small format crossbreds (fattening and dual-purpose). Increased body mass of adult animals had no significant effect on bovine females reproductive results. It was confirmed that higher daily yield of milk by 1 kg in first-calf heifers can influence the increase of the number of servings per pregnancy by 0.017 times and longer duration of the first service-period by 1.08 days.

According to the results of Veselinović et al. (1991) during studying hormonal preparations in estrus synchronization in heifers, besides recommendations given for using a specified preparation it was concluded that the application of vitamin preparations in combination with hormones can have positive effects as well.

For the purpose of shortening the duration of service-period, the effect of postpartum improved nutrition is also applied what can influence better milk yield and therefore calves live weight gain as well. For earlier possible occurrence of estrus postpartum certain restrictive methods can be applied such as inserting the nose rings to calves in order to prevent their excessive suckling by which we can influence the maintenance of cow condition. Morris et al. (1993) in New Zealand investigated reproductive and maternal performances of purebred cows and crossbred cows meant for meat production. In experiment were used cows of Aberdin-Angus breed and Hereford which were mated with the bulls of 11 breeds (Aberdin-Angus, Holstein, Hereford, Jersey, Blonde d'Aquitaine, Charolais, Chianina, Limousine, Maine-Anjou, Simmental and South Devon). They studied reproductive and maternal performances of purebred and crossbred cows during first 4 calvings with the first serving in the age of 14-16 months. They analyzed in total 7575 records on mating obtained from 2109 cows. Differences between the animals per locations at which they were raised were more distinctive for the traits of reproduction than for the traits of growth and resulted in interaction of genotype and environment. At all the locations Friesian crossbreds were weaned at largest body mass per animal but they were equalized or surpassed by crossbreds of Jersey breed regarding an efficiency of production of bull calves. Majority of European crossbreds had relatively much higher productivity obtained in the most favorable conditions what was especially expressed in Simental crossbreds. Heterosis effect in crossbreds in relation to average values obtained by pure breeds were as follows for the rate of pregnancy (0.12), productivity (0.21) and the rate of production efficiency (0.16). Heritabilities for body mass and age in puberty were 0.34 for both traits. Repeatability and heritability for cow reproductive traits were low (0.0-0.10), but the values were higher for body mass of bull calves up to weaning taken as bovine female traits (0.09-0.38). Generally speaking, large European breeds which distinguished themselves regarding growth and carcass production produced female offspring which reached puberty at older age, had poorer reproductive performance (especially in less favorable conditions) and larger body mass at mature age.

Silva et al. (1994) monitored the effects of restrictive procedure (inserting the nose rings) in calves suckling period on reproductive parameters of cows in cow-calf system. Reproductive results were registered in first-calf–dual breed crossbreds of Hereford x Friesian and Simmental x Friesian breeds. The effects before and after suckling restriction were monitored. Cows were distributed on 2 pastures during day. Suckling was suppressed or stopped throughout 7 days by inserting nose rings to 46 day old animals in the group on restrictive suckling (RS), while the second group was on continued (control) normal suckling (NS). The cows on restrictive suckling had shorter interval from calving to conception than the cows which suckled normally NS (76.0 ± 5 days, 84.1 ± 6 days, $P<0.05$). The cow breed and nutritional regime had no effect on interval from calving to conception. However, a useful effect of suckling restriction on the days from calving to conception was observed only in cows fed ample grass rations in the middle of gestation. Bullcalves with restrictive suckling (RS) spent more time on pastures during the period when they had nose rings inserted and differences in behavior remained also in the next 5 days after the nose rings were removed (62.4 ± 7 minutes, 38.6 ± 7 minutes on pasture; $P<0.001$), and were lighter at weaning (146.7 ± 3 kg, 162.4 ± 3 kg; $P<0.01$). In spite of lower body mass of bull calves at weaning they came to the conclusion that suckling manipulation by inserting nose rings to bull calves can be used in order to stimulate at an earlier time repeated pregnancy.

Sinclair et al. (1998) studied metabolic status and reproductive parameters in four groups of cattle meant for meat production such as follows: Aberdin-Angus cattle (small size cattle with low dairy potential), Welsh Black breed (small size cattle with high dairy production potential), Charolais breed (large format and body mass cattle with low milk potential) and Simmental breed (large cattle, high milk potential). Over two years the heifers were placed in stable and fed rations made in such a way so as to represent energy intakes from hilly or small hills pastures (Aberdin-Angus and Welsh Black), or from small hills or lowland pastures (Charolais and Simmental) in Great Britain. Average daily intakes were equivalent to 705 and 820 kJ M 0.75 for every breed. For both offered levels of ration energy intake, Welsh Black and Charolais breeds had relatively high rates of weight gain and longest duration of postpartum anovulatory periods. Charolais cows also had the lowest rate of conception in relation to all other breeds. Average interval from calving up to the first ovulation differed significantly among breeds (56.9, 72.7, 73.1 and 52.7 days for Aberdin-Angus, Welsh Black, Charolais and Simmental, $P<0.001$). Postpartum anovulatory period showed tendency of shorter duration from the first up to the second calving in cows with low milk potential while the same anovulatory period showed tendency of increase in cows with high milk potential. Cows with average score for body condition in the value of 2.5 units during calving were sensitive regarding duration of anovulatory period, up to the loss of body mass during early postpartum period, especially when levels of glucose in blood were low, while cows with score for body status in the value higher than 2.5 units were not sensitive during calving.

Goonewardene et al. (1999) analyzed the records for 3 synthetic lines of cattle from a cattle farm in Canada (Kinsella, Canada) obtained during 11 year period. The first line consisted of 60% dairy breeds (Holstein, American Brown and Simmental) and 40% meat type breeds; second line consisted of 33% Aberdin-Angus, 33% Charolais, 20% Galloway and 14% other meat type breeds; third line consisted of 60% Hereford and 40% other meat type breeds. Reproductive traits and the traits of growth of genetically horned and hornless cattle were compared. Number of observations varied from 2663 to 4263

depending on the trait observed. No differences were observed between horned and hornless cattle in all lines for rate of pregnancy, calving and weaning, body mass of calves at birth and body mass at weaning, average daily live weight gains of calves before weaning, frequency of incidence of difficult calvings, results for body mass of cows and body condition of cows during birth of calves and weaning of bull calves. Regardless the absence of significant differences hornless cattle were given an advantage for using in breeding stock.

Correa et al. (2001) analyzed reproductive performance of cows raised within Brazilian Agricultural Research Corporation (Embrapa cattle). The average rate of pregnancy, calculated on the basis of all breeding females (2-3 years old heifers and cows) was 75.9%. Average rate of conception for first-calf heifers was 62.1%. The average rates of total mortality and mortality until weaning were 2.2 and 6%. Mortality rate in bull calves was 53.7% out of total mortality rate in the herd and the sex had a significant effect ($P<0.01$) on rate of mortality in bull calves, the mortality of male animals being higher than mortality of female animals. Average interval between calvings was 463.45 days (15.2 months) and it was influenced significantly ($P<0.05$) by the year of cow birth. The authors suggested further research with the aim of solving higher rate of mortality in males whose commercial value is deemed higher than that of females.

Possibilities of improving the fertility traits in cow-calf system

Fertility traits in cows represent a common problem in all production systems and directions. Improvement in the production technology, in the first place, by proper balancing of rations in some bovine production phases does not seem to be sufficient for obtaining optimal bovine females fertility at an annual level.

A positive effect of improved nutrition on the occurrence of estrous is well-known in breeding females of all kinds of domestic animals. The results of research reported by Morris et al. (1993) can confirm this. The results of other authors (McClure, 1961., Staples et al., 1990., Beam and Butler, 1998), cited by Vuković et al. (2011) show that fertility disorders can occur as a consequence of poor nutrition (especially energy deficient rations) and that there is an effect of negative energy balance and high milk yield postpartum on the state of cow fertility and the occurrence of ovarian cysts. Glucose contained in ration is necessary both for milk synthesis and for normal functioning of central nervous system (CNS-a) including hypothalamus and hypophysis where GnRH, that is, FSH and LH are being synthetized. Meeting the needs for milk synthesis, particularly postpartum, has a priority in relation to other needs what results in a state of sexual inactivity (Lotthammer 1985).

The occurrence of estrous in cows, after uterus involution, depends a good deal on cow body condition. Besides nutrition the state of cow condition in cow-calf system is highly influenced by cow milk yield in the first months postpartum as well as by daily length of suckling. The cows with higher milk yield will certainly have an effect on higher gains in calves in suckling period but a body condition of those cows will at the same time be worse in comparison with the cows with lower milk yield if they are compared in the same breeding conditions.

As they grow up, calves needs for food intake are greater so milk as nutrient is not sufficient for meeting their daily needs in nutritional materials. Because of that older calves try to follow the cows and suckle the cow`s milk constantly in order to satisfy their needs and that has a significant harmful effect on cow condition. In practice the inserting of nose rings to calves proved justified in order to prevent excessive suckling. Silva et al. (1994) monitored the effects of restrictive procedure (inserting nose rings) in calves suckling period after the age of 46 days on cow reproductive parameters. Cows with restrictive suckling had shorter service period compared to cows which suckled normally (76.0±5 days, 84.1±6 days, $P<0.05$).

In the conditions of intensive cattle production, like milk production, the success is mostly dependent on good fertility in breeding stock. However, in cow-calf system which is in its nature either semi-intensive or extensive, the success in production can even depend more on normal fertility in cows. Taking into account that one calf obtained per cow is the only annual income, besides manure, it seems to be imposed as an aim to have interval between consecutive calving of 365 days what would be considered as an optimal fertility in cow-calf system.

Realization of mentioned aim in cow fertility, meaning a length of interval between calvings of 365 days is today almost impossible to obtain without induction and estrus synchronization by means of hormonal preparations. In our climate conditions the induction and estrus synchronization in cows in cow-calf system should be coordinated with the season as well. The aim is to obtain calves (calving) in the period of a year with best conditions , primarily regarding nutrition and reduction in fattening cost and that is at the start of spring.

Preparations of prostaglandin and its analogues in several different protocols are used in induction and estrus synchronization, while for the synchronization of preovulatory follicle development and induction of ovulation we use preparations based on GnRH and progestin in combination with prostaglandin.

Prostaglandin $PgF_2\alpha$ and its synthetic analogues are used in regulation of estrous cycle in cows. As reported by Vakanjac and Maletić (2013) the effect of prostaglandin depends on the stage of cycle in which it is applied, i.e. the phase of development of corpus luteum. Corpus luteum reacts on treatment with prostaglandin from 5th (heifers), i.e. 7th day (cows) of estrous cycle to 17th day when spontaneous luteolysis by endogenous prostaglandins originating from endometrium begins. For these reasons it seems unjustified to apply hormone in the period before 5th day and after 17th day of cycle. The occurrence of estrus after application of prostaglandin or its analogues depends on the stage of development of dominant follicle in the moment of hormone application. If the preparation is applied in the time of growth of dominant follicle of the first follicular wave we can expect the occurrence of ovulation in 2-3 days. If the preparation is applied in later phase when follicle has lost its dominance the ovulation can be expected only in 4-5 days when dominant follicle, which is going to ovulate, separates from the second follicular wave.

DeJarnette et al. (2001), as well as Dogan et al. (2008) report the results of hormonal treatments on the incidence of estrous, that is, application of various protocols in induction and estrus synchronization in cows.

Vakanjac and Maletić (2013), citing the results of other authors state following protocols for induction and estrus synchronization by applying prostaglandin preparations:

Prostaglandin administered once: This method means administration of hormones to only those cows on whose ovaries, by clinical examination or by ultrasonography, as well as by measuring concentration of progesterone in milk (> 2 ng/ml) or in blood (> 5ng/ml) functional corpus luteum was confirmed. Advantages of this method are reflexed in its economical aspect and a number of breeding females that in 5-7 days after intensive monitoring enter estrous and become inseminated. The authors state that 30% breeding females do not enter expected estrous after hormone application.

Prostaglandin administered twice: In this method the breeding females in which estrus was not observed after the first application of prostaglandin get another application of the second dose in the interval of 11 to 14 days. All the cows that have normal duration of cycle, should, within 3 to 5 days after the second application of prostaglandin, show the signs of estrous regardless the phase of estrous cycle in which they were after the first dose of prostaglandin. There is also a variant of this method in which prostaglandin is administered twice to breeding females which do not show signs of estrous and at the same time do not become pregnant 60 or more days after calving. They get two doses of prostaglandin applied ($PgF_2\alpha$) in the interval of 11 to 14 days even regardless whether there are or there are no visible signs of estrous and insemination is being performed 72 to 96 hours after the second application of prostaglandin.

Several times given prostaglandin: In this method $PgF_2\alpha$ is administered three times in the intervals of 11 to 14 days during which the animals are being controlled between the application of hormones and breeding females that enter estrous gets inseminated. Breeding females which do not show the signs of estrous even after the third dose of $PgF_2\alpha$ are subjected to fixed-time artificial insemination 70 to 80 hours after administration of the third dose.

The results of fertility during cow insemination after the application of prostaglandin for purposes of estrous induction are by 20-30% lower compared to common way of discovering estrous in breeding stock. It happens because prostaglandin can induce estrous but leads to no synchronization of the growth of follicles and LH peak what reduces the fertility.

Therefore if the synchronization of ovulation is the aim we can use prostaglandin ($PGF_2\alpha$) and gonadotropin-releasing hormone (GnRH) in exactly determined time, i.e. so-called "Ovsynch" program. There are several modifications of "Ovsynch" program, such as Co-Synch, Pre-Synch, Select-Synch, Hybrid- Synch. Details regarding each modified "Ovsynch" program can be read in the paper by Vakanjac and Maletić (2013) as well as in the papers of some other authors. In addition, the same authors state the application of progestagene in bovine estrus synchronization - CRESTAR and CIDR program. CIDR (controlled internal drug release) technology means using the progesterone impregnated vaginal sponges (1.38 gr) where via vaginal mucus the progesterone is being resorbed. In this way luteal phase of estrous cycle is being prolonged, the occurrence of estrous prevented and all treated breeding females brought into the same phase of estrous cycle.

Conclusion

On the basis of the results reported in previous research papers on production and reproductive traits of cows raised in cow-calf system it can be concluded that reproduction, that is, poorer expressed traits of fertility in cows can be a limiting factor in cow-calf production system.

In order to improve fertility traits various improvements and modifications of production technology are recommended. The application of hormones for the induction and estrous synchronization in cows with the aim of increasing production economy seems to be essential nowadays. Besides all protocols used for induction and estrous synchronization in cows a special emphasis was given to the importance of the application of prostaglandin (PGF$_{2\alpha}$) which is most widely used today both in dairy cows breeding stocks and in cow-calf system.

Regarding tendencies in beef industry in majority of European countries (surplus of produced milk quantities and unsolved placement of milk, reduction in the number of dairy cattle, reduction in total produced quantities of beef meat as a consequence of reduction in total number of cattle, primarily, reduction in the number of dairy cattle) a cow-calf system should certainly be an alternative and one of the ways to solving current problems and negative tendencies.

Acknowledgement

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Invited paper

MONTHLY AND DAILY VARIATION OF TEMPERATURE-HUMIDITY INDEX (THI) ON DAIRY CATTLE FARMS

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Abstract

The aim of this study was to analyze the variability of temperature-humidity index (THI), depending on the influences of farm, year, month and daily hour of measurement. A systematic measurement of the temperature (°C) and relative humidity (%) was carried out in the stall-barns for dairy cattle housing, in the period from March 2014 to September 2016. More than 230000 individual values of THI from nine dairy cattle farms were analyzed. To analyze the variability of THI, a fixed model with the effects of the farm, year, month and hour of the measurement was used. Observed throughout the year, the influence of month and daily hour of measurement were highly significant. The most critical months with average THI above 72 were June, July and August. September also had average THI very close to 72, while in May an average THI was above 68. High determination of the model for THI (75%), with high significance of all the factors included in the model, indicates that the farm with all its determined and undetermined effects, together with the month and daily hour of measurement, represents very important sources of variation that may affect the more frequent occurrence of heat stress in dairy cattle and, consequently, to jeopardize milk production. All of this suggests that when designing the stall-barns for dairy cattle housing, special attention should be paid to technological solutions for proper maintenance of the microclimate in them, especially taking into account present changes in climate and the increasing occurrence of short-term, but extreme, weather events.
Keywords: Milk production, heat stress, temperature, relative humidity, source of variation

Introduction

The negative effect of heat stress on domestic animals has been very well known since the ancient time. Expression of this negative impact is varying a lot among species, breeds and individual animals but the most important effect is associated with the decreasing of production and reproduction traits (De Rensis et al., 2015). Among the numerous farm husbandry conditions that have more or less significant influence on dairy cattle and milk production, the most important challenges in modern dairy cattle management is to maintain appropriate microclimate (Herbut and Angrecka, 2012), especially in the light of the present changes in climate and the increasing occurrence of short-term, but extreme, weather events (Bogdanović et al., 2015). Among the all microclimate   factors

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that may have influence on dairy cattle and milk production the most important are air temperature and relative humidity as well as their relationship expressed as so-called temperature-humidity or thermal-humidity index (THI).

The estimated range of temperature for highest efficiency of energy utilization in dairy cattle is about 13-18°C. Nevertheless, significant changes in feed intake or in numerous physiological processes will not usually occur within the range between 5 and 25°C (National Research Council, 1981). Moreover, the temperature range from -0.5 to 20°C has little effect on milk production (Herbut and Angrecka, 2012). However, every increasing in air temperature above 25°C, especially in joint combination with higher relative humidity, approaching surrounding environment to the upper critical temperature, which can be reached even at the 25-27°C (Herbut and Angrecka, 2012). In such environmental conditions heat stress is being very easily expressed, consequently leading not only to the health and welfare problems in dairy cows but also to the decreasing of the overall profitability of milk production.

The most common indicator for the assessment of heat stress in dairy cows is temperature-humidity index (THI) (Kučević et al., 2013). The combined effect of temperature and relative air humidity is used to calculate the temperature-humidity index and in relation to established values provides a satisfactory assessment of potential heat stress (Akyuz et al., 2010). It is considered that milk production is endangered if the THI values exceeds 72, which corresponds to an air temperature of 22°C and relative humidity of 100%, air temperature of 25°C and relative humidity of 50% or air temperature of 28°C and relative humidity of 20% (Gantner et al., 2011). In the range of THI of 72-78 the milk production is seriously affected and cooling of the animals is necessary. In the dangerous range at THI of 78-82 production and reproduction traits are severely affected, while in the emergency range at THI values of 82 and above, deaths of cows may easily occur and cooling of the animals is absolutely essential (Brouček et al., 2007).

Previous researches have been more focused on identifying the relationship between the THI and milk production and the negative effect of increasing THI on milk yield and milk quality has been very well documented (Ravagnolo et al., 2000; West et al., 2003.; Freitas et al., 2006; Brouček et al., 2007; Brouček et al. 2009; Bertocchi et al., 2014). In contrary to the relationship between the THI and production or reproduction traits, the relatively smaller research attention has been paid so far to the variability of THI and source of variation for THI. Having in mind that the production of cow's milk in Serbia is organized on farms with very different capacities, which mutually differ in relation to agro-ecological, zootechnical and socio-economic conditions (Bogdanović et al., 2015), the aim of this study has been to analyze the effect of the month and daily hour of measurement on the overall variability of THI values.

Material and Methods

In order to determine the monthly and daily variability of the temperature-humidity index (THI), a systematic measurement of the air temperature and relative humidity was carried out on 9 dairy cattle farms in the period from March 2014 to September 2016. This study included dairy cattle farms of different capacities that are located in the vicinity of Velika Plana (two farms), Čačak (two farms), Šabac (one farm), Bačko Gradište (one farm), Pančevo (one farm) and Loznica (two farms).

The measurement of the temperature and relative air humidity in dairy cattle stall-barns was carried out using the device for automatic registration of microclimate parameters, i.e. Data logger AMTAST, AMT-116. The measurements were carried out continuously every 60 minutes during all months included in the research period.

The temperature-humidity index (THI) is calculated as follows:

$$THI = (1.8T + 32) - ((0.55 - 0.0055RH) \times (T - 26.8)) \text{ (Dunn et al., 2014)}$$

where is: T = temperature of the air (°C) measured in cattle stall-barns, RH = relative humidity of the air (%) measured in cattle stall-barns.

After initial data review and preparing, a set of total of more than 230000 individual values of THI were analyzed. To analyze the variability of THI, a model with the effects of the farm, year of the measurement, month of the measurement and hour of the measurement as fixed factors was used. This model was used for analysis not only the variability of THI but also the variability of temperature and relative humidity. The model equation was as follows:

$$y_{IJKL} = \mu + F_I + Y_J + M_K + H_L + e_{IJKL}$$

Where is: y_{IJKL} = the observed trait, μ = population mean value for given trait, F_I: fixed effect of the farm (1,..., 9), Y_J: fixed effect of the year of the measurement (2014, 2015, 2016), M_K: fixed effect of the month of the measurement (1,..., 12), H_L: fixed effect of the hour of the measurement (0,..., 23), e_{IJKL}: other non-determined effects or random error with characteristics N (0, σ2). This model was chosen because it had the highest coefficient of determination by keeping the significance of all the included factors. The variability of THI was analyzed using statistical procedures PROC FREQ and PROC GLM of the SAS statistical package (SAS 9.1.3, 2007).

Results and Discussion

Table 1 shows the descriptive statistic parameters for the values of the air temperature, relative humidity and temperature-humidity index in relation to the year, month and daily hour of measurement.

Table 1. The values for temperature, relative humidity and THI (Mean ± SD (min-max))

Factor: Year, Month and Time	Temperature (°C)	Relative humidity (%)	Temperature-humidity index (THI)
2014	19,5 ± 6,6 (-7,7 – 40,3)	72,5 ± 13,9 (9,6 – 100)	68,1 ± 11,2 (20,6 – 98,6)
2015	14,4 ± 7,01 (-14,2 – 45,4)	72,1 ± 18,6 (0,0 – 100)	59,5 ± 11,9 (20,4 – 109,7)
2016	15,1 ± 7,3 (-11,9 – 41,0)	66,9 ± 21,9 (0,0 – 100)	61,1 ± 12,2 (13,2 – 99,5)
January	8,8 ± 4,2 (-11,9 – 23,1)	74,8 ± 25,5 (0,0 – 100,0)	50,4 ± 7,5 (13,2 – 74,6)
February	10,1 ± 3,8 (-0,8 – 23,9)	78,1 ± 18,1 (0,0 – 100,0)	52,0 ± 6,9 (32,2 – 76,3)
March	12,3 ± 3,5 (0,0 - 26,9)	73,8 ± 15,2 (0,0 – 100,0)	56,1 ± 6,5 (36,9 – 80,4)
April	16,4 ± 4,9 (1,9 – 31,5)	62,6 ± 14,4 (18,5 – 97,5)	63,4 ± 8,2 (36,4 – 86,8)
May	19,9 ± 4,5	69,5 ± 13,5	68,8 ± 7,6

	(6,8 – 34,9)	(18,9 – 98,7)	(46,0 – 91,6)
June	23,0 ± 3,8	67,3 ± 13,8	73,9 ± 6,2
	(-0,5 – 41,0)	(5,9 – 100,0)	(43,3 – 99,5)
July	25,5 ± 3,6	66,7 ± 12,5	78,0 ± 5,8
	(13,0 – 40,3)	(18,5 – 100,0)	(57,1 – 98,5)
August	24,8 ± 3,7	68,0 ± 14,3	76,9 ± 6,0
	(11,5 – 45,4)	(18,5 – 100,0)	(53,6 – 109,7)
September	21,4 ± 3,9	72,6 ± 14,5	71,1 ± 6,6
	(7,8 – 38,7)	(9,6 – 100,0)	(46,6 – 96,5)
October	16,4 ± 4,4	72,1 ± 17,7	63,1 ± 7,6
	(1,8 – 30,3)	(15,9 – 100,0)	(36,6 – 85,4)
November	12,1 ± 4,6	72,6 ± 18,2	56,0 ± 8,1
	(-0,3 – 27,8)	(16,8 – 99,0)	(32,5 – 81,9)
December	8,8 ± 3,8	78,1 ± 20,2	49,9 ± 6,9
	(-14,2 – 19,5)	(18,5 – 99,7)	(20,6 – 68,7)
00:00 h	15,6 ± 6,7	74,3 ± 16,8	61,6 ± 11,4
	(-10,5 – 31,0)	(0,0 – 100,0)	(14,9 – 86,8)
02:00 h	15,0 ± 6,6	74,8 ± 17,1	60,6 ± 11,2
	(-10,9 – 29,9)	(0,0 – 100,0)	(14,3 – 85,2)
04:00 h	14,5 ± 6,5	75,3 ± 17,4	59,6 ± 11,2
	(-11,9 – 29,5)	(18,5 – 100,0)	(13,2 – 84,6)
06:00 h	14,2 ± 6,7	76,0 ± 17,1	59,1 ± 11,5
	(-11,6 – 41,0)	(13,9 – 100,0)	(14,2 – 99,5)
08:00 h	15,1 ± 6,9	74,6 ± 16,5	60,8 ± 11,8
	(-10,8 – 31,9)	(18,5 – 100,0)	(15,9 – 87,9)
10:00 h	16,4 ± 7,3	71,1 ± 17,2	63,0 ± 12,4
	(-9,3 – 35,6)	(18,5 – 100,0)	(19,6 – 93,3)
12:00 h	17,7 ± 7,6	67,8 ± 17,9	65,1 ± 12,8
	(-9,8 – 37,1)	(9,6 – 100,0)	(23,5 – 95,3)
14:00 h	18,3 ± 7,8	65,9 ± 18,3	66,2 ± 12,9
	(-7,1 – 39,3)	(18,4 – 100,0)	(22,1 – 98,2)
16:00 h	18,4 ± 7,9	66,1 ± 18,2	66,3 ± 13,1
	(-8,0 – 44,7)	(18,5 – 100,0)	(20,0 – 108,2)
18:00 h	18,1 ± 7,8	68,5 ± 17,3	65,7 ± 13,0
	(-10,0 – 45,4)	(18,5 – 100,0)	(20,7 – 109,7)
20:00 h	17,2 ± 7,3	71,3 ± 16,6	64,3 ± 12,4
	(-8,8 – 33,4)	(0,0 – 100,0)	(18,5 – 89,8)
22:00 h	16,3 ± 6,9	73,2 ± 16,7	62,8 ± 11,7
	(-13,0 – 32,4)	(0,0 – 100,0)	(17,4 – 89,1)

It can be noticed from Table 1 that in all 3 analyzed years an average value of the THI was below the critical value of 72. However, extremely high maximum values of THI have been recorded in all years which indicate that the potential for occurrence of heat stress existed over multiyear period. The same can be observed for THI in relation to the month of measurement. Three critical months, when the occurrence of heat stress is not only highly probable, but represents a real danger, are June, July and August. During all of these three months, the average values of the THI were above 72. Moreover, potentially dangerous months were also September in which recorded values of the THI were close to critical threshold of 72 and May in which recorded values of the THI (above 68) were in the range that indicates the possibility for occurrence of heat stress. In relation to the

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daily hour of measurement, the attention should be paid rather on trend of daily variation of THI than on their absolute values. Despite that an average daily value of THI did not exceed 66, over all hours during the day there is a possibility for recording the extremely high values of THI. This is mainly depending on farm and its husbandry conditions, as a very powerful source of variation, as well as on month of measurement. These results indicate that possibility for occurrence of heat stress is constantly present on dairy farms regardless their technological features. The results of this study coincide with results previously published by Ravagnolo et al. (2000), Bouraoui et al. (2002), Akyuz et al. (2010), Gantner et al. (2011) i Herbut and Angrecka (2012).

The impact of all analyzed factors involved in the applied fixed model was statistically highly significant (Tables 2).

Table 2. Analysis of variance for temperature, relative humidity and THI

Model / Factor	Dependent variable	R^2	df	Significance
Model	Temperature	0,76	44	***
	Relative humidity	0,24	44	***
	THI	0,75	44	***
Intercept	Temperature	---	1	***
	Relative humidity	---	1	***
	THI	---	1	***
Farm	Temperature	---	8	***
	Relative humidity	---	8	***
	THI	---	8	***
Year of measurement	Temperature	---	2	***
	Relative humidity	---	2	***
	THI	---	2	***
Month of measurement	Temperature	---	11	***
	Relative humidity	---	11	***
	THI	---	11	***
Hour of measurement	Temperature	---	23	***
	Relative humidity	---	23	***
	THI	---	23	***
Error	Temperature	---	231483	---
	Relative humidity	---	231483	---
	THI	---	231483	---

Coefficient of determination of 75% for THI and 76% for air temperature, with high significance of all the factors included in the model, indicates that the farm with all its determined and undetermined effects, together with the month and daily hour of measurement, represents very important sources of variation that may affect the occurrence of heat stress in dairy cattle. Moreover, high determination as well as pattern of expression of THI and air temperature indicates that certain similarity in origin of variability in these two variables exists. On the other hand, significantly lower coefficient of determination for relative humidity (24%) indicates that variability of this parameter is under influence of other undetermined effects not included in model. All of this suggests that when designing the stall-barns for dairy cattle housing, special attention should be paid to technological solutions for proper maintenance of the microclimate in

them, especially taking into account present changes in climate and the increasing occurrence of short-term, but extreme, weather events.

Conclusion

In order to determine the variability of the temperature-humidity index (THI) on dairy cattle farms, a systematic measurement of the temperature and relative humidity in the dairy cattle stall-barns, was carried out in the period from March 2014 to September 2016. This study included nine dairy cattle farms of different capacities which are primarily engaged in milk production. The most farms had an average value of the TH index below the critical value of 72, but also all farms recorded extremely high maximum values which indicate that the potential for occurrence of heat stress existed in all analyzed farms. Observed throughout the year, the impact of month and daily hour of measurement were highly significant. High determination of the model for temperature (76%) and THI (75%), with high significance of all the factors included in the model, indicates that the farm with all its determined and undetermined effects, together with the month and hour of measurement, represents very important sources of variation that may affect the occurrence of heat stress in cattle. All of this suggests that when designing the stall-barns for dairy cattle housing, special attention should be paid to technological solutions for proper maintenance of the microclimate in them, especially taking into account present changes in climate and the increasing occurrence of short-term, but extreme, weather events.

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Invited paper

EFFECTS OF ZINC, COPPER AND SELENIUM SUPPLEMENTATION ON DAIRY CATTLE LACTATION AND REPRODUCTIVE PERFORMANCE

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Abstract

Trace minerals are indispensable for preserving health and improving fertility and lactation performances in dairy cattle. Adequate levels of some microelements, such as zinc, copper and selenium added to feeds for dairy cows in lactation period can increase milk production and composition and also reduce the incidence of diseases which are frequent in a postpartum period (mastitis, metritis, retained placenta). Microelements are important for proper functioning of a number of enzymes and proteins which are involved in physiological, biochemical and metabolic processes that contribute milk production. Organic forms of microelements in animal nutrition compared with those from inorganic sources show better biological availability and can be retained longer in the organism. Insufficient quantities of these microelements, inadequate absorption and interaction with other microelements may lead to the impairment of the immune response due to metabolic and oxidative stress.

Keywords: copper, dairy cattle, lactation, reproductive performances, selenium, zinc

Introduction

Trace elements are required in small amounts, usually less than 100 mg/kg dry matter (NRC, 2001) and are present in very minute quantities in animal serum usually less than 2 ppm: zinc 0.8-1.2 ppm, copper 0.57-1 ppm and selenium 50-220 ng/L (Suttle, 2010). Though required in minute amounts, they contribute to immunity and improving reproductive and lactation performances in dairy cow. Dairy cows require zinc, copper and selenium to maintain antioxidant activity of their immune system (Weiss and Hogan, 2005; Joksimović Todorović and Davidović, 2007a; Nemec et al., 2012). These microelements act as cofactors of enzymes like superoxide dismutase, glutathione reductase, glutathione peroxidase, thioredoxin reductase, ceruloplasmin and catalase (Joksimović Todorović et al., 2005; Ran et al., 2010; Hussein and Staufenbiel, 2012). Copper is the essential element in two enzymes that are important for immune competence: copper/zinc-superoxide dismutase and ceruloplasmin. Selenium is involved in the antioxidant system via its role in the enzymes glutathione peroxidase (GSH-Px) and thioredoxin reductases. These trace elements act as antioxidants and prevent oxidative stress by neutralizing oxidants (free radicals like peroxides, super oxides or hydroxyl ions) produced under different stresses like environmental, production or stress

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related to diseases (Joksimović Todorović and Davidović, 2014). Selenium supplementation improves neutrophils phagocytic capacity, while low copper status results in reduced neutrophil phagocytic capacity (Cortinhas et al., 2010; Joksimović Todorović et al., 2016). In dairy cattle, zinc concentrations are decreased during the acute phase response whereas plasma copper concentration may increase (Hayes,1994).

Traditionally, microminerals have been supplied as inorganic salts (oxide, sulfate) in animal feed. In recent years, mainly organic forms of microelements (complexed, chelated amino acids, proteinates), have been administered in animal nutrition because they are more bioavailable and better resorption, can be retained longer in the organism, can be an important tool in maximizing milk production and maintaining health, its antioxidative effect, as well as its lower toxicity (Spears, 2003; Joksimović Todorović and Davidović, 2007b). Consequently, organic forms of the element have been used with increasing frequency by the feed industry. Bioactivity of selenium from selenized yeast is estimated to be 20% higher than in inorganic selenium. Some researcher found that absorption was essentially identical for inorganic and chelated zinc, being about 40%, but that the chelated form of zinc was retained better than the inorganic form of zinc (Hassan et al., 2011). Micronutrients concentrations often decrease around calving and extra supplementation is recommended in some dairy herds. Recommendations given by the National Research Council (2001) are that microelements be added for dairy cow feeds during pre-partum and lactation: zinc in the quantity from 40 to 60 mg/kg dry matter DM (660 and 902 mg/day, respectively), copper intake 10-20 mg/kg DM (252 and 372 mg/day, respectively), and selenium intake 0.1 do 0.3 mg/kg DM (4.3 and 5.7 mg/day, respectively). However, maximum tolerant level in the total diet of cows is 0.5 ppm daily selenized yeast, 35 mg/kg for copper and 150 mg/kg for zinc (Commission Regulation, 2003). Resorption of microelements is further dependent on the quantity thereof in the feed, on age, and interaction with other elements. Low zinc content in earth and feeds, high intake of phosphates and phytates, deficiency of carbohydrates, vitamin A and amino acids in feed conditions the occurrence of hypozincemia. Copper deficiency in cattle is generally due to the presence of dietary antagonists, such as sulfur, molybdenum and iron that reduce copper bioavailability.

Provision of microelements supplements has the effect of increasing the concentration of these micronutrients in the colostrum and milk as well as influencing the production and quality of milk. Supplementation of inorganic and organic sources of microelements in rations for dairy cows can significantly affect the quantity and content of milk while supplementation of organic forms can have a cumulative effect on the yield of milk over a number of lactations. Rabiee et al. (2010) in a comprehensive meta-analysis noted that feeding organic forms supplements as replacement for inorganic sources before calving and then during the following full lactation was the best strategy for increased milk production and improved reproductive performance (reduced days open and number of services per conception). On the other hand, Kincaid and Socha (2004) and Chester-Jones et al. (2013) compared inorganic to a combination of inorganic and organic sources of microelements in Holstein dairy cows beginning 21 d prepartum until 150 DIM and they observed that no differences in milk yield and composition.

Some authors reported that mineral matters supplementation can have either a negative, neutral (Vanegas et al., 2004) or positive effect (Sales et al., 2011) on reproductive performances. Increasing supplementation organic forms postcalving improved first

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service conception rate and reduction in days to first estrus (Ahola et al., 2004; Chester-Jones et al., 2013). Toni et al. (2007) and Siciliano-Jones et al. (2008) partially replaced inorganic sulfate forms (zinc, coper and manganese) with amino-acid complexes microelements and observed improved lactation performance and reduced culling rates.

Effects of selenium, zinc and copper on lactation performance in dairy cows

Copper and zinc requirements of cattle, on a mg/kg DM basis, are highest in early lactation (NRC, 2001). Zinc is an essential trace element involved in general protein metabolism and in the catalytic, structural and regulatory processes of keratinization. For that reason, zinc plays important role in teat canal keratin synthesis, maintaining structural integrity and health of the udder. The reduction of SCC with quick formation of keratin in teat canal provided by the supplementation of Zn (Kinal et al., 2007). Feeding organic zinc may have enhanced resistance to pathogens that cause mastitis because of improved skin integrity and keratin lining of the teat canal. Like zinc, copper is important for keratin formation. Risks of metritis, mastitis, locomotion problems or diarrhea in calves are increased when zinc or copper levels are either marginal or deficient (Enjalbert et al., 2006). Feeding specific amino acid complexes of zinc, copper and selenium reduces the incidence of new subclinical and clinical mastitis, decrease somatic cell count (SCC), increased milk production (Griffiths et al., 2007; Machado et al., 2013; Nayeri et al., 2014), but does not alter the concentration of serum superoxide dismutase, glutathione peroxidase or ceruloplasmin (Cortinhas et al., 2010). Zinc, copper and selenium supplementation has been associated with higher antioxidant capacity resulting in reduced somatic cell count during the first 140 days of lactation, and improvement of milk production performances in dairy cows.

Impacts of the supply of organic trace minerals on milk yield and composition are variable. Partial substitution of zinc and copper sulfates with organic trace minerals during the dry period and lactation resulted in higher immunoglobulin levels in both colostrum and blood serum of newborn calves and lower calf mortality at calving (Formigoni et al., 2011; Nayeri et al., 2014). Griffiths et al. (2007) and Siciliano-Jones et al. (2008) reported significantly effects of trace mineral supplementation with organic sources when compared with inorganic sources on milk yield, but without changes on milk component. Ashmead et al. (2004) following trace mineral supplementation during gestation and the first 180 d of lactation reported a cumulative effect on milk yield during three lactations with organic mineral supplementation. Rabiee et al. (2010) observed that organic forms of microelements increased daily milk production by 0.93 kg, milk fat by 0.04 kg, and milk protein by 0.03 kg. Also, Hasan et al. (2011) founded that the milk yield and fat corrected milk were significantly increased for zinc methionine rations compared with inorganic zinc ration. Production of milk and milk energy increased 6.3% and 5.6%, respectively, in response to complexed organic sources supplementation (Griffiths et al., 2007). Cope et al. (2009) reported a 1.4 kg/day increase in milk yield of cows when they received organic zinc sources as compared with inorganic sources, at the level recommended by NRC (2001), but when the supplementation was at a lower level than recommended, the source had no effect. The use of organic minerals increases the fat-corrected milk yield and the milk fat content without changing dry matter intake, suggesting higher efficiency in the use of energy for milk production (Del Valle et al., 2015). Nocek et al. (2006) observed effects of organic mineral sources on both milk yield

and composition and reported an increase in fat production without changes in the SCC during the first lactation evaluated. Changes in milk production and composition regardless of SCC could be due to the involvement of Zn, Se and Cu in other physiological processes. These authors noted that during the first lactation cows fed inorganic microelements had the least milk production, while greater milk production had cows in the second lactation when fed organic trace elements or combination of organic and inorganic minerals versus cows fed inorganic minerals or those fed a complex organic trace elements at 75% of NRC (2001). Fat and protein levels were variable in both lactations. On the other hand, some authors did not determine any significant differences in milk yield and composition in individuals fed inorganic and organic source of zinc, copper and selenium (DeFrain et al., 2009; Nemec et al., 2012; Cortinhas et al., 2012).

Trace elements are also important for hormone synthesis which plays an important role in growth and milk production (Suttle, 2010). Zinc is involved in hormone secretion and function (somatomedin-c, osteocalcin, thyroid hormones, insulin, and growth hormone). Glucagon and zinc stimulated glycogenolysis by increasing the phosphorylation of glycogen phosphorylase but acted oppositely on glycolysis (Hassan et al., 2011).

Adequate mineral nutrition may be used as a strategy to optimize immune system function, and therefore it may have a positive effect on the defense mechanisms of mammary gland against mastitis (Davidović et al., 2012; Joksimović Todorović et al., 2012). Adequate copper and/or zinc input optimize immune system function by the reduction of the metabolic and oxidative stress. Copper supplementation of a diet reduced the peak clinical response during *Escherichia coli* mastitis.

Milk and milk products are an important source of dietary minerals. Cows milk concentration of zinc ranges from 2-6 mg/l, copper 0.1-0.6 mg/l and selenium 2-60 µg/l (Knowles et al., 2006). The possibility for increasing concentration micronutrients in milk is limited by the biochemical mechanisms of animal homeostasis. Organic selenium is much more efficient than inorganic one at increasing the selenium concentrations in milk what is the result of its better bioavailability (Knowles et al., 2006; Davidović et al., 2014; Joksimović Todorović et al., 2014). Ran et al. (2010) established that the cows fed diets supplemented by organic selenium (5 mg daily) had on 60th, 70th and 90th day after calving 43, 39 and 53% higher concentration of selenium in milk than cows fed with SS. Zinc in cows milk binds primarily to colloid calcium phosphate in casein micelles and 53.7% of copper binds to casein. The zinc concentration in milk is higher than its blood serum concentration (Davidović et al., 2014 and 2015), while copper and selenium concentrations in milk are lower compared to concentration to blood (Davidović et al., 2014; Joksimović Todorović et al., 2015). Concentrations of microelements are generally higher in colostrum than mature milk (Pavlata et al., 2004). Weiss and Hogan (2005) determined that in both groups of cows fed 0.3 mg Se/kg feed selenium yeast or sodium selenite the concentration of selenium in colostrum was 3.8 times higher than in milk. Selenium and zinc concentrations in milk during winter and spring were significantly higher than those during summer and autumn, while the content of copper remained approximately constant during the year (Rodriguez et al., 2001).

Role of zinc, copper and selenium in improving reproductive characteristics and preventing postpartum ailments

Trace minerals are important for reproductive performance in livestock because their supplementation improves reproduction (Chester-Jones et al., 2013) and also via contributing to the normal health of reproductive organs and reproductive cycles (Karkoodi et al., 2012). The results of the research into the effects of microelements supplementation on reproductive performances are controversial.

Chester-Jones et al. (2013) observed that cows receiving supplemental zinc and copper from 100% polysaccharide complexes (POLY) had superior reproductive performance to cows supplemented with 100% inorganic sulfate (SULF) or combination of organic and inorganic trace minerals (67% SULF:33% POLY and 67% SULF:33% specific amino-acid complex SULF-AA). Some authors reported a positive response to the complex organic-inorganic microelements compared with inorganic sources on first-service conception rates: 27.4 versus 18.4% (Ballantine et al., 2002), and 27.0 versus 21.0% (DeFrain et al., 2009). In contrast, Chester-Jones et al. (2013) and Siciliano-Jones et al. (2008) found a combination of inorganic and organic microelements reduced days to first service versus inorganic trace elements. Number of days from parturition to estrus, the insemination interval, the service period and number of cows pregnant by 150 days into lactation was lower in cows fed the inorganic sources of microelements than dairy cows fed the organic minerals (Rabiee et al., 2010; Chester-Jones et al., 2013). Campbell et al. (1999) observed that feeding cows additional Zn, Mn, Cu, and Co in the form of Co glucoheptonate and specific amino acid complexes of Zn, Mn and Cu, starting at calving, reduced days to first estrus and tended to reduce days to first luteal activity. Furthermore, reproductive response to additional trace minerals was magnified if cows retained their placenta.

Feeding organic trace elements increased conception rates in cattle, reduced days open (13.5 less days open) and number of services per conception (0.27 less services per conception) (Rabiee et al., 2010) and increased ovarian activity in dairy cows (Boland, 2003). Manspeaker et al. (1987) concluded that supplementation of dairy cow diets with chelated minerals during late gestation and lactation increased fertility because of increased ovarian activity, more effective involution and regeneration of endometrial tissue response, fewer persistent bacterial infections, and less periglandular fibrosis, endometritis and embryonic mortality.

Microelements involved in synthesis of hormones that are important for reproduction (impacting on excretion of gonadotrophins, androgens and prostaglandins, sharing in prolactin release, playing a role in contraction of myometrium during birth). Their deficiency affects both steroid and thyroid hormone production (Boland, 2003). Copper and zinc play an important role in regulating progesterone production by luteal cells via involvement of superoxide dismutase (Sales et al., 2011). Zinc is also involved in the reorganization of ovarian follicles which are the source of progesterone. In dairy cows was established positive correlation between serum progesterone level and copper-zinc (Yildiz and Akar, 2001). Inadequate serum levels of zinc and copper may induce decreased follicular growth and fertility, abnormal estrus, anestrus, and abortions (O'Donoghue and Boland, 2002).

Periparturient period in dairy cows is accompanied by numerous physiological, metabolic and nutritive changes. The way in which they occur and develop have a great influence on lactation performances, subclinical and clinical postparturient diseases, reproductive and immune system function disorders (immunosuppression) (Joksimović Todorović and Davidović, 2007c and 2013a). Antioxidative status may be one of the factors which influences the reproductive functions in dairy cows. Trace mineral supplementation at 230 and 260 days of gestation and 35 days postpartum significantly decreased the incidence of stillbirths, metritis, endometritis and retained placenta, conditions which are known to impair reproductive performance. Systemic trace mineral supplementation decreased the proportion of cows with intrauterine contamination by *Fusobacterium spp.* and *Trueperella spp.*, which are bacteria associated with uterine diseases, especially metritis and clinical endometritis. This was due to some of the trace minerals, particularly selenium and copper, being stored in the liver after supplementation and available during periods of deficiency or high demands (Machado et al., 2013). Retained placenta is one of the major causes of endometritis in cows, reduced fertility, and therefore reduced fertility rate, more difficult conception and can prolong calving interval. Adequate selenium levels have been significance for postpartum uterine involution and reduce the incidence of mastitis and retained placenta (Joksimović Todorović and Davidović, 2013b). Selenium promote the function of neutrophiles affect their migration and hemotoxic activity, elevate the number of neutrophiles in placenta and affect the weakening of links between fetomaternal joint (cotyledon and caruncle). Selenium performs its biological role in organism through enzyme gluthatione peroxidase (GSH-Px). Cows with retained fetal membranes had lower glutathione peroxidase activity in maternal and placental tissues than cows without retained placenta. At certain concentrations of selenium, the activity of GSH-Px reaches plateau, so that further increase of the selenium level results in no increase of the activity of this enzyme. High selenium levels added into food (> 5 mg/kg) lead to no linear increase of the activity of this selenoenzyme. In the first days the selenium activity increases in animal blood plasma, but only 10 days later a significant fall occurs therein (Joksimović Todorović and Jokić, 2005; Joksimović Todorović et al., 2005).

Marginal copper deficiency resulted in reduced neutrophil killing and decreased interferon production by mononuclear cells. Insufficient intake or disturbance in the copper and zinc absorption can weaken the immune system due to metabolic and oxidative stress and development of mastitis, metritis and disorder in muscle - skeletal system (Cortinhas et al., 2010).

Conclusion

In this review, resent researches on micronutrients supplements for improving the milk and reproductive performances of dairy cows were considered. Intensification of production and increase of the milk yield of cows requires not only full coverage of the requirements for proteins end energy, but also an appropriate balancing of the microelements with regard to physical form and interaction between the components of the feed provided. While inadequate intake and absorption of certain trace elements may result in in a weakened immune system functions, lower milk production and decreased fertility, unjustified supplementation can be expensive and lead to health problems.

Acknowledgment

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Oral presentation

FIXED EFFECTS IN MODELS FOR GENETIC EVALUATION OF GILTS FROM FIELD TEST IN VOJVODINA

Mirkov M.[1], Radović I., Škorput D[2], Luković, Z.[2]

Abstract

Fixed effects in the model for backfat thickness and time on test in performance tested gilts were evaluated. Data records from 138 large-scale farms in Vojvodina in the period from January 2001 to December 2015 were analyzed. In total, 71,531 data records from field test of gilts were included in the study. Analysis included three breeds (Landrace, Large White, Pietrain) and reciprocal crossbreds of Landrace and Large White. Least square method implemented in SAS GLM procedure was used to analyze fixed effects on traits obtained in field test of gilts. Two statistical models were developed: one for each trait analyzed. Effects tested in the models were: genotype, farm, season of the test, and weight at the end of the test. The model for time on test was simpler than model for backfat thickness, containing only fixed effects of genotype, farm and season of the test. The weight at the end of the test was included in the model for backfat thickness. Choice of the model and effects in the model was based on coefficient of regression and degrees of freedom. All effects in the models were statistically significant. The coefficient of determination of the model for backfat thickness was 0.44, while the coefficient of determination for model for time on test was also 0.44. Obtained results are the first step in setting up statistical model for genetic evaluation using best linear unbiased prediction and will be useful in future development of the statistical model for prediction of breeding values for economically important traits in gilts in performance test in analyzed population.
Keywords: pigs, backfat thickness, time on test, performance test, fixed effects

Introduction

Field test presents the basis for selection of gilts for production traits in Vojvodina (Mirkov et al., 2015). Selection of the pigs in Vojvodina is currently based on selection index, taking into account economically important productive traits, such as growth rate, time on test, backfat thickness, or lean mean content. However, due to comparative advantages over the selection index (Keele, 1998; Vincek et al., 2004), BLUP (Best Linear Unbiased Prediction) procedure might become the method of choice it the future for pig breeding program in Vojvodina. BLUP procedure is based on the mixed model methodology developed by Henderson (1975). Countries around the world use different statistical models and effects in the models to fit the data. Effects like genotype, sex, season, body weight, herd-year-season interaction, interaction between genotype and season, interaction between weight and genotype are usually applied   in the models for

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field test traits. Specific production conditions in Vojvodina require determination of appropriate effects in the statistical model for prediction of breeding values for traits such as backfat thickness and time on test. Choice of the effects in model is the critical point in development of models used for genetic evaluation of the pigs (Satoh *et al.*; 2002; Gorjanc *et al*, 2001). Thus, the aim of the study was to determine fixed effects in the model for genetic evaluation of backfat thickness and time on test in gilts in field test.

Material and Methods

Data records for gilts were provided by Main breeding organization of Vojvodina (Serbia) in the period between January 2001 and December 2015 for 138 large scale farms. Data contain 71,531 data records in the analysis. Only gilts weighting from 80 to 120 kg were included in analysis. Traits measured in the field test of gilts were backfat thickness (BF) and time on test (TT). Time on test presents age at the end of the test, approximately at 100 kg. Genotype, season, and farm were defined as class effects, while weight at the end of the test was defined as linear regression. Analysis included three pure breeds: Landrace, (L) Large White (LW), Pietrain (P) and reciprocal crossbreds of Landrace and Large White (LxLW; LWxL). Season was defined as combined effect year-month. There were totally 102 levels for 15 years. Procedures MEANS and GLM (SAS, 2004) were used for statistical analyses. Development of the fixed part of the model was started by testing single effects of genotype and season for both traits and weight only for backfat thickness, and combining effects mutually. The most complete were final models for both traits. Choice of the model and effects was based on coefficient of determination (R^2) and degrees of freedom (DF) for model and effects separately. Comparison among all LSMEANS was made using the pdiff option and the Sheffè adjustment method.

Results and Discussion

Different statistical models for backfat thickness and time on test were tested (Table 1). Comparison among models was based on the coefficient of determination and degrees of freedom. Analysis showed that models with following effects should be used for traits analyzed: effects of genotype, farm, and season and weight for backfat thickness, and effects of genotype, farm and season for time on test. Coefficient of determination was 0.51 for backfat thickness and 0.59 for time on test when final models were applied. Although all analyzed effects were statistically significant, the largest increase in R^2 was achieved by including farm effect into the model.

Table 1. Development of the fixed part of the model for backfat thickness and time on test

Trait Model	BF Genotype	Season	Farm	Weight	R^2	DF	TT Genotype	Season	Farm	R^2	DF
A	+				0.03	5	+			0.02	5
B	+	+			0.08	106	+	+		0.06	106
C	+	+		+	0.15	107	+	+	+	0.44	243
D	+	+	+	+	0.44	244	/	/	/	/	/

Analysis of the effect of the breed showed that the Large White gilts had the highest value for backfat thickness, while Pietrain gilts had the lowest value (Table 2) for the same trait. There was significant statistical difference in backfat thickness among Pietrain all other purebreds (P<0.05), as well as difference between Landrace and other breeds, where Landrace gilts had significantly higher backfat thickness then Pietrain gilts and significantly lower backfat thickness then Large White and both reciprocal crossbreds. Gogic et al (2012) also suggested higher backfat thicknes in Large White then in Landras and Pietren gilts. The Pietrain gilts spend the longest time in the test, while crossbred between Large White and Landrace stayed shorter in test than other breeds. However, this difference was not significant when compared to other breeds.

Table 2. Effect of the genotype on backfat thickness and time on test

Genotype	Backfat thickness, mm	Time on test, days
Large White	14.25±0.12 [A]	207.41±0.79[A]
Landrace	13.99±0.12[B]	206.83±0.82[B]
Pietrain	10.61±0.29[C]	210.12±0.12[C]
Large White x Landrace	14.39±0.12[A]	205.20±0.80[B]
Landrace x Large White	14.36±0.13[A]	206.04±0.84[B]

A, B, C and D within column means differences between LSMEANS for P<0.01

The regression of body weight to backfat thickness is shown in Figure 1.

The effect of body weight on the backfat thickness is usually included in models for evaluation as covariable. According to Whittemore (1998), feed intake is key factor for the carcass fatness of meat production pigs, and not the rules relating to the time and weight of the body. However, backfat thickness and body weight have a strong linear association which is confirmed by Smith et al. (1992).
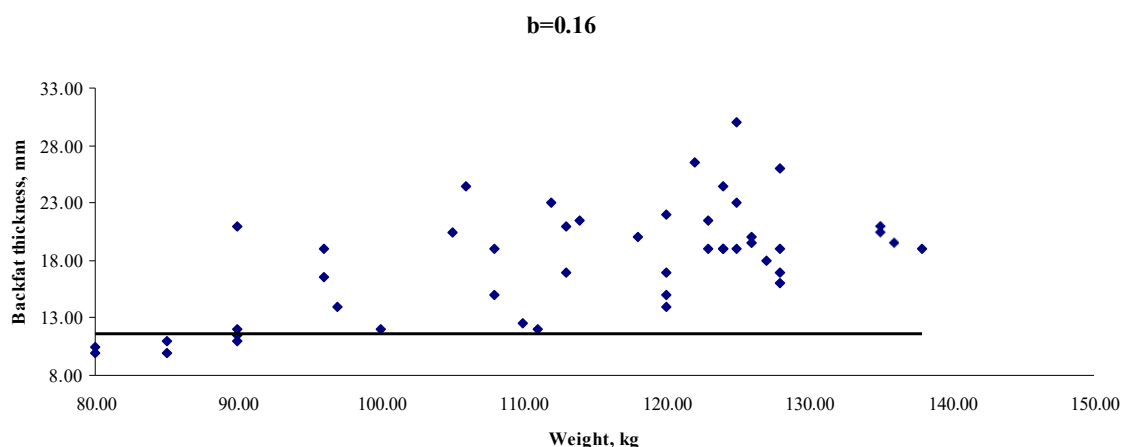
Figure 1. Effect of the weight on backfat thickness at the end of the test

Such linear association is confirmed by this study, where increase in body weight resulted in increase of backfat thickness.

Season had a large effect on backfat thickness (Figure 2). This can be especially observed in short-termed changes, which can be explained by climate changes within year. However, long term changes in backfat thickness can also be observed; but their trend is not continuous trough the whole analyzed period. Favorable decreasing trend after 2012 can be observed, which might be continued with further efforts to improve genetic basis and production conditions.
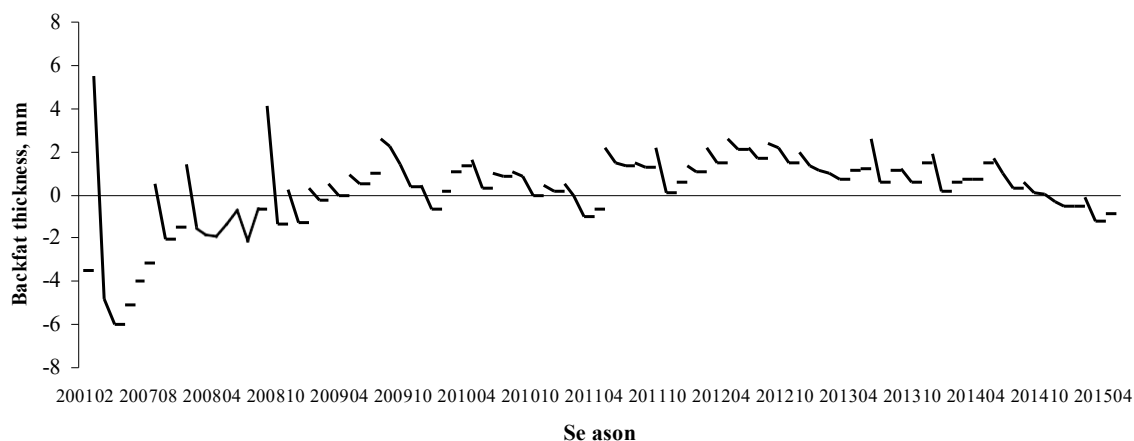
Figure 2. Effect of the season on backfat thickness

Except seasonal changes within year caused by climate conditions, it was obvious that long term changes existed in the time on test existed from the beginning of the analyzed period (Figure 3). Long term changes are also not continuous trough analyzed period, with periods of increase and decrease of the analyzed trait. However, in the end of the analyzed period favorable decreasing trend can be seen. This favorable trend can be consequence of efforts made to improve genetic basis and production conditions.
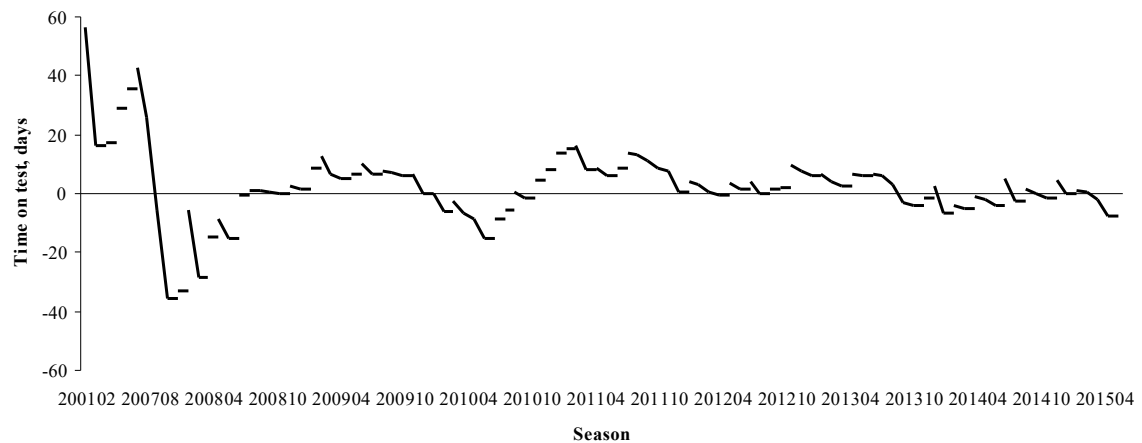
Figure 3. Effect of season on time on test

With inclusion of effect of the farm in the statistical models for both analyzed traits, the highest increase in R^2 was observed. Figures 4 and 5 show big differences between farms in the analysis.
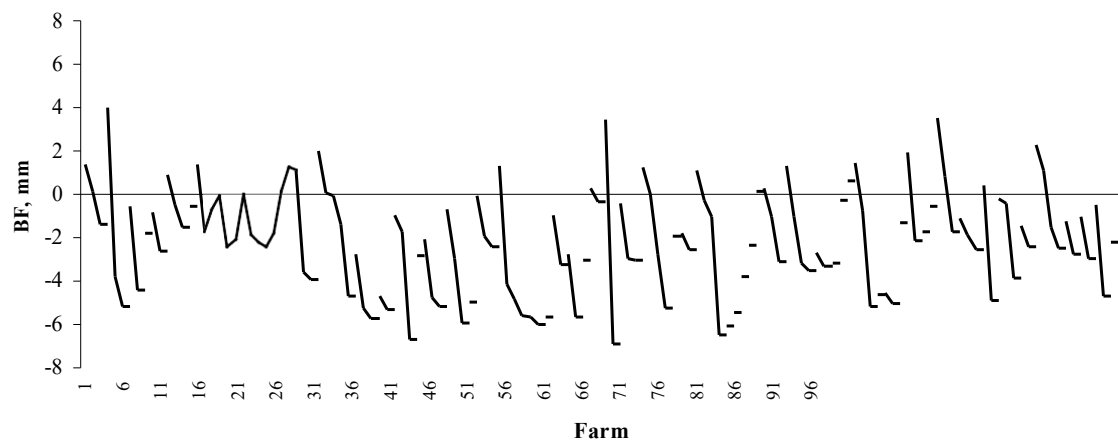
Figure 4. Effect of the farm on backfat thickness

The largest difference between farms in backfat thickness was 14 mm (Figure 4). Such big difference between farms is probably caused by differences in management and production conditions. This difference is expected, given that the farm are trying to increase the number of test heads to increase the selection pressure.
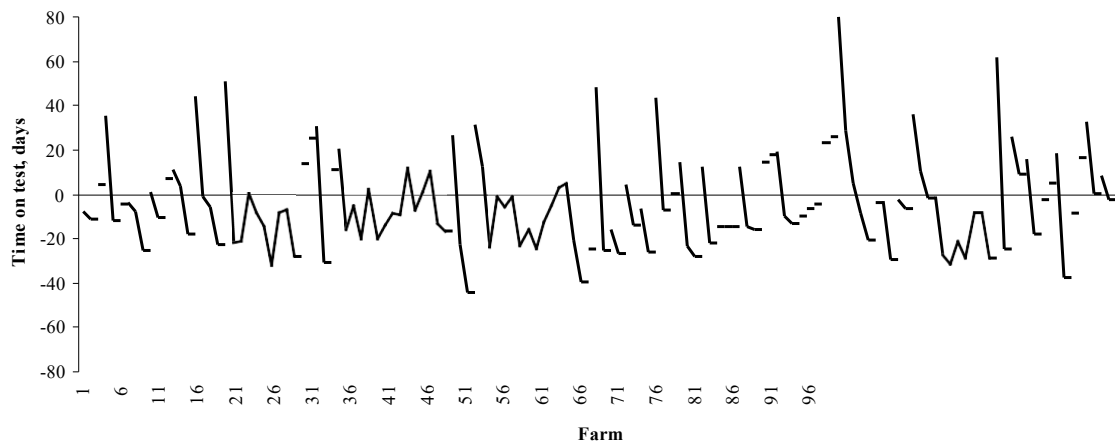
Figure 5. Effect of the farm season on time on test

Big differences between farms included in the analyses can also be seen in time on test. (Figure 5) The biggest difference between farms for this trait was even 125 days. Different management and technological solutions, variable production conditions are probable reasons for large variability between farms.

Conclusion

Analysis of fixed effects in statistical models for genetic evaluation of backfat thickness and time on test showed that all tested effects should be included in the model for prediction of breeding values of gilts on field test, due to their obvious effect on analyzed traits. Especially strong effect of the farm on both analyzed traits was observed. Following step in the development of statistical model for genetic evaluation of productive traits is development of the random part of the model. Further efforts to achieve data quality are required in order to obtain reliable estimates of effects influencing economically important traits.

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Oral presentation

THE 1/29 ROBERTSONIAN TRANSLOCATION IN SOME ANDALUSIAN INDIGENOUS BREED CATTLE

Miguel Moreno Millán[1]*, Delia Saleno[2], Sebastian E. Demyda[2]*

In Memoriam Prof. Dr. Ingemar Gustavsson (2016)

Abstract

In 1985, first data about the incidence of 1/29 robertsonian translocation in indigenous Andalusian Retinta cattle breed (Moreno Millán et al.) came to the fore being detected ever after in all Spanish cattle breeds. In our laboratory were studied cytogenetically more than 5.600 animals belonging to most of them - 4.206 animals until now, to the Retinta breed and the rest to different other breeds (De Lydia, Berrendo en Negro, Berrendo en Colorado, Pajuna, Negra Andaluza and Cárdena) - all of them indigenous from Andalucía.

Since 1989 Retinta breed cattle has been studied cytogenetically. At the beginning, the incidence of 1/29 translocation was 32%, now it stands at around 4.5%. This change is the result of the elimination of bulls carrier for the translocation, both in heterozygote or homozygote stage, as reproducers. In 1989 this decision was taken by the owner's association (Asociación Nacional de Criadores de Ganado Vacuno Selecto de Raza Retinta). Regarding its fertility it was estimated in 1989 to be around 69 % and today it is estimated to be over 80%. Obviously this increase in fertility does not correspond only with the established decision, but corresponds also to complete actions within the Official Selection Program established by the Ministry of Agriculture in which the mandatory cytogenetic analysis is carried out.

Regarding the other Andalusian cattle breeds, all of them endangered, we have initiated the systematic cytogenetics study in Berrendo en Colorado and Berrendo en Negro since 2009 and having convinced the owners to take the same decision as Retinta Association, the incidence decreased from 28,5% to 14,6 % in Berrendo en Colorado; from 49,3% to 23,3% in Berrendo en Negro, both in 2016; as regards the rest of breeds, we started to study them in recent years and we have only limited information and data on them. The incidence of 1/29 translocation in Cárdena breed is now 14,3%; in Negra Andaluza 19.4% and 34.5 % in Pajuna breed. The exception was the incidence in De Lidia breed (Bullfighting breed) in which we observed 0%.

Keywords: chromosomes,1/29 translocation, cattle

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Introduction

Gustavsson and Rockborn discovered in 1964 the robertsonian translocation 1/29 (Figure 1). This is the only translocation in domestic animals with a direct effect on the reproduction of carrier animals in heterozygous stage because this chromosomal alteration produces selection zygotic causing embryonic loss and subsequent reduction of fertility. From 1964 this translocation was found in all European breeds of cattle (*Bos taurus*, L.). On the other hand, there is no evidence of production ex novo of this translocation in cattle. If the founder animal from a herd was not a carrier, the translocation not appears and in the case of carrier animals that ever lived, we must consider that the genetic drift is involved.

The most important effect of this chromosomal alteration is the reduction of fertility just highlighted by Gustavsson himself in 1969. In our Research Group we are studying this reduction and its economic impact in our breeds.

Aims

As Andalucia is a region with a great wealth of indigenous cattle breeds (Retinta, Berrendo en Colorado, Berrendo en Negro, Cárdena, Negra Andaluza, Pajuna and De Lidia), some of them endangered according to FAO, the main objective and interest of this study is to analyze the prevalence of 1/29 robertsonian translocation in these breeds both homozygous and heterozygous stages and to analyze their effects on fertility thereof.

Materials and Methods

Cytogenetic analysis was performed on microscope preparation of metaphase chromosomes obtained after a routine culture of peripheral blood lymphocytes according De Grouchy et al. (1964) method slightly modified. Chromosome spreads were obtained by dropping 100 µl of the cell suspension onto wet slides and flame dried. Giemsa-stained karyotypes were performed in a Cytovision platform (Leica, Madrid, Spain) chromosome using the *Bos taurus* standardization (ISCNDA, 1991).

Results and Discussion

More than 5.600 animals were cytogenetically analyzed in order to identify the carrier of 1:29 robertsonian translocation in five indigenous Andalusian cattle breeds. The Retinta is the most important breed in our region and Figure 2 shows the in incidence of the translocation along the time since 1989 till now. The evolution was a slowly decrease from more than 30% until around 4.5% actually. The decline in the prevalence of translocation was the result of not allowing the use carrier bulls in either heterozygote or homozygote stage as future reproducers. This was a political decision of the Owner's Association (Moreno Millán and Rodero Franganillo, 1992). Gustavsson establish in 1969 that this translocation has a direct effect on the fertility of cows having reduced fertility. In our research group we study this effect on this breed and we have found incidence in calving interval, days at calving and days at first calving (Saleno et al. 2016).

Regarding the other Andalusian indigenous cattle breeds, there is a reduced number of animals analyzed until now because we started its systematic cytogenetically study few

years ago and we have no enough information. Furthermore the effective number of animals in all these breeds is also much reduced. Nevertheless we have obtained results concerning the current situation of the translocation in these breeds (Table I). This was possible because the Owner's Associations are interested to know in what situation their breeds are and, of course, the possible negative effects of the translocation on then. Pajuna and Berrendo en Negro breeds have the highest incidence and especially in both we have stablish a systematic study. It is interesting to observe that in De Lidia breed we have no found any case and we think that it will be the result of an intensive breeding selection by the owners themselves. With these results the Associations established the policy of eradication and we hope the decrease of the incidence of this translocation in these breeds and subsequent increase of their fertility.

Figure 1: Karyotypes of two cows carrying 1/29 translocation. A) Heterozygote stage. B) Homozygote stage.
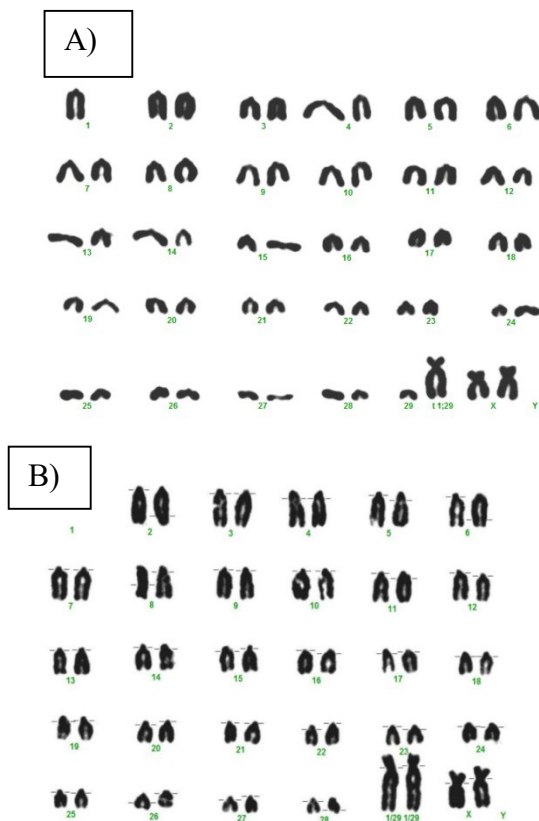
Figure 2: Evolution of the Robertsonian translocation 1/29 in Retinta breed cattle since 1989 till 2016 (%)
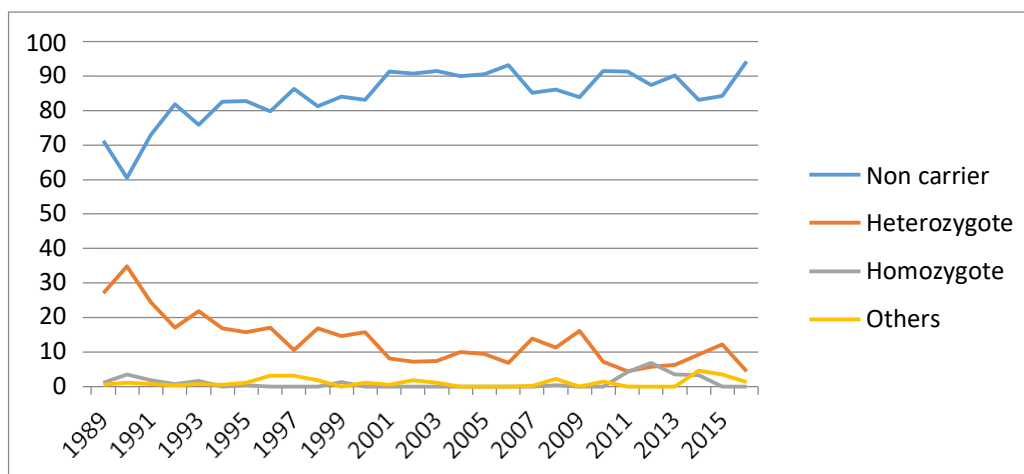
Table I: 1/29 Robertsonian translocation in other Andalusian indigenous cattle breeds (%)

Berrendo en Negro	Berrendo en Colorado	Pajuna	Cárdena	Negra Andaluza	De Lidia
23,3%	14,6%	34,5%	14,3%	19,4%	0%

Conclusion

1-In all Andalusian indigenous cattle breeds the 1/29 translocation is presents except in Lidia breed.

2-With the exception of Retinta and De Lidia Breeds, the effective number of animals in the other breeds is under of 1.000 considering then endangered.

3-Along time the effects of drift, artificial selection and/or adaptation to different environments and the type of reproduction may have eliminated the translocation in Lidia breed.

4- A political decision to avoid the use of carrier Bulls as reproducers allows the Associations to reduce the incidence of the translocation and to increase the reproduction rate.

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Oral presentation

MHC CLASS II DRB1 GENE POLYMORPHISM IN LORI SHEEP BREED

Nanekarani Sh.[1], Khosravi M.[2] , Goodarzi M.[3]

Abstract

The present study aimed at analyzing of ovine major histocompatibility complex class II (Ovar II) DRB1 gene second exon in Lori Sheep breed. The MHC plays a central role in the control of disease resistance and immunological response. Genomic DNA from blood samples of 124 sheep was extracted and a 296 bp MHC exon 2 fragment was amplified using polymerase chain reaction. PCR products were characterized by the restriction fragment length polymorphism technique using Hin1I restriction enzyme. The PCR-RFLP patterns showed three genotypes, AA, AB and BB with frequency of 0.282, 0.573 and 0.145, respectively. There was no significant ($P > 0.05$) deviation from Hardy–Weinberg equilibrium for this locus in this population. The results of the present study indicate that exon 2 of the Ovar-DRB1 gene is highly polymorphic in Lori sheep and could be considered as an important marker assisted selection, for improvement of immunity in sheep.

Keywords: MHC-DRB1 gene, Polymorphism, PCR-RFLP, Lori Sheep

Introduction

Knowledge of the genes underling the expression of a trait will allow researchers to search for novel combinations of alleles to make further improvements in desired traits. The behavior of genes that control a trait is likely to be dependent on the genetic background. Some of these novel allele combinations may result in the improvement of particular traits beyond that which would be possible by selecting phenotypically superior animals within a population. It is therefore important to maintain a diverse range of genetic backgrounds to provide sources of variation (Williams, 2005). The major histocompatibility complex (MHC) class II genes, named Ovar, has been mapped to the sheep chromosome 20 and are considered best candidates for disease resistance. Ovar-DRB1 was chosen as the immune response gene in this study because it is highly polymorphic, and more than 160 alleles have been identified by DNA sequencing of exon 2 from various sheep breeds (Konnai et al., 2003). Accordingly, several studies have been performed in the last years on the MHC-DRB1 gene using of different methods including single-strand conformational polymorphism (SSCP) method in Tibetan Sheep (Shaobin et al., 2015), Mehraban and Lori-Bakhtiari sheep (Zamani et al., 2016), and PCR-restriction fragment length polymorphism (RFLP) analysis in Heath and Lowland Sheep breeds of Polish (Gruszczyñska et al., 2005),

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Rohilkhandi goats (Shrivastava et al., 2015), Sangsari sheep (Jamshidi et al., 2011), Afshari and Zel Sheep (Sohrabi et al., 2013), Makuie sheep (Ashrafi et al., 2014), Lori-Bakhtiari, Shaul, and Zandi sheep (Nikbakht etal., 2012), of Iran and Chinese Merino, Kazakh and Duolang sheep of China (Li et al., 2011). In addition to disease resistance, Ovar-MHC genes have been found to be associated with other economical traits such as weight traits in sheep (Zamani et al., 2016; Ashrafi et al., 2014; Li et al., 2011). Previous studies have reported the use of various restriction enzymes for polymorphism detection of second exon of the DRB1 gene in Iranian sheep breeds; RsaI restriction enzyme in Sangsari sheep (Jamshidi et al., 2011), Shaul, Lori-Bakhtiari and Zandi breeds (Nikbakht et al., 2012), Makuie sheep (Ashrafi et al., 2014), and HaeIII restriction enzyme in Afshari and Zel breeds (Sohrabi et al., 2013). Also, five restriction enzymes, MvaI, HaeIII, SacI, SacII, Hin1I, were used in Chinese breeds (Merino, Kazakh and Duolang sheep) (Li et al., 2011).

In present study, the polymorphism of the class II Ovar-DRB1 exon 2 was detected by PCR-RFLP analysis using Hin1I restriction enzyme in Lori sheep breed. The Lori sheep breed is one of the largest mutton breeds in Iran and the existence of polymorphism in Ovar-DRB1 gene may play a role in selection for resistance to diseases.

Material and Methods

Animals and data collection
In this study, the blood samples of 124 Lori sheep were randomly collected from Lorestan Province of Iran. Approximately 5 ml of blood were collected from each animal in vacutainer containing EDTA and stored at 4°C. Genomic DNA was extracted by salting out method according to (Miller et al. 1988) with minor modifications. Also, DNA quantity and purity of each sample were assessed by spectrophotometer and agarose gel electrophoresis, which were proper for a PCR protocol application.

PCR-RFLP analysis
Based on the method described by (Konnai et al., 2003; Li et al., 2011), we used the Primer sequences OLA-ERB1 (5'-CCG GAA TTC CCG TCT CTG CAG CAC ATT TCT T-3') and HL031 (5'-TTT AAA TTC GCG CTC ACC TCG CCG CT-3') for the first stage of PCR. Reactions were performed in a total volume of 20 µl containing 1.5 mM of MgCl$_2$, 120 µM of each dNTP, 0.2 mM from each primer, 50-100 ng of ovine genomic DNA, and *1.5* U of Taq DNA polymerase. The amplification conditions for primers of the Ovar-DRB1 gene were as follows: a single cycle of 5 min at 94 °C, followed by 15 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s, with a final extension at 72 °C for 10 min. The second stage of PCR was performed with primers OLA-ERB1 and OLA-XRBI (5'-AGC TCG AGC GCT GCA CAG TGA AAC TC-3'). In this stage cycling conditions were: a single cycle of 5 min at 94 °C, followed by 30 cycles of 94 °C for 30 s, 63 °C for 30 s, and 72 °C for 60 s with a final extension at 72 °C for 10 min. PCR products (10 µl) from the second step were digested for 4 h at 37 °C with 5 U of Hin1I in a total volume of 20 µl. The products of enzyme digestion were analyzed by a 1.5 % agarose gel electrophoresis.

Statistical analysis
Genetic diversity parameters, genotype and allele frequencies of Ovar-DRB1 gene were calculated using POPGEN software (version1.32). Hardy-Weinberg equilibrium for the population was also analyzed using Chi-square (X2) test (Yeh and Yang, 2010).

Results and Discussion

The primers used in this study (OLA-ERB1, OLA-HL031, and OLA-XRBI) successfully amplified the 296 bp fragment in sheep (exon 2, of Ovar DRB1 gene) (Figure 1). We analyzed the status of the Ovar-DRB1 polymorphism in Lori breed and restriction enzyme analysis with Hin1I, produced restriction patterns and allele frequencies in accordance with that reported by (Konnai et al., 2003). The Hin1I digestion of the PCR products revealed digestion fragments of 178 and 118 bp (B allele) and non-cutting fragment 296 bp (A allele). The sizes of the bands and genotypes observed in the different Hin1I patterns are illustrated in Figure 2.
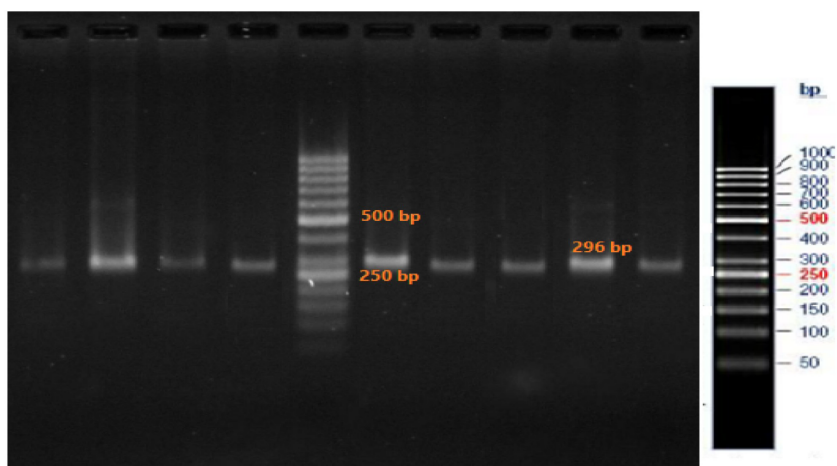
Figure 1. Amplified product of 296 bp of the second exon MHC Class II DRB1
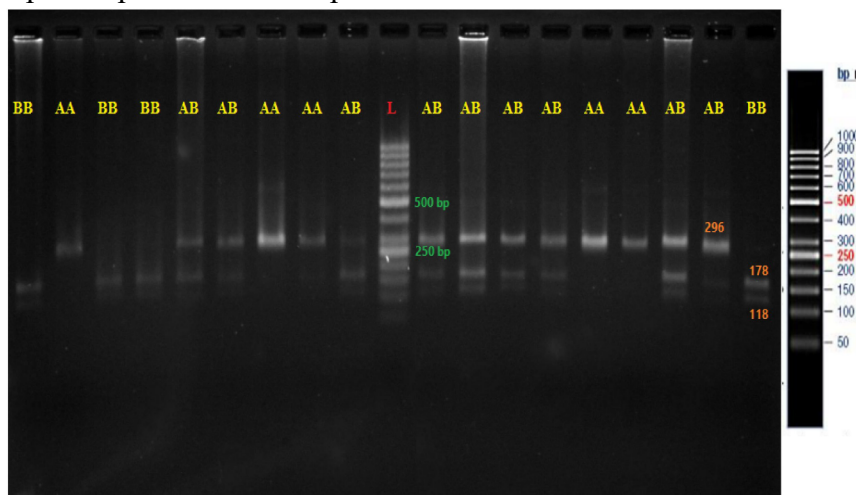
Figure 2. RFLP patterns of PCR amplification of the second exon MHC Class II DRB1 gene, (AA): 296 bp, and (AB):296, 178 and 118 bp and (BB): 178 and 118 bp.

Considering digestion patterns of the studied exon 2 with the Hin1I enzyme, Figure 2 depicts the all possible genotypes were seen. Hin1I alleles were similar to those previously recognized and reported (Konnai et al., 2003; Li et al., 2011).
In addition, the frequency distribution of the patterns are shown in Table 1. The genotypic frequencies for AA, AB and BB in Lori sheep were 0.282, 0.573 and 0.145, respectively. The Hardy-Weinberg test indicated that the patterns observed do not deviate significantly from the theoretical proportions of the study population (P = 0.07). Previous reports have also shown that this population that is breeding randomly within the herd tends to be in HWE

(Yeh and Yang, 2010). In contrast to this report, the patterns produced by Hin1I were not in Hardy-Weinberg equilibrium in Chinese Merino sheep (Li et al., 2011).

Table 1. Allelic and Genotypic Frequencies of the Second Exon OVAR-DRB1 Gene in Lori Sheep Breed

Locus	Allele frequency		Genotype frequency		
	A	B	AA	AB	BB
OVAR-DRB1	0.5685	0.4315	0.282	0.573	0.145

Further analysis of marker data was done to obtain heterozygosity statistics are given in Table 2. The observed number of alleles at this locus was two and the mean effective number of alleles was 1.963. Shannon's information index revealed high genetic diversity within studied population. The mean Shannon's information index value was 0.684 (Table 2). The observed and expected heterozygotes were 0.573 and 0.493, respectively. Also, the F_{IS} value at Ovar-DRB1 (Hin1I) locus was -0.167. The negative value interpret an excess of observed heterozygotes relatively to expected heterozygosity. The structure of the ovine MHC and its association with resistance to various diseases in sheep has received increasing attention during recent years. Study of genetic polymorphism of the Ovar-DRB1 gene were performed in various sheep breeds. Almost all of the results revealed extensive polymorphism at this locus. It should be noted that the level of heterozygosity is one of the most important indexes of genetic diversity in the population. In this study, rate of observed heterozygosity was high. Exactly, as shown in Table 1 and 2 the framework of genetic parameters is appropriate, and this work reports within-breed genetic variation in this population.

Table 2. Genetic Diversity Parameters of the Second Exon OVAR-DRB1 Gene in Lori Sheep Breed

Locus	N_A	N_E	I	H_O	H_E	Nei	F_{IS}
OVAR-DRB1	2.00	1.963	0.684	0.573	0.493	0.491	-0.167

N_A= Observed number of alleles; N_E= Effective number of alleles; I= Shannon's Information index; H_O = Observed heterozygosity; H_E= expected heterozygosity; Nei= Nei's (1973) expected heterozygosity: F_{IS}= Wright's (1978) fixation index

Although meat production deserves the first priority in all regions and all of the Iranian sheep breeds are used as meat producer but some of other traits such as resistance against disease is very important for health of animal and then human. In this context can be pointed to an association between some genotypes and hydatidosis resistance in some breeds (Sohrabi et al., 2013; Salles et al., 2011).
MHC genes combined with microsatellite loci behave as highly polymorphic molecular markers and are useful for breeding and conservation programs and potentially linked with resistance against disease (Yasmeen et al., 2014; Salles et al., 2011). Whereas the usefulness of genetic markers depends upon the heterozygosity coefficient and their polymorphism. The new information from genetic markers mainly affects the accuracy of selection. Hence, in MAS schemes, genetic gain is mainly increased by enhancing the accuracy of selection.

Then, the strategy of MAS for susceptibility traits of infectious diseases has many advantages. The acquire time and capital, Elusion of drug use and to avoid side effects are additional advantages of MAS for resistance to diseases traits (White and Knowles, 2013). However, in studies of variation in disease susceptibility, it is important to have the ability to characterize MHC polymorphism and determine its relationship to immune responsiveness (Konnai et al., 2003). Immunological role performed by MHC depends upon the maintenance of highly polymorphic levels within these genes at the level of population. Generally, there is no doubt that second exon MHC-Class II DRB1 plays an important role in resistance against disease, but other major genes related to this trait may also be present. Therefore, the resistance or susceptibility to diseases could not be totally accurately predicted by this region of gene alone. The more wide screening is need to quite reveal the mechanisms underlying the ability to deal with disease-causing germs in sheep. Beside, Relationships between molecular polymorphism in the exon 2 region of Ovar-DRB1 and weight traits in Iranian Makui sheep have been investigated, that no correlation was detected (Ashrafi et al., 2014). Finally, the critical role of MHC genes and molecules in immune system make these genes a candidate for use in marker assisted selection (MAS) of diseases resistant animals (Behl et al., 2012).

Conclusion

The use of genetic markers from within the Ovar-MHC may be useful, via marker-assisted selection, for increasing resistance to various diseases provided they do not impact negatively on other economically-important traits.

Acknowledgment

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RESULTS OF OUT-OF-SEASON BREEDING IN NULLIPAROUS SAANEN GOATS

Milovanović A.[1], Barna T.[1], Apić J.[1], Bugarski D.[1], Maksimović N.[2], Jović Ž.[3]

A group of sixty seven nulliparous goats postponed mating because of inadequate body development at their 10-12 months of age. They were exposed to "male (buck) effect" to provoke out of season breeding at age of 16-20 months. Twenty out of 67 reacted by mating but only 10 become pregnant. Forty seven goats that shows no heat were subjected to hormonal synchronization and 37 mated, resulting with 27 pregnant goats (pregnant rate of 57.45%).

The total effect of out-of-season breeding shows that only 66% of mated goats become pregnant, 16.41% remained anestric, from which 7 goats were diagnosed with hydro/pyo-metra (10.45%) at pregnancy check.

Results are indicating that adequate rearing of goat kids for replacement is of a paramount importance for future reproductive performance of nulliparous goats.

Keywords: goat, reproduction, synchronization, out-of-season, hydrometra

Introduction

According to national database for 2015, in R. of Serbia is registered 219.000 goats, but, only few percent are located on farms with 100 and more animals (Stat.Yearb.Serb. 2015). Goat farming in Republic of Serbia can be prosperous branch of livestock production. Number of pedigree animals is slowly increasing, but total number of goats is constantly decreasing. Limiting factors for more intensive propagation are economic instability of dairy goats' products, expensive initial investment in breeding animals, low communication with scientific centers and high mortality rate in kids. Regardless, new farms populated with imported, high producing goats are appearing and several small milking factories are established.

Hormonal synchronization is common practice in intensive goat farming. This procedure is used in large scale by goat breeders in France, where about 10% of the French goat population is inseminated. Expected kidding rate is about 60% (Leboeuf et al., 1998).

Recent efforts are made to use out of season non-hormonal cycle control with use of light regime and buck effects (Pellicer-Rubio et al., 2008). Other protocols are exclusively based

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on different combinations of hormones, as they are progestagen, cloprostenol, GnRH, PMSG or hCG i/m injections (Holtz, 2005; Andrabi et al., 2015).

Dominant method of estrus control in goats in Serbia is taken from French breeders' protocols by the use of progestagen-impregnated vaginal pessaries (polyurethane sponges) with an injection of equine chorionic gonadotropin and prostaglandins 48 hours before sponge removal (Leboeuf et al., 1998).

According to Baril et al. (1993) the efficiency of this treatment has allowed for the development of an AI (artificial insemination) genetic improvement program and settings when estrus detection is not very efficient or when there is no time to be carefully conducted.

In Serbia, AI is randomly used. Thus, hormonal synchronization is dominantly utilized for planned breeding, synchronizing kidding and lactation period in the way to meet continuous or seasonal demand in milk production and rational use of labor force.

Hormonal treatment in nulliparous goats often is not implemented (needed). Exceptions are made for out of season breeding or AI. In this article, synchronization is more used as a corrective measure of management feeding strategy omission in young, replacing goats.

Material and Methods

Experiment was carried out on a 280 milking goat farm in North part of R. of Serbia (coordinates: 45°04′N 19°20′E). Animals were kept in stalls, feed and milked twice a day. Average production was 730 liters of milk in 280 days of lactation. Average pregnancy rate in lactating goats was 89% in 2013. Annual buck fertility control was done before breeding season by clinical tests and semen control after electroejaculation. Semen was evaluated by CASA method, flow cytometry (viability test and sperm chromatin structure assay) and cyto-morphology. Number of goats per buck are determined according to breeding value, and then adjusted to scrotal circumference, body condition and semen quality. Pregnancy check was done by ultrasound scanning 1.5 months after breeding period. For continuous milk supply of their local milk plant, an out of season breeding group of lactating goats was formed in April-May, as a routine biotechnical procedure. Average fertility rate after hormonal synchronization was about 65-70%.

A group of 67 nulliparous goats born in November 2012 had postponed seasonal mating because of inadequate body development (physically immatured animals-underfed) at their 10-12 months of age. Their body weight was only 30-30 kg, and farm manager decided to prolong their growing period. April/May 2014 was chosen to be optimal period for grouping these goats with bucks when their body weight reached 55-65kg within 13-16 months of age.

Our first intention was to test of out-of-season "buck effect" (or "male effect") on nulliparous goats. For this purpose bucks were fenced in boxes nearby goats for 10 consecutive days and after they were joined for breeding. Bucks were closely monitored for mounting activities twice a day (between 9-11h and 14-16h) by two teasers buck equipped with aprons. Mounted goats, according to breeding plan were taken to appropriate buck, (so called hand-mating). Supervision of mountings were controlled and evidenced by experienced herdsman. After one unsuccessful mating, goats were paired with second buck in next breeding cycle.

After 45 days (equivalent to two cycles of 21 days), all goats that weren't in heat were sponged with 30 mg flugestone acetate polyurethane vaginal sponges (Synchro-part, Ceva, France) for 11 days. At ninth day after sponging, a 400 I.U. of PMSG (Sugonal, VZ Subotica, Serbia), together with 5 mg dinoprost (Enzaprost, Ceva, France) were applicated i/m, 48 hours before sponge retirement. Buck were joined to goats 42 hours after sponge removal and left in group for next few days. Buck activities were monitored and recorded. One buck had about 6 goats available for mating. If buck was not active on goat detected by teaser, goat was paired with second available buck.

All goats were scanned with WED 3000 Vet Palm hand held veterinary animal ultrasound scanner at 2 months after the end of the breeding period (WELD, China).

Results

From 67 nulliparous goats, only 20 goats (29,85%) reacted by heat induced with buck effects and allowed to be mounted in non-season period. Ten goats become pregnant (50.00%) and delivered 17 kids (kidding rate 1.7).

Remained 47 goats that shows no heat were subjected to hormonal synchronization and 38 mated (80.85%), resulting with 27 pregnant goats (pregnant rate 71.05% from mated goats). From 47 sponged goats, 57.45% become pregnant. Kidding rate was 1.44.

The total effect of out of season breeding (natural and hormonal) shows that only 66% of mated goats and 55.22% of total goats get pregnant. Number of goats mated in May but remained open was 19/67 (28.35%) and number of anestric goat in May (goats not reacted on natural mating or buck effect) was 11/67 (16.41%). Interestingly, incidence of hydrometra was very high; seven of 67 goats (10.45%) were detected with hydrometra.

Table 1. Results from natural and hormonal synchronization of nulliparous goats (end of May beginning of June)

Cycle induction	No. of goats in group	Mated		Non-mated		Pregnant (n)	Pregnant (%) of total	Pregnant (%) from mated
		number	%	number	%			
Natural	67	20	29.85%	47	70.14%	10	14.92%	50.00%
Hormonal	47	38	80.85%	9	19.15%	27	57.45%	71.05%
Total effect:	67	56	83.58%	11	16.42%	37	55.22%	66.07%

Table 2. Kidding results after natural and hormonal synchronization of nulliparous goats

Type of breeding	Kidding rate (goats/litter)	Offspring				
		Total number	Females	Males	% of females	% of males
Natural	1.70	17	7	10	41.20%	58.80%
Hormonal	1.44	39	21	18	53.80%	46.20%
Total effect:	1.51	56	28	28	50.00%	50.00%

After ultrasound scanning, a group of 30 non-pregnant animals were left to wait for their natural breeding season (September/October 2014, when goats achieved 24 months of age).

Seven goats that were diagnosed with hydro/pyometra at pregnancy check by ultrasound scanning were treated with 8 ml benzyl penicilline-dihydrostreptomycine antibiotic for 3 days (Neostrep, FM Pharm, Serbia), with AD3E vitamin (Neovit, FM Pharm, Serbia) and two injections of 5 mg dinoprost (Enzaprost, Ceva, France), i/m, 11 days apart. These goats were remated in November.

From 19 goats that mated in May but remained open, 11 become pregnant and 8 left barren. From 11 goats that were anestric in May, 6 get pregnant and 5 remained open.

Interestingly, incidence of hydrometra in nulliparous goats was very high-10%. After mentioned treatment and natural breeding, three goats become pregnant; four goats remain barren and were culled.
Finally, 54/67 (80.60%) of nulliparous goats with delayed body development and postponed mating became pregnant and 13/67 (19.40%) were culled or died as unpregnant.

Table 3. Fate of non-pregnant goats at next breeding season – September/October 2014 (nulliparous goats were already 2 years old at that time)

Type of breeding	Number of goats	% of all goats (n=67)	Final result	
			Pregnant	Barren
Mated in May but remained open	19	28.35	11	8
Anestric in May	11	16.41	6	5
Total effect:	30	44.76	17	13
Hydrometra (treated and rebred)	7	10.45	3	4

Picture 1 and 2. Pseudopregnancy (*hydrometra*); the uterine wall is thin, tensed with clear or slightly turbid fluid, absence of caruncles on fetal membranes.
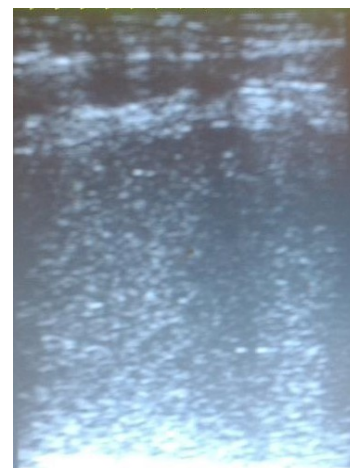
Picture 3. Pseudopregnancy (*pyometra*); distinctly turbid fluid in uterus.

Table 3. Ranking of buck according to conception

Serial and tattoo number	Age of buck (year)	Offered for mating		Served (N-S)	Conceived	Conception per services (%)
		Synch.(S)	Natural (N)			
1. TB 0510	4	7	1	6	5	83,3%
2. TB 2110	4	12	9	20	16	80,0%
3. TB 15312	2	5	2	6	4	66,7%
4. TB 23114	2	2	0	8	5	62,5%
5. TB 02914	1	5	5	10	6	60,0%
6. TB 21912	2	5	1	4	1	25,0%
7. TB 21410	4	6	0	2	0	0,0%
8. 945.152	6	5	2	0	0	0,0%
Total/average ♠.	3,13♠	47	20	56	37	66,07%♠

Regarding buck activities, it may be noted very different individual results of conception per pregnancy for some bucks, although they had good breeding history and fertility results on regular annual control 6 months before last breeding season (6 month ago).

Discussion and Conclusion

Expected pregnancy rate in synchronized and AI in lactating goats according to Leboeuf et al. (1998) is about 60%, but 25% of flocks had a kidding rate lower than 50%.
It can be expected that fertility rates of nulliparous does after AI are lower than in lactating goats. This is more obvious in goats with lower body weights at AI. For example, pregnancy rate was 37.1% and 36.9% for Saanen goats less than 32 kg of BW and 33-55 kg of BW (<24 months), respectively. For older nulliparous goats (≥24 months), pregnancy rate of lighter group (24-32 kg of BW) was 44.1%, comparing to heavier group (33-50 kg BW), where pregnancy rate was 53.6% (Leboeuf, 1992).

Our trial, although not fully comparable to mentioned references for fertility because of different approach (booth type of synchronizations were used to manage improper raise of young, replacing goats instead of programmed genetic progress), lower fertility rate after "male effect" achieved in this trial may be a consequence of anovulatory pattern of first estruses induced by buck presence. It is obvious that "male effect" was not enough signal for out-of-season goat synchronization, neither in provoking heat, but, probably also in ovulation, because only 10 of 20 mated goats become pregnant. Beside this, only 20/67 (29.85%) reacted on buck presence as the only stimulus (no use of photoperiodic regime, no hormones). Obviously, treatment with artificial long days and progestagens is needed.

In hormonal synchronization estrus induction is in 80.85% of goats (Leboeuf, 1998). According to Baril et al. (1993) estrus was observed in 98.1% of the treated goats 24 and 72 hours after sponge removal.

Estrus induction in synchronized group was 10-15% lower than in adult goats (80.85%). Pregnancy rate of mated nulliparous goats was solid - 71.05%. Pregnancy rate of total goats included in trial (24/47; 57.22%) can be classified as appropriate result for nulliparous Alpine goats, as it is stated in article of Leboueuf (1992). From this results, we can conclude that deep anoestrus was a main problem for underweight goats, with problems of ovulation/early conception.

Also, sponge type cannot be addressed as a reason of some lower pregnancy rate in synchronized groups. In our country only one type of progesterone sponges is available on market with 30 mg of progesterone. Standard progesterone sponges are impregnated with 45 mg fluorogestone acetate (FGA). A reduction of the progestagen load from 45 to 20 have no detrimental effects on synchronization, fertility and prolificacy (Leboeuf et all, 2003).

Undernutrition act with different mechanism on pregnancy establishment (embryo survival): abnormalities of the ovum or the embryo, luteal inadequacy and failure of the supply of progesterone to the uterus, and failure of the systems of maternal recognition of pregnancy trough IFNτ secretion from the conceptus and of PGF2α production from the uterus (Rhind, 1992; Abeceia et al., 2006).

Restricting feed intake in early post-natal life (to 75 % of ad libitum intake) in prepubertal ewe lambs should not have any effect on reproduction, lactation performance, or survival of ewes through four years of age (Borwick et al., 2003; Thomas et al., 2015). According to Borwick et al. (2003), undernutrition of ewe lambs in utero and in early post-natal life does not affect hypothalamic–pituitary function in adulthood.

According to Rosales Nieto et al. (2013), the reproductive efficiency of the entire sheep flock could be improved if ewe lambs go through puberty early and produce first lamb at one year of age, rather than the "traditional" two years in Australian Merino. Ewe lambs that were heavier at the start of mating were more fertile and had higher reproductive rate.

Opposite, Rhind et al. (1998) noted that effects on the lifetime reproductive performance of female sheep of two levels of nutrition before they were weaned or during their adult life had a significantly lower lifetime incidence of multiple births than those on a high plane of nutrition (P<0.05) but not when restriction took place in adult life. Authors suggest that food

resources should be diverted, where possible, to this phase of the production cycle to ensure that subsequent reproductive performance is not compromised.

Presence of high percent of hydrometra and pyometra complex in nulliparous goats in this trial is very evident (pseudopregnancy in 10.45% of animals). Ultrasound check of goats was not done before sponging or breeding season, so, initial prevalence of pseudopregnancy remain unknown. Normally, pseudopregnancy affects 3 to 4% of females, with varying frequencies between herds and within a herd, but for some A.I. groups, pseudo- pregnancy may attain 20% of animals (Mialot et al., 1991; Smith and Sherman 1994). It is characterized by the presence of a persistent corpus luteum and a sterile liquid in the uterus (Pierterse and Taverne, 1986), the origin being unknown.

Two bucks revealed zero conceptions, in spite of solid results on semen evaluation and previous breeding history 6 months ago. This can be one of the reasons of some unexpected results of bucks ranged on 6 to 8 position. Check of semen quality can be advisable for lowering risk with male animals.

Proper rearing and breeding, above all in young period has to be a part of integral farm technology. Our breeding results are indicating that adequate rearing of goat kids for replacement is of a paramount importance for future reproductive performance of nulliparous goats. Risk for culling because of infertility of replacing goats can be high if they are not raised properly.

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Oral presentation

THE FUTURE OF BUFFALO BREEDING IN BULGARIA – INNOVATIONS IN CRYOPRESERVATION OF SEMEN FROM BUFFALO BULLS

Ivanova M.[1], Gradinarska D.[1], Kirilova I.[1], Tsvetkov T.[1], Daskalova D.[1]

Abstract

The aim of this study was to analyze proteins contained in the seminal plasma of buffalo bulls, which are responsible for the protection of the sperm plasma membrane and the good freezability of the spermatozoa. Semen from Bulgarian Murrah buffalo bulls was frozen in straws with addition of an antioxidant mix (L-Glutathione, N-Acetyl Cysteine, vit. E, vit. C, Calcium, Selenium, and Zinc).

Differences in the chromatographic profiles of ejaculates with good (Group A) and poor (Group B) cryotolerance of the spermatozoa were demonstrated through High–Performance Liquid Chromatography. In Group A, low molecular weight proteins with a molecular mass of between 6.4 and 14 kDa were established, as compared to Group B where such proteins were almost absent. The number of spermatozoa with progressive motility in Group A was significantly higher than that of static or non-progressive spermatozoa ($p < 0.05$). The thermal resistance test of spermatozoa after thawing showed that the spermatozoa with progressive motility in Group A were 43.58 ± 2.23, while in Group B they were only 10.6 ± 1.35 ($p < 0.001$).

An adequate antioxidant protection of the spermatozoa was achieved through the addition of the antioxidant mix. The current research presents an innovative approach for freezing semen from buffalo bulls in practice.

Keywords: antioxidants, buffalo bulls, freezing, seminal plasma proteins

Introduction

In the recent years the worldwide production of buffalo milk is growing at a much higher rate in comparison with the production of cow milk. The topicality of buffalo breeding is justified by the global trends for the development of animal husbandry. The main reason for this change is related to the buffalo breeding reconstruction towards production of more milk and meat. In Bulgaria this reconstruction ended with the complete replacement of the aboriginal Bulgarian Buffalo used for work, meat and milk, with the new breed Bulgarian Murrah, which owns high productive qualities for milk and meat.

Another important reason for this tendency is related to the climate changes and global warming. In the next 10-15 years it is expected 20% of the cattle in the world to be replaced by buffaloes. Overall, the major prerequisites that justify buffalo breeding on a global and national scale may be presented in the following order:

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- Organic production (milk, meat, etc.) with specific qualities and very high market value;

- Secure niche and virtually unlimited market in the conditions of missing or relatively weak competition in EU countries at present and in perspective;

- Qualitative indicators of the obtained food products, in relation to human health and welfare;

- Centuries-old buffalo breeding traditions in various regions of the world and in Bulgaria;

- Suitable soil and climatic conditions for the breeding of this domestic animal species.

All this makes it necessary to change our viewpoint towards more promising and distant possibility for breeding, reproduction and selection of this animal species.
There is an obvious tendency for gradual reduction or temporary stabilization of the number of cattle in Bulgaria (Table 1). This reduction is mostly related to the gradual decline in the number of dairy cows with 23.7% from 2003 to 2012. At the same time, there is a tendency for increase in the number of buffaloes with 16.5% and buffalo-cows with 26.7%. Most buffaloes were bred in 2011 – almost 10,000 heads (Table 1)

Table 1. Dynamic changes in the number of cattle and buffaloes (in thousands) in Bulgaria for the period 2003-2012 (Source: Ministry of Agriculture and Forestry of Bulgaria, direction "Agrostatistics")

	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Cattle	728.3	671.58	621.8	628.27	602.06	564.9	539.56	544.46	557.64	526.11
Dairy cows	378.2	368.72	347.75	350.14	335.89	314.67	296.76	308.17	306.84	228.75
Buffaloes	7.9	8.0	8.2	8.2	9.0	9.2	8.3	9.2	9.9	9.2
Buffalo-cows	4.5	4.1	4.7	4.7	5.2	5.3	5.0	5.4	6.3	5.7

At the same time, there is a tendency for reduction to stabilization of cow milk production. In previous years the average yield was about 1100 thousand tonnes. This occurred against the background of an increase in milk productivity with about 400 kg for 10 years. In 2012 the milk productivity reaches average 3 785 kg per cow. Good news is the growing production of buffalo milk. In a 10-annual reference period this increase is about 60%. The peak of production was in 2011 when the average milk production in buffalo-cows increased. In 2012 it reaches 1408 liters (Table 2).

Table 2. Dynamics of production of cow and buffalo milk (in thousands of tonnes) for the period 2003-2012 (Source: Ministry of Agriculture and Forestry of Bulgaria, direction "Agrostatistics")

	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Cow's milk	1309	1345	1287	1299	1148	1143	1073	1124	1126	1093
Buffalo milk	5	6	7	7	7	7	7	8	9	8

It is obvious from the presented data that the implementation of an effective breeding activity, based on controlled selection through innovations is an important condition for the development of high-quality stock-breeding. In this way the advancement of buffalo breeding presents great opportunities to find a worthy place in the overall livestock gene pool in Bulgaria. It is particularly important to point out that Bulgaria fulfils all criteria that

are favorable and are related to climate changes, environmental protection, globalization of the market, as well as with social and demographic changes relevant to human health.

It is important to draw attention to the management and preservation of genetic resources - gametes and embryos. Bulgaria has traditions and scientific developments that have proven their effectiveness in the years of implementation in practice. Through the implementation of innovations in the biotechnologies for collection, evaluation, preservation and freezing of spermatozoa, oocytes and embryos, Bulgaria has proven visibility at a national and international level. By the end of 2012, there were 3,146,280 stocked doses of deep frozen semen from 22 cattle breeds and 3 buffalo breeds. These data demonstrate the need for increasing the buffalo genetic resources for future use in expanding and developing the buffalo breeding.

Reproductive biotechnology for preservation of spermatozoa is an important structural unit in buffalo breeding and selection. Innovations in this direction have a number of advantages and guarantee the success of the implementation of controlled reproduction in buffaloes. These findings have led us to focus our efforts on solving some of the unsettled issues related to cryopreservation of semen from buffalo bulls.

The present studies have examined the role of seminal plasma proteins (SPPs) related to the cryotolerance of buffalo bull spermatozoa. It is known that some SPPs are related to basic structural and functional changes of the plasma membrane (PM) of the spermatozoa. It is assumed that they can participate in the activation of intracellular signaling pathways, thus preparing the spermatozoa for fertilization of the oocyte. There are other opinions, claiming that some of the SPPs have decapacitation effect and stabilize the sperm PM after ejaculation. This stabilization is achieved through concealing of some surface-membrane receptors by SPPs and inhibiting the primary cholesterol efflux (Caballero et al., 2009; Daskalova et al., 2012).

The effect of cooling on the sperm PM integrity is well studied (Franciska et al., 1990). It has been proven that capacitation-like membrane changes are observed in male gametes. It is believed that cooling leads to efflux of phosphatidylcholine of the PM, which is referred to as cryocapacitation (Thomas et al., 2006). Same as in capacitation, certain acceptor molecules extract membrane cholesterol, some phospholipids and surface-membrane proteins. All this leads to destabilization of the PM and accordingly to an increase in membrane fluidity and permeability (Thomas et al, 2006). Studies show that the reduced quantity of cholesterol in the PM is one of the causes of low cryotolerance of the spermatozoa (Manjunath et al., 2002). It has been proven that cryopreservation reduces up to 60-70% the binding capacity of some SPPs to the sperm PM. The authors associate this data with lower cryotolerance of the sperm membrane structures (Nauc and Manjunath, 2000).

During low-temperature preservation, the presence of some SPPs contributes to restore basic surface-membrane structures (Selvaraju et al., 2016). There is evidence of proteins which protect the spermatozoa from PM cryodamage and so stabilize the biological parameters of spermatozoa after thawing (Maxwell et al, 2007). A positive correlation between contents of certain proteins in the seminal plasma (SP) and the possibility of freezing semen of bulls (Patel et al., 2016) and boars (Daskalova et al., 2014; Daskalova et al., 2015). For example, the presence of acidic proteins with molecular weight (MW) 13–16 kDa could be used as a marker for good cryotolerance of the spermatozoa. On the other hand, the presence of other

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proteins with MW 25-26 kDa is associated with low cryotolerance of the spermatozoa (Jobim et al., 2004).

The functional and biological role of the BSP proteins was studied in *in vitro* experiments. Some of these proteins have a protective effect on the motility of the spermatozoa. It is suggested that the mechanism of this protection is expressed in priority binding of the BSP proteins with choline-containing phospholipids, which are presented on the external surface of the PM. In this way the movement of phospholipids in the bilayer is prevented. In this fashion the authors explain the stabilization effect of the BSP proteins on the sperm PM (Gwathmey et al., 2006).

Information on the SPPs is extremely rich, but also contradictory. For example, it is known that in *in vivo* conditions BSP proteins have both helpful and harmful effects on the sperm PM integrity. These effects are important for the maintenance of the spermatozoa a variety of conditions. In this way the successful implementation of their physiological function – fertilization of the oocyte is ensured. *In vitro*, some functional properties of the BSP proteins can be used in the field of reproductive biotechnologies. These proteins could be candidates for the protective biosubstances during low-temperature preservation of gametes.

The aim of the present study is to analyze proteins contained in the SP which are related to the cryotolerance of buffalo bull spermatozoa. An attempt was made to optimize the cryopreservation of semen from buffalo bulls through the addition of a mix of antioxidants.

Material and Methods

The experiments were conducted with ejaculates from buffalo bulls, delivered by Executive Agency for Selection and Reproduction in Animal Breeding in Sofia and Sliven.

Isolation of seminal plasma
SP was isolated by double centrifugation of the semen. The first centrifugation was performed at 2500 rpm for 10 min at 4°C (K24D centrifuge). For the second centrifugation the supernatant was used at 12000 rpm for 5 min. The SP received was filtered through 0.22 µm membrane (Milipore®) and stored at -80°C.

HPLC separation of proteins from SP
SPPs were separated using High-Performance Liquid Chromatography (HPLC) system with semi-preparative column for size exclusion chromatography (TSK gel G3000SW, 21.5 mm x 300 mm, 10 - 500 kDa, TOSOH BIOSCIENCE®). 1000-µl SP samples were injected into the column and eluted with phosphate buffered saline (9g NaCl, 0.19g NaH_2PO_4, 1.09g Na_2HPO_4 x $12H_2O$, 1l d.H_2O, pH 7.0) at 6 ml/min flow rate for 20 min.

Comparative analysis of SP from buffalo bulls with proven good and low cryotolerance of the spermatozoa was made. The protein content of each sample was determined by spectrophotometry.

Semen processing
After collection and evaluation, the semen was diluted to 1:15 with cryoprotective medium. The purpose of this dilution is to achieve $5x10^6$ motile spermatozoa per dose, which guarantees good artificial insemination results. The medium used for cryopreservation was the following: 2.32 Sodium citrate dehydrate, 2.42 Tris (gm), 1.34 Citric acid monohydrate,

1.0 Glucose or fructose, 7 ml Glycerol, 20 ml egg yolk, 100 UI penicillin, d. Water to   100 ml. The so diluted semen was loaded in 250-µl straws.

The next step was equilibration (3 hours on 4°C) and freezing of the semen. Semen was frozen with an initial speed of 3-5 sec to -79°C, and then the straws were immersed in liquid nitrogen.

Semen thawing
Straws were thawed in water bath at 39°C. Immediately after thawing, 1 mg/ml antioxidant mix (AOM) was added to the cell suspension. The AOM contains: L-Glutathione, N-Acetyl Cysteine, vitamin E, vitamin C, Calcium, Selenium and Zinc.

Computer-assisted sperm analysis (CASA) of buffalo bull semen

Total motility, progressive motility and velocity of the spermatozoa were assessed using CASA System Sperm Class Analyzer® (Microptic®, Spain), analytical software module "Motility and concentration". The analysis was made on Leja chambers with 2-µl droplet volume. The following parameters were analyzed: VCL (curvilinear velocity; time-averaged velocity of a sperm head along its actual curvilinear path), VSL (straight-line velocity; time-averaged velocity of a sperm head along the straight line between its first detected position and its last), and VAP (average path velocity; time-averaged velocity of a sperm head along its average path, computed by smoothing the curvilinear trajectory according to algorithms in the SCA Motility module).

After the filling of the chamber, the samples were allowed to settle for 1 min before the capture. The analysis was made on at least 1000 spermatozoa at 3-5 microscopic fields. For the present study the spermatozoa from each sample were assessed twice: after collection and after thermal resistance test.

Results and Discussion

Protein content and CASA analysis of spermatozoa with differences in cryotolerance
The analysis of the HPLC profiles demonstrates specific differences in the chromatographic profiles of ejaculates with good (Group A) and low cryotolerance of the gametes (Group B). Five distinct peaks are differentiated and the proteins in those peaks range from 10 to 500 kDa (Figure 1).
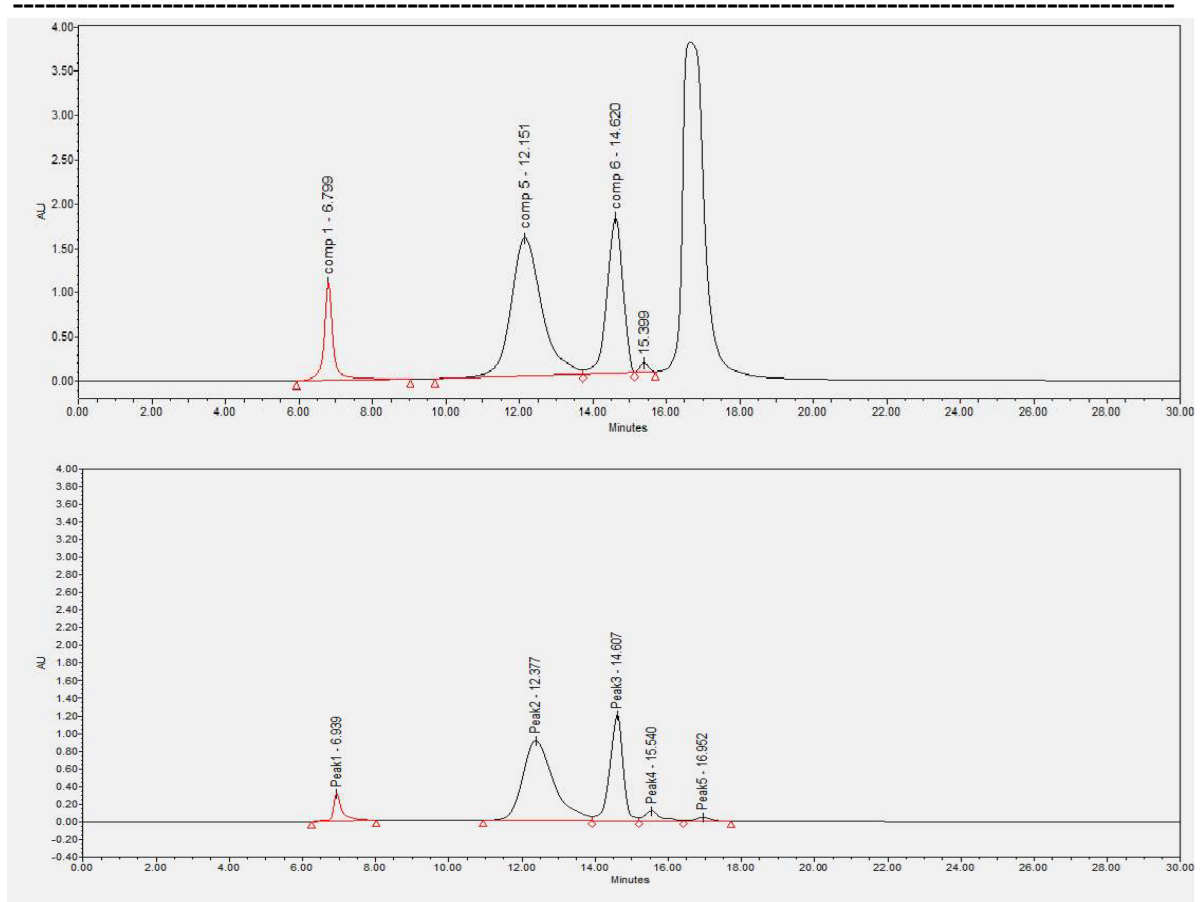
Figure 1. Chromatographic profile of separated SPPs from buffalo bull (λ=280 nm): (Up) ejaculate with good cryotolerance of the spermatozoa; (Down) ejaculate with low cryotolerance of the spermatozoa.

Differences in the quantity of certain SPPs were established. In Group A there are very pronounced protein peaks on 12.151 min and on 14.620 min, while in Group B these proteins have lower absorption, which speaks of lower concentration. In Group A there is a larger amount of proteins separated on 17 min. These are proteins with low MW between 6.4 and 14 kDa. It can be assumed that these proteins belong to the group of calmodulin-binding proteins (15 and 16 kDa) (bSVSP15), spermadhesins (15-18 kDa), ubiquitin (8 kDa) or seminalplasmin (5.4 kDa). Maybe they are acidic proteins (BSP-A1, BSP-A2, BSP-A3) which have MW between 15 and 17 kDa. In the semen of bulls the presence of acidic BSP proteins (13-16 kDa) was proven, which are markers for good cryotolerance of the spermatozoa (Jobim et al, 2004).

Our interest is aimed at the function of calmodulin, which is related to sperm capacitation. These proteins are involved in the changing and concealing of some components of the PM surface, in increasing the permeability of the PM for metal ions and water, leading to acrosome reaction (Colás et al., 2009). Also particularly important is the functional role of spermadhesins which have high affinity to bind to heparin, phospholipids, carbohydrates and protease inhibitors. They participate in the membrane transformation of the spermatozoa after ejaculation, in the formation of a reservoir of spermatozoa in the oviduct before fertilization (via carbohydrate interactions), and are associated with capacitation, gametes interaction and uterine immunomodulation (Calvete et al., 1996; Jonáková et al., 2007).

CASA results for the biological parameters of buffalo bull spermatozoa after freezing are presented in Graph 1. There are obvious differences in the percentage of spermatozoa with progressive motility on the 10th min, which is almost three times greater than the percentage of the static or non-progressive spermatozoa in ejaculates with good cryotolerance (Group A). In Group B, the static spermatozoa are 31.25% and the spermatozoa with non-progressive motility consist the highest percentage (43.37%). The thermal resistance test showed that the most significant differences in motility are registered on 240 min after thawing. In Group A the spermatozoa with progressive motility are 43.58 ± 2.23, while in Group B they are only 10.6 ± 1.35 ($p < 0.001$).
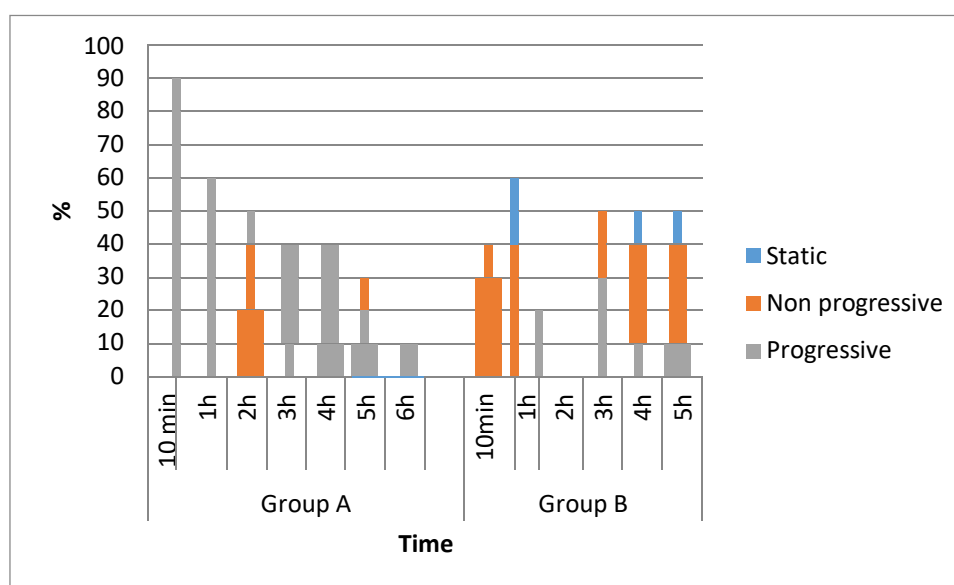
Graph 1. Comparative analysis of the cryotolerance of buffalo bull spermatozoa after freezing in straws - progressive motility (n = 8). (Group A) Ejaculates with good cryotolerance; (Group B) ejaculates with reduced quality parameters after thawing.

In conclusion, the presence of low MW proteins between 6.4 and 14 kDa in the SP of buffalo bulls is a criterion of good cryotolerance of the spermatozoa and can be applied in the evaluation of ejaculates.

Role of AOM on biological parameters of buffalo bull spermatozoa during cryopreservation
The results on the effect of AOM on the freezability and survival of buffalo bull spermatozoa are presented in Graph 2. The analysis of the results shows that the post-thaw supplementation of a combination of L-Glutathione, N-Acetyl Cysteine, vitamin E, vitamin C, Calcium, Selenium and Zinc (AOM) protects the motility and kinematic parameters of the spermatozoa. Significantly higher values are proven when compared to the controls. The presence of AOM preserves the motility and survival of the spermatozoa for up to 6 hours after thawing. Data on the percentage of progressively motile spermatozoa are in the range of 11.2 ± 2.02 %, while in the controls it is only 0.5 ± 0.02 % ($p < 0.001$). The post-thaw supplementation of AOM protects the motility and kinematic parameters of the spermatozoa at times more as compared to the control sample.
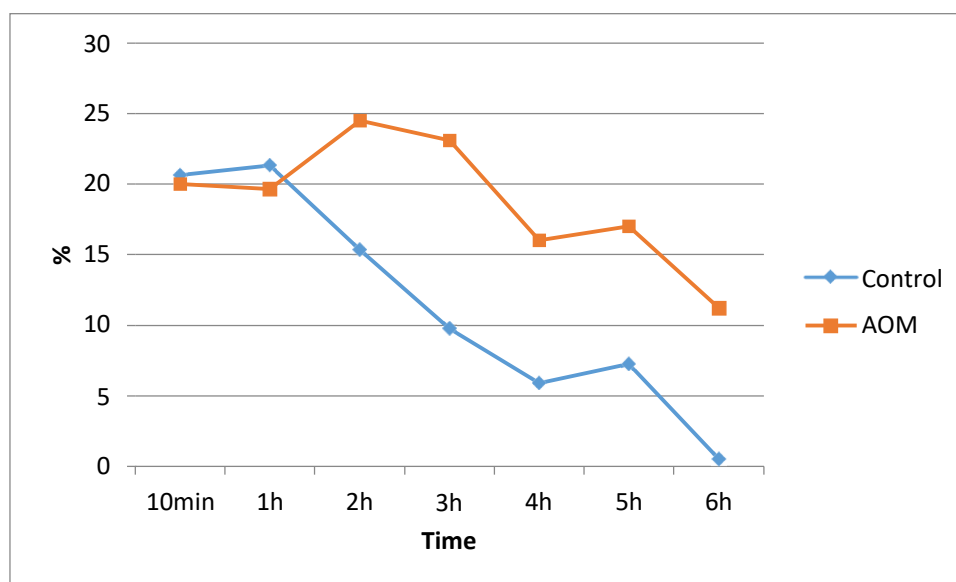
Graph 2. Comparative analysis of the progressively motile spermatozoa, frozen in straws (n= 8).

The data on the kinematic parameters VCL, VSL and VAP demonstrate that the supplementation of AOM preserves significantly higher percentage of spermatozoa with progressive motility. This protection is well pronounced in time, compared with the control (Graph 3).
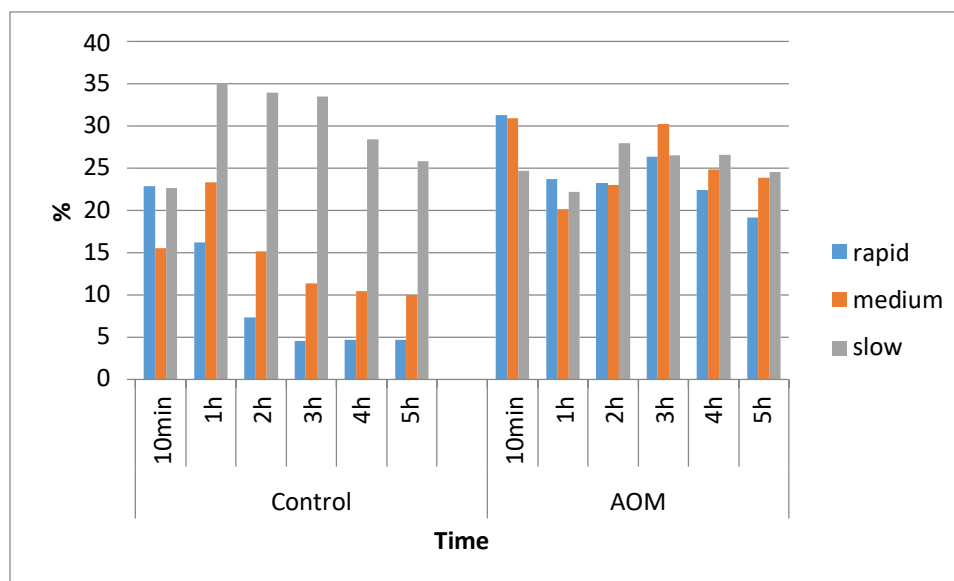
Graph 3. Analysis on the role of antioxidants on the kinematic   parameters of buffalo bull spermatozoa frozen in straws (n = 8).

Conclusion

The present study presents a new approach to freezing of semen of Buffalo bulls through an adequate antioxidant protection of the spermatozoa. The supplementation of AOM (combination of L-Glutathione, N-Acetyl Cysteine, vitamin E, vitamin C, Calcium, Selenium and Zinc) inhibits oxidation processes accompanying cryopreservation. The antioxidant substances in the AOM protect the spermatozoa from peroxidation and formation of free radicals. The mechanism of protection is based on neutralization by biochemical interactions of the toxic metabolic products in the process of freezing.

In the biotechnology of freezing of buffalo bull semen in straws, the addition of AOM (L-Glutathione, N-Acetyl Cysteine, vitamin E, vitamin C, Calcium, Selenium and Zinc) in a dose of 1mg/ml to the thawing medium is a guarantee for good results and can be used in the practice of artificial insemination.

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Oral presentation

ANALYSIS OF FIRST FIVE LACTATIONS CURVES OF HOLSTEIN COWS IN SERBIA

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Abstract

Lactation curves for 4854 Holstein cows from ten farms in province of Vojvodina, Serbia in first five lactations were analyzed. Data included in trail were on the bases of daily milk recording from 7th till 500 days of lactation. Aim of this study was to find out the peak of lactation (1 to 5) for the purpose of improving individual feeding management of dairy cows. Primiparous cows reached 20 days later (with 80 days) peak of lactation then cows in second, third, fourth and fifth lactation (with 60 days). Obtained results can assist farmers for the proper nutrition balancing of milking cows in order to makes milk production more profitable in the first place of cows after first calving.

Keywords: daily milk production, Holstein cows, lactation curve, parity

Introduction

Lactation is the period between calving and drying of the cows. Milk yield from calving to a peak of lactation increases rapidly. After the peak, milk production decreases gradually. Generally, cows reach the peak of lactation between 50 and 80 days. Some authors (Park and Lindberg, 2004; Čobić and Antov, 1996) found the peak of lactation between 4 and 8 weeks. Piccardi at al., (2014) investigated the productive and reproductive performance of purebred Holstein cows in first lactation. They showed that peak of lactation was around 90 days. Milk production is influenced by lot of factors (e.g. breed, nutrition, season of calving, cow health etc.). In the initial phase of lactation cows can't consume as many nutrients as they need. The day of reaching peak of lactation is therefore very important to properly balance the diet ratio of dairy cows. Time (day) to reach peak of lactation and lactation curves can be tools for farmers for management decision-making and selection. There are several mathematical equations which allows prediction of total milk yield in lactation: Wood model (Wood, 1967), Wilmink model (Wilmink, 1987), Ali and Schaeffer model (Ali and Schaeffer, 1987), Dijkstra model (Dijkstra at al., 1997), and Gompertz, Schumacher and Morgan equations (Thornley and France, 2007). The objective of the current paper is to find out, on the basis of real data of daily milk yield of Holstein cows in Serbia, when they rich the peak of lactation in order to help farmers to manage the nutrition in that phase of lactation.

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Material and Methods

For the study, data from 10 commercial farms of 4854 Holstein cows were obtained. The data consisted approximately 41000 daily milk yield records, corresponding to 16311 first, 12042 second, 7495 third, 3510 fourth and 1859 fifth parity of individual lactation curves. Data were used for lactation length from 7th days and no longer then 500 days. Polynomial equation was used for graphic presentation of lactation curves. Number of cows and lactation production by parity are presented in Table 1.

Table 1. Milk production of cows used in data analyzing by parity

Lactation number	Number of cows	305 Days lactation production (kg)
I	1910	7868
II	1410	8681
III	881	8685
IV	431	8412
V	222	8227

Cumulative milk production was calculated from daily milk yield records.

Results and Discussion

All data from five lactations shows similar ascending lactation curves. Cumulative milk production curves are shown in Fig. 1.
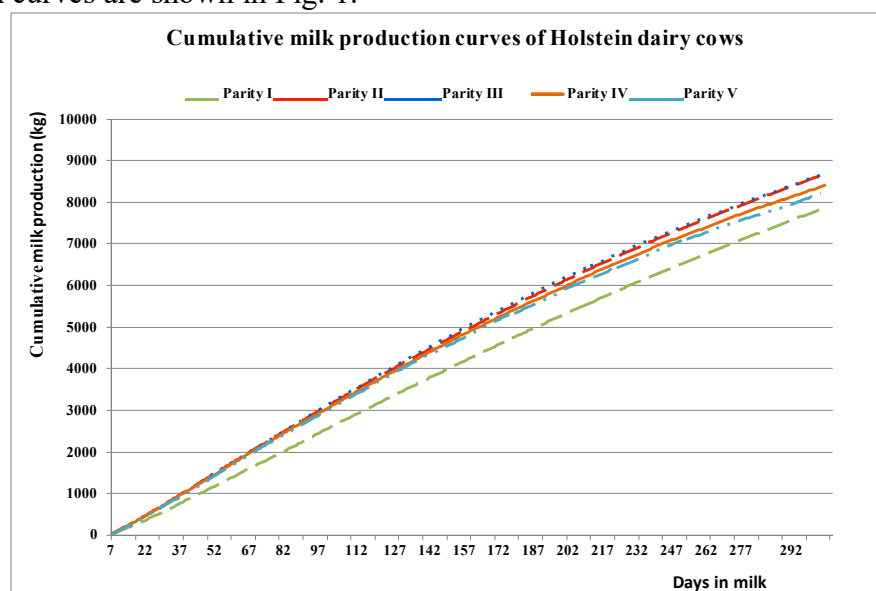
Fig 1. Cumulative milk production curves of Holstein dairy cows by parity

As it can be seen (Fig. 1), cumulative milk production for first parity was the smallest, increasing till third and after decreasing till fifth parity. Those results are similar with conclusions of Lopez at. al., 2015, who were investigated first three lactation curves.

Reaching the peak of lactation is very important for farmers because they have to make a proper decision for feeding management in the first phase of lactation.
For the first parity of our study, lactation curve was shown in Fig. 2.
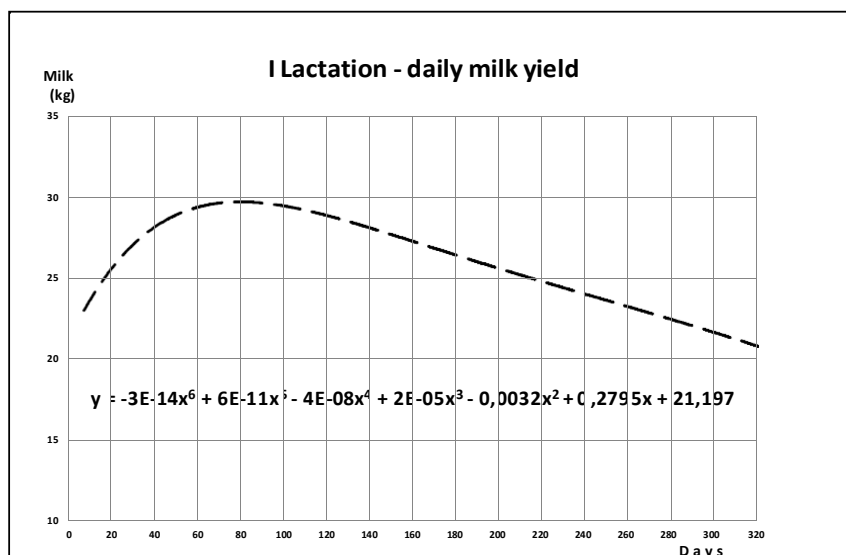
Fig. 2. First lactation curve of Holstein dairy cows with peak at 80 days

Generally, it is known that cows reach peak of lactation between 4 and 8 weeks. As it can be seen (Fig.2.), first parity (16311 measurements) in our trait shows that peak was reached around 80 days. Difference between 80 and 57 days (8 weeks) seems not so high, but if farmer has medium or large number of cows on his farm, 20 days of inadequate feeding can make a lot of costs, and increase a total cost of milk production. In comparison with other researchers, results in experiment are close to results obtained by Lopez, at.al. (2015) and Piccardi, at. al. (2014). On comparable Fig. 3 can be seen daily milk yield curves. Even more, Piccardi, at.al. (2014) shows on their figure, that peak of lactation curve was around 100 days.
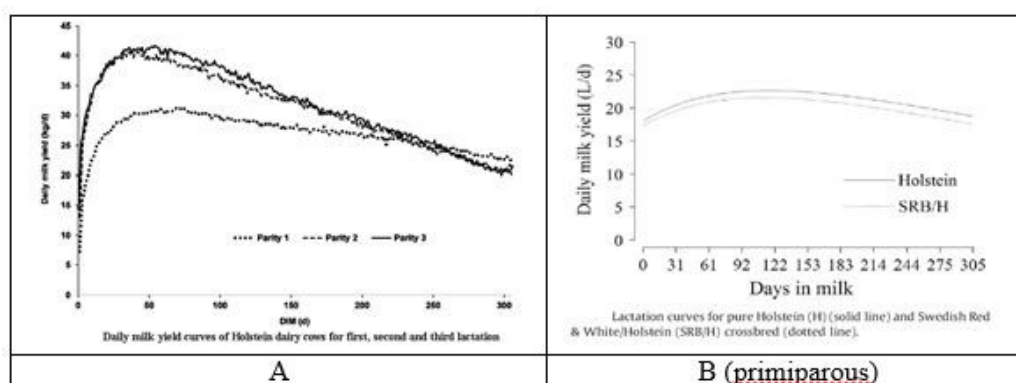
Fig. 3. Lactation curves of Lopez, at.al. 2015. (A) and Piccardi, at. al. 2014. (B)

Lactations curves from second till fifth lactation shows different results in comparison with first parity. All four parity (from 2nd till 5th) shows earlier peak of lactation than the first one. Differences were around 20 days earlier, even the peak of lactation for the fifth parity was lower than 60 days. The results for lactation curves of second, third, fourth and fifth parity are shown In Fig.4.
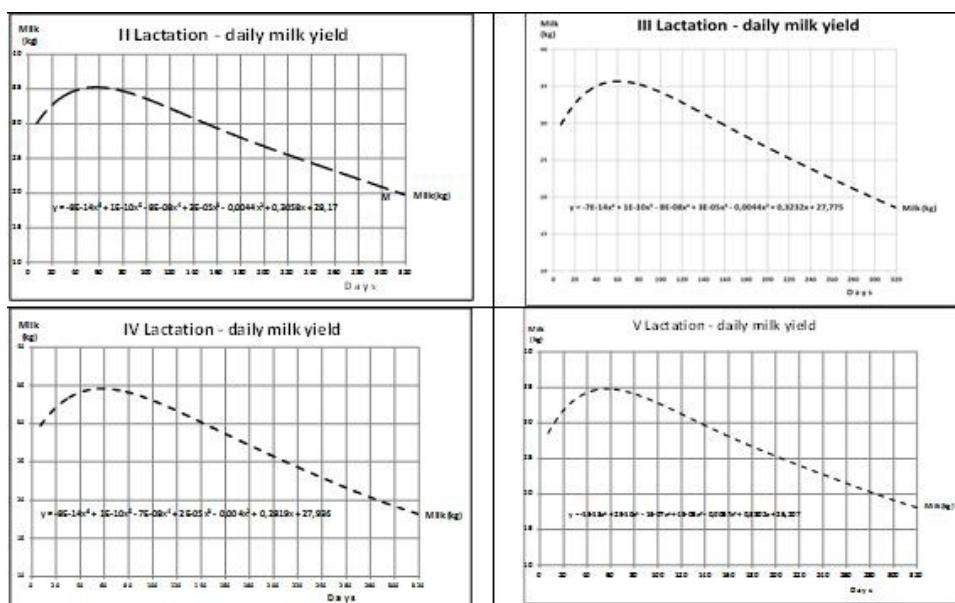
Obtained results shows that primiparous reach peak of lactation earlier then cows in higher parity, what can be sign to farmer in feeding management to reduce ratio after 80 days of lactation for primiparous, and for cows from second and more lactation, reduction can be made 20 days earlier.

Conclusion

In this study, the main goal was to show, on the basis of real data of daily milk recordings, lactation curves from first to fifth parity of Holstein cows in Serbia. Many of researchers have investigated the ability to predict lactation curves of dairy cows (Stanton at. al. 1992; Pollott, 2000; Tekerli at. al., 2000; Kamidi, 2005; Macciotta, 2005; Fathi Nasri at. al. 2008; Strucken at. al. 2011; Jeretina at. al. 2013; Lopez at. al., 2015). Some of them wanted to, in the absence of daily milk recording data using several mathematical equations mentioned before, predict milk production in aim that farmer could properly balance the daily ration of cows. Providing detailed information during different stages of lactation, on the basis of real daily milk recordings, could be used for a more individual-specific feeding management. Improving individual feeding management can increase production level of milking cow and profitability of whole herd.
Further investigation of lactation curves with analysis milk urea samples would be of interest in the context of faster detection of unbalanced ratio of dairy cows.

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Oral presentation

BODY MASS INDEX OF MALE PEKIN DUCKS OBTAINED FROM DIFFERENT BREEDER AGES

Onbaşılar E.E.[1]

Abstract

This aim of this study was to determine the body mass index of male Pekin ducks obtained from different breeder ages. For this purpose, 72 male Pekin ducklings (Star 52) obtained 37 and 135 weeks of breeder ages were used in this study. Body weight and body length were determined from hatch to 6 weeks of age. Body length was measured between the first cervical vertebra and the pygostyle. BMI was calculated as a ratio of body weight to body length (g/cm^2). Body mass index from hatch to 6 week of age was 0.41, 0.63, 0.72, 0.69, 0.84, 0.88 and 0.94 of male Pekin ducks obtained from 37 week of breeder age and that was 0.44, 0.67, 0.67, 0.70, 0.86, 0.90 and 0.95 of male Pekin ducks obtained from 135 week of breeder age. Body mass index was higher in examined weeks of male Pekin ducks obtained from older breeder except the 2nd week of age, but this difference was not statistically significant. At the 2nd week of age male ducks obtained from younger breeder gained more weight than that from older breeder, and body mass index was higher in male Pekin ducks obtained from younger breeder at this week. Effect of breeder age on body mass index was statistically significant only at 2nd week of age. The present study showed that breeder age has limited effect to the body mass index in male Pekin ducks.

Keywords: Pekin duck, breeder age, body mass index.

Introduction

Pekin duck is more adapted to cold weather than any of the other poultry species, because of its thick skin and largest subcutaneous fat layer. The ducks' weight gain is higher at the early stage than that of broilers (Shalev, 1995). Pekin ducks have a growth curve with initial higher growth rates and slower later. Growth is controlled by genetic factors such as genotypes and sexes. Breeder age has positive effects on hatching body weight (Applegate and Lilburn, 1998). However Onbaşılar et al. (2011) reported that breeder age effects in Pekin ducks were not stay till later ages. Body mass index (BMI) is a measure of relative weight based on individual's mass and length (Kabir et al., 2015). The objective of this research was to compare the effects of the breeder age on BMI.

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Material and Methods

Seventy two male Pekin ducklings (Star 52) were used in this study. Ducklings were obtained 37 and 135 weeks of breeder age. They were leg-banded after hatching and then were reared in groups of 12 ducklings in floor pens (170X94, width X length, respectively). One bell drinker and one hanging suspended feeder were used in the floor pen. It was covered with wood shavings as a litter. Birds were fed a starter diet (18 % crude protein and 2800 kcal/kg metabolizable energy) until 3 weeks age and then they were fed a grower diet (17 % crude protein and 2700 kcal/kg metabolizable energy). Food and water were provided *ad libitum* during the experiment.

Ducks were weighted and body length were taken weekly from hatch to 6 week of age. Body length was measured between the first cervical vertebra and the pygostyle (Saatçi and Tilki 2007). BMI was calculated as a ratio of body weight to body length (g/cm^2).

Statistical analysis was assessed by using SPSS Version 21.0 for Windows (SPSS, Inc., Chicago, IL, USA). BMI was analyzed with one way ANOVA. *P*-value $\leq$ 0.05 was considered significant.

Results and Discussion

BMI of male Pekin ducks obtained from different breeder ages was shown in Table 1. At hatch; BMI was 0.41 and 0.44 g/cm^2 for male Pekin ducks obtained from 37 and 135 week old breeder flocks, respectively. And this values were not statistically significant between breeder age groups. Then at 1st week of age, BMI was 0.63 and 0.67 g/cm^2 for male Pekin ducks obtained from 37 and 135 week old breeder flocks, respectively. At these ages, BMI was higher of male Pekin ducks obtained from older breeders. But this was not statistically significant. Then at 2 week of age BMI decreased in male Pekin ducks obtained from older breeders (P$\leq$0.05). BMI base on individual's weight and body length (Kabir et al., 2015). Then this may be due to the ducks obtained from younger breeders gained more weight at this week than older breeders. From 3 to 6 week of age again BMI increased in male Pekin ducks obtained from older breeders. But this difference between breeder ages was not statistically significant. At 6 week of age; BMI was 0.94 and 0.95 g/cm^2 for male Pekin ducks obtained from 37 and 135 week old breeder flocks, respectively. Body mass usually has a fairly strong correlation with fat mass and in many cases explains more than 50% variation in fat mass (Labocha and Hayes, 2012). Decreased fat content may be desired in meat products and this can be provided by decreased BMI (Kabir et al., 2015). Researchers showed that, BMI is varies not only among species but also between sexes (Castro and Myers, 1990).

Conclusio

The present study was showed that breeder age was limited effect the BMI in male Pekin ducks.

Table 1. Body mass index (BMI, g/cm^2) of male Pekin ducks obtained from different breeder ages

Time	BMI of male ducks obtained from 37 week old breeder	BMI of male ducks obtained from 135 week old breeder	P
At hatch	0.41	0.44	0.157
1th week of age	0.63	0.67	0.131
2nd week of age	0.72	0.67	0.053
3rd week of age	0.69	0.70	0.746
4th week of age	0.84	0.86	0.499
5th week of age	0.88	0.90	0.370
6th week of age	0.94	0.95	0.709

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Oral presentation

EFFECT OF BREEDER AGE ON CORRELATIONS BETWEEN SOME BODY MEASUREMENTS AT HATCH AND AT 6 WEEK OF AGE IN MALE PEKİN DUCKS

Onbaşılar E.E.[1]

Abstract

This aim of this study was to determine the correlation between some body measurements at hatch and at 6 week of age in male Pekin ducks obtained from different breeder ages. For this purpose, 72 male Pekin ducklings (Star 52) obtained 37 and 135 weeks of breeder ages were used in this study. Body weight, lengths of beak, head, neck, body, tibia and metatarsus, diameters of beak and head, and also depth and width of chest were determined at hatch and at 6 weeks of age and correlations between different breeder ages of male Pekin ducks were determined. Correlation coefficients between some body measurements at hatch and at 6 week of age were ranged from 0,001 to 0.566 in male Pekin ducks at 37 week old breeders. And the highest correlation coefficient was between body weight at hatch and body weight at 6 week of age, while the lowest correlation coefficient was between metatarsus length at hatch and head diameter at 6 week of age. However, correlation coefficients between some body measurements at hatch and those at 6 week of age in male Pekin ducks obtained from 135 week old breeders ranged from 0.005 to 0.473. The highest correlation coefficient was between neck length at hatch and body length at 6 week of age, while the lowest correlation coefficient was between chest width at hatch and metatarsus length at 6 week of age. The present study was showed that breeder age affected the correlations between some body measurements at hatch and at 6 week of age in male Pekin ducks.

Keywords: Pekin duck, body measurement, correlation

Introduction

Ducks are raised generally for meat production. They have rapid growth during the first week of life (Onbaşılar et al., 2011). Some body measurements are important factors for poultry breeders (Adeniji and Ayorinde, 1990). Researches (Meijerhof, 2005; Wolanski et al., 2007; Petek et al., 2008) showed that the quality of the day-old chick had a big influence on the growth and final performance of the poultry. Wolanski et al. (2006) found a positive relationship between hatchling length and body weight at 35 days of age in male broilers, respectively. Breeders need some techniques to select animals for their breeding aims (Saatçi and Tilki, 2007).

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Breeder age is important because it has positive effect to ducklings growth (Applegate and Lilburn, 1998). However, the relationship between body measurements at hatch and later performance in ducks obtained from different breeder age are not well known. The present study aimed to determine the correlation between some body measurements at hatch and at 6 week of age in male Pekin ducks obtained from different breeder ages.

Material and Methods

Seventy two male Pekin ducklings (Star 52) were used in this study. Ducklings were obtained 37 and 135 weeks of breeder age. They were leg-banded after hatching and then were reared in groups of 12 ducklings in floor pens (170X94, width X length, respectively). One bell drinker and one hanging suspended feeder were used in the floor pen. It was covered with wood shavings as a litter. Birds were fed a starter diet (18 % crude protein and 2800 kcal/kg metabolizable energy) until 3 weeks age and then they were fed a grower diet (17 % crude protein and 2700 kcal/kg metabolizable energy). Food and water were provided *ad libitum* during the experiment.

Ducks were weighted and body measurements were taken just after each weighing during the 6 weeks. Beak length was defined as length of the upper beak rim, and head length as the distance between the end of the beak and the end of the *condylus occipitale*. Beak and head diameter were measured using calipers. Neck length was measured between the first and the last cervical vertebrae and body length between the first cervical vertebra and the pygostyle. The length of the tibia and metatarsus was measured on the right leg. Chest depth was measured between the first back vertebra and the sternum. Chest width was measured as the distance between the right and left glenoid cavity (Saatçi and Tilki 2007).

Statistical analysis was assessed by using SPSS Version 21.0 for Windows (SPSS, Inc., Chicago, IL, USA). Body weight and some body measurements of ducks obtained two different breeder ages were analyzed separately. Pearson's correlation tests were performed for correlations between some body measurements at hatch and at 6 week of age in male Pekin ducks. *P*-values less than 0.05 were considered significant.

Results and Discussion

Correlation coefficients between some body measurements at hatch and those at 6 week of age in male Pekin ducks obtained from 37 week old breeders ranged from 0,001 to 0.566 (Table 1). And the highest correlation coefficients was between body weight at hatch and body weight at 6 weeks of age, while the lowest correlation coefficients was between metatarsus length at hatch and head diameter at 6 weeks of age. However, correlation coefficients between some body measurements at hatch and those at 6 week of age in male Pekin ducks obtained from 135 week old breeders ranged from 0.005 to 0.473 (Table 2). The highest correlation coefficients was between neck length at hatch and body length at 6 week of age, while the lowest correlation coefficients was between chest width at hatch and metatarsus length at 6 week of age. However, only correlation between body weight at hatch and body weight at 6 week of age of male ducks obtained from 37 week old breeder age was statistically significant (P<0,01). Wolanski et al. (2004) found a positive relationship between hatchling weight and body weight at six weeks of age, without taking gender into account. In our study, correlation coefficient of male ducks obtained from younger breeder was higher than that from older breeder. This result showed that breeder age needs to be taken into account when hatchling weight is used for the prediction of subsequent performance.

Correlation coefficients between head length at hatch and beak diameter at 6 week of age,

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neck length at hatch and head, beak and body lengths at 6 week of age, body length at hatch and beak length at 6 week of age, chest width at hatch and beak diameter at 6 week of age, tibia length at hatch and head, beak and neck lengths at 6 week of age, metatarsus length at hatch and body weight at 6 week of age in male ducks obtained from younger breeder were statistically significant. Longer head at hatch caused the wider beak at 6 week of age (P<0,05). Among neck length at hatch and head, beak and body lengths at 6 week of age were found negative correlation. Therefore, longer neck at hatch caused the shorter head, beak and body (P<0,05). Between body length at hatch and beak length at 6 week of age was determined the negative correlation (P<0,01). Chest width at hatch was only correlate with beak diameter at 6 week of age and wider chest at hatch was caused to wider beak at 6 week of age (P<0,05). Tibia length at hatch with head (P<0,001), beak (P<0,001) and neck (P<0,05) lengths at 6 week of age had negative correlations and longer tibia at hatch caused to shorter head, beak and neck at 6 week of age. Length of metatarsus at hatch had positively correlate with the body weight at 6 week of age (P<0,05).

However, correlations in examined parameters of male ducks obtained from 135 week old breeder were very limited and they were different from 37 week old breeder. Only significant correlation was found head diameter at hatch with chest width (P<0,05), beak length at hatch with beak length at 6 week of age (P<0,05), neck length at hatch with beak diameter, body and tibia lengths (P<0,05) at 6 week of age. In male ducks obtained from 137 week of age, wider head at hatch was linked with wider chest at 6 week of age. Longer beak at hatch was caused shorter beak at 6 week of age. And longer neck at hatch was caused to narrowed beak, shorter body and shorter tibia at 6 week of age.

Conclusion

The present study showed that breeder age affected the correlations between some body measurements at hatch and at 6 week of age in male Pekin ducks. Breeder age needs to be taken into account when hatchling traits are used for the prediction of subsequent performance. And this knowledge is undertaken to select the animals.

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Table 1. Correlation coefficients between some body measurements at hatch and at 6 week of age in male Pekin ducks obtained from 37 week old breeders

Variable at hatch					Variable at 6 wk of age						
	BW	Head length	Head diameter	Beak length	Beak diameter	Neck length	Body length	Chest depth	Chest width	Tibia length	Metatarsus length
BW	0,566**	-0,022	0,006	0,070	-0,056	0,007	-0,001	0,213	-0,132	-0,154	-0,083
Head length	0,141	0,090	0,091	0,077	0,295*	0,141	0,076	0,107	-0,137	0,106	0,049
Head diameter	0,105	0,178	0,270	-0,224	-0,155	-0,033	-0,095	-0,230	-0,022	0,150	-0,151
Beak length	-0,078	-0,149	0,049	-0,266	-0,119	0,092	-0,162	-0,027	-0,066	0,145	0,163
Beak diameter	0,088	-0,321	0,137	-0,151	-0,180	-0,076	-0,212	-0,137	0,026	0,118	0,156
Neck length	0,054	-0,306*	0,067	-0,340*	-0,215	-0,068	-0,353*	-0,004	-0,015	-0,048	-0,091
Body length	0,017	-0,109	0,032	-0,410**	0,000	-0,131	-0,104	-0,003	-0,089	0,048	-0,042
Chest depth	0,042	0,058	0,169	-0,072	0,219	-0,046	0,073	0,085	0,140	-0,029	-0,138
Chest width	0,128	0,271	0,275	0,171	0,319*	-0,063	-0,048	0,300*	0,098	-0,038	0,149
Tibia length	0,006	-0,379***	-0,234	-0,520***	-0,264	-0,315*	-0,265	-0,106	0,125	-0,079	-0,033
Metatarsus length	0,312*	-0,106	0,001	-0,205	-0,026	-0,063	-0,072	0,143	0,211	-0,003	-0,011

*: P<0,05 **: P<0,01, ***: P<0,001

Table 2. Correlation coefficients between some body measurements at hatch and at 6 week of age in male Pekin ducks obtained from 135 week old breeders

Variable at hatch	Variable at 6 wk of age										
	BW	Head length	Head diameter	Beak length	Beak diameter	Neck length	Body length	Chest depth	Chest width	Tibia length	Metatarsus length
BW	0,327	-0,290	-0,197	-0,131	-0,015	-0,135	-0,230	0,310	0,273	-0,289	0,068
Head length	0,177	-0,013	-0,225	-0,075	0,023	-0,148	0,065	0,054	-0,179	0,114	0,076
Head diameter	-0,193	0,058	0,163	-0,161	0,199	-0,039	0,047	0,346	0,433*	-0,280	-0,148
Beak length	-0,081	-0,040	-0,145	-0,450*	-0,266	-0,246	-0,216	-0,335	-0,309	0,054	0,099
Beak diameter	0,054	-0,012	0,166	0,030	0,051	-0,157	-0,050	0,080	-0,264	0,155	-0,021
Neck length	-0,153	-0,330	0,120	-0,297	-0,514*	-0,299	-0,473*	0,148	0,291	-0,410*	-0,286
Body length	-0,105	00055	-0,300	-0,091	-0,098	-0,278	0,075	0,053	0,376	-0,073	-0,107
Chest depth	0,123	-0,025	0,189	-0,049	0,198	-0,231	-0,099	0,290	0,074	-0,161	-0,047
Chest width	0,273	-0,125	-0,060	-0,173	0,300	-0,289	-0,136	0,327	0,126	-0,300	-0,005
Tibia length	-0,083	-0,051	-0,227	-0,330	-0,141	-0,144	0,029	-0,233	0,110	0,043	-0,109
Metatarsus length	0,206	0,249	-0,165	-0,215	-0,128	-0,251	-0,083	-0,194	0,076	-0,019	0,077

*: $P<0.05$

Oral presentation

STUDY OF GENETIC DIVERSITY IN *HIPPODAMIA VARIEGATA* (COL: COCCINELLIDAE) (GOEZE) ON LORESTAN PROVINCE USING RAPD MARKERS

Yari R[1]*., Hashemi M[2]., Jafari R[3].

Abstract

Lady beetle *Hippodamia variegata* (Goeze) is one of the predators on aphids that have a significant role in their biological control. Despite the importance of these efficient predators, limited study has been done on the effect of different hosts on the biology of this lady beetle in Iran. In order to evaluate genetic diversity within and between species of *H. variegata* was evaluated using 9 RAPD primers (A-03, A-07, C-02, C-12, C-14, K-01, K-02, K-19, F-09) in 11 samples collection from different parts of Lorestan province. All primers repeatable bands clearly established. Length of amplified fragments by all primers were varied from 150 to 2200 base pairs. Primer A-07 (27 bands) produced the highest and primer C-02 (15 bands) produced the lowest number of bands. Two populations Doroud and Khorramabad constructed the first cluster close to 0.43 Nei & Li coefficient was calculated using UPGMA method. Other clusters formed with a very low percentage of similarity. This results show the susceptibility of ecology and geography of Lorestan province despite the small size of the province to establish and maintain genetic diversity in the province Lady beetles. The results of cluster analysis indicated high genetic differences among populations studied in Lorestan province.

Keywords: Hippodamia variegata, RAPD-PCR, genetic variation, Loresta

Introduction

Coccinellids have been widely used in biological control for over a century and the methods for using these predators have remained virtually unchanged. The causes for the relatively low rates of establishment of coccinellids in importation biological control have not been examined for most species. Augmentative releases of several coccinellid species are well documented and effective; however, in effective species continue to be used because of ease of collection. For most agricultural systems, conservation techniques for coccinellids are lacking, even though they are abundant in these habitats. Evaluation techniques are available, but quantitative assessments of the efficacy of coccinellids have not been done for most species in most agricultural crops. Greater emphasis is needed on evaluation, predator specificity, understanding colonization of new environments and assessment   of

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community-level interactions to maximize the use of coccinellids in biological control (Obrycki and Kring, 1988).

H. variegata is an Old World ladybird, occurring also in Africa. There is much variation in Old World *H. variegate* in color and pattern of the elytra (wing covers) (Dobzhansky, 1933, Hodek, 1973). But none has been noted among New World forms. The larvae and adults of *H.variegata* are important predators of aphids and other plant parasitic insects (Obrycki and Orr., 1990). Like most members of Coccinellidae, adult *H.variegata* are able to exploit habitats where prey exist and to move on to new, unexploited patches when local prey abundance dwindles. Thus *H.variegata* was considered to be a valuable agent for biological control of plant pests, and it was reasoned by the U.S. (Krafsur et al. 1996).

[22]*RAPD-PCR*

RAPD markers consist of relatively short DNA fragments (about 200-2000 base pairs long), amplified via PCR by small (usually 10 bases in length) arbitrary (with a G + C content > 50%) primers. Typically, primers are used singly and must anneal to priming sites in opposite orientations in order for amplification to occur. Following convention, we term the pair of inverted priming sites, plus the intervening sequence of nucleotides, a RAPD locus, and the amplified product from a particular locus a RAPD marker. The resulting amplification product(s) can be size-separated electrophoretically on an agarose gel and visualized by SYBR green staining (Grosberg et al., 1996).

Because RAPD-PCR primers are not designed to amplify a specific target sequence, the amplified loci are anonymous and presumably scattered throughout the genome (Williams et al., 1990; 1991; Tinker et al., 1993). RAPD loci carry the advantages that (1) there is no need for prior nucleotide sequence data for the taxa under study and (2) many of the loci may be acting as neutral markers. On the other hand, in the absence of information concerning the nature of the DNA being amplified by RAPD-PCR, there is no guarantee that any single primer will produce usable markers or that the variation is, in fact, neutral (Grosberg et al., 1996).

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[22] Randomly Amplified Polymorphic DNA

Material and Methods

Sample collection

To identify of genetic variation in nine natural populations and two trade populations of this lady, in the march of 2014 samples from Lorestan province of Iran (Pole-e Dokhtar, Azna, Boroujerd, Khorramabad, Aleshtar, Doroud, Aligoudarz, Nourabad and Kouhdasht) regions collected(Fig. 1). In order to study of genetic diversity 9 decameric random primers used. Samples were stored in 96% ethanol separately.

Fig.1: *H. variegate* populations studied in Lorestan province. Standard1=S1,Standard2= S2, Azna=Az,Khorramabad= KH, Nourabad=N, Aligoudarz=Al,Kouhdasht= K,Pole-Dokhtar= P, Doroud=D,Boroujerd=B, Aleshtar=Als

Table 1
Code and sequence nucleotide primers used in the RAPD analysis

Primer	Primer sequence 5′ to 3′
K-01	5′-CATTCGAGCC-3′
K-02	5′-GTCTCCGCAA-3′
K-19	5′-CACAGGCGGA-3′
C-02	5′-GTGAGGCGTC-3′
C-12	5′-TGTCATCCCC-3′
C-14	5′-TGCGTGCTTG-3′
A-03	5′-AGTCAGCCAC-3′
A-07	5′-GAAACGGGTG-3′
F-09	5′-CCAAGCTTCC-3′

DNA Isolation

The genomic DNA was extracted from abdominal. DNA was extracted with Kit (takapouzist.com). DNA stored at -20 ºC until used. Total genomic DNA concentration was measured by spectrophotometer at 260nm wavelength. Quality of DNA was checked by running 5 µl of extracted DNA on 0.7% agarose gle prepared in 0.5X TBE buffer. The DNA samples giving smear in the gel were rejected.

RAPD Amplification AND Data Analysis

RAPD-PCR amplifications were performed in a total volume of 25 µl. RAPD analysis were done using random decamer primers synthesized by Gen Fanavaran Company, Iran. Total of 9 RAPD primers [Operon Technologies] (Table 1) of 4 different series (A, C, F and K) were used to amplify the genomic DNA of 11 *H. variegata*.

The RAPD-PCR reaction was performed by using 10X PCR buffer with MgCl2, dNTP (dATP, dCTP, dGTP, dTTP), decamer oligonucleotide primer and Taq polymerase. RAPD-PCR was optimized containing 2.5µl of 10X buffer, 1µl of MgCl$_2$, 0.5µl dNTPs, 2µl RAPD-primer, 0.4µl Taq and 2U of genomic DNA. The genomic DNA amplification was done by using thermal cylcer (convergent-Germany) using the following program: 5 minutes initial denaturation at 94°C followed by 40 cycles comprising 30 Second denaturation at 94°C, 40 Second primer annealing at 40°C and extension at 72°C for 40 Second and then final extension at 72°C for 5 minutes. PCR products were separated on 1.5% agarose gel. Gels were run at 5 V/cm for one hours, stained with SYBR green and photographed under UV light. All results were not shown (Fig. 2).

Results and Discussion

   The reproducibility of the RAPD techniques can be influenced by variable factors, such as sequence of primer, template quality and quantity, the type of thermal cycle and polymerase employed. The extreme band obtained from A-07 primer with 27 bands and the least bond belonged to C-02 primer with 15 bands. A total of 179 fragments were amplified. The number of amplification products produced varied between15 to 27 with an average of 19.80 per primer. Two populations Doroud and Khorramabad constructed the first cluster close to 0.43 Nei & Li coefficient. Other clusters formed with a very low percentage of similarity. The results of cluster analysis indicated high genetic differences among populations studied in Lorestan province, Iran.
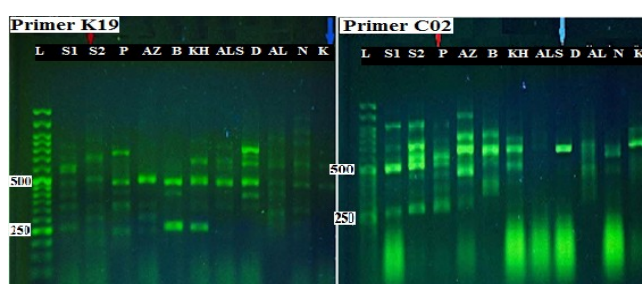
Fig. 2: RAPD patterns generated using Nine decameric primers and DNA from *H. variegate* primers K-19, C-02.

Amplified fragments were scored from top to bottom of the lane as presence (1) or absence (0) and bivariate 1-0 data was used for genetic analysis. Only visible and unambiguous amplified fragments were scored. Genetic similarity among 11 sampled was calculated using Nei's similarity indices by analyzing the data in UPGMA method by [23]MVSP ver. 3.2 software. The similarity index was used to calculate the genetic distance values and to construct the dendrogram. The dendrogram provides a visual representation of the differences in the population of *H. variegata*. The molecular weights of bands were estimated based on the standard bands from Gene Ruler DNA Ladder Marker. The presence of band was scored from the photograph. Only clear and reproducible bands were given consideration (Fig. 3 and Fig. 4).

[5] Multi Variate Statistical Package
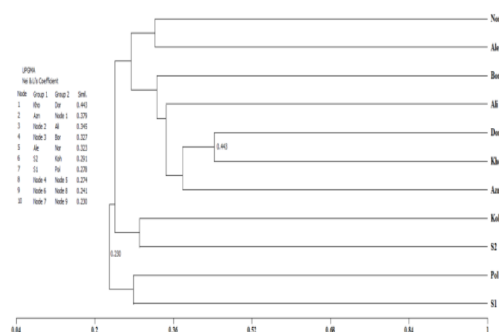
Fig. 3: Dendrogram of *H. variegata* populations generated by UPGMA analysis using Nei and Li coefficient.
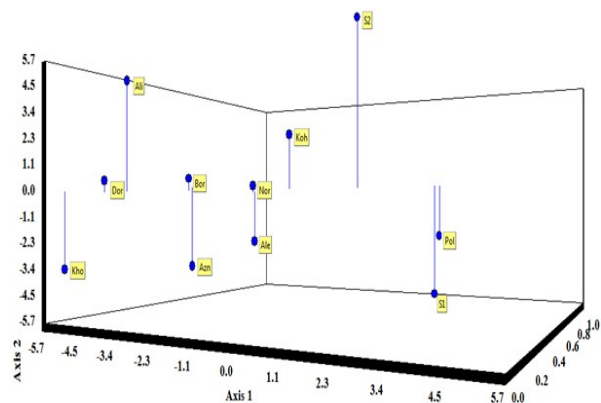
Fig. 4: 3D Ordination [24]PCoA

While RAPD analysis is clearly an approach of merit for such studies, some characteristics of RAPD reactions were encountered. First, RAPD band patterns must be empirically determined to be reproducible before their use as markers is justified. This could be made after repeated reactions to confirm the absence or presence of the band. Second, preferential amplification during RAPD reactions has little effect on the use of RAPD markers in diagnostic studies, but could be of serious importance in studies requiring independent markers, such as hybrid zone analysis, pedigree analysis or relatedness estimates based on band sharing. Conclusively, on the bases of ease, cost, technical labor, speed and amount of DNA needed, the RAPD marker system is preferentially used in taxonomic and classification studies.

The following table compared the results of this study with the results of other scientists in other regions (table 2). Quality results with RAPD-PCR method depends on the type of DNA sample (animal, plant,..), the DNA extraction method, the number and type of primers, PCR program, components of the PCR reaction and even thermocycler model are used.

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[6] Principal Coordinates Analysis

Table 2.
Results comparison of this study with other researches

	This study	(Bamehr et al., 2014)	(Haitham et al., 2009)	(Haubruge et al., 2002)
Population	Hippodamia variegata	Cryptolaemus montrouzieri	Coccinella undecimpunctata	Coccinella septempunctata
Locations	Lorestan	mazandaran	Egypt	Belgium
Number primers	9	22	5	16
Primers kind	(C,K,A,F)	(OPB01,10,OPA01,10,OPW01-2)- B-09 no band -21 primers	(C07,C15,C16,C18,K15)	(OPBE-01, OPBE-16)
Cycle PCR	40	45	-	-
Total bands	179	437	80	-

Conclusion

The results showed that the Lorestan province with a small area have a high potential in creating or maintaining genetic diversity in population of predators. This is necessary to preserve and protect natural growth and development regions by the relevant authorities.

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Poster presentation

ASSESSMENT OF DAIRY TRAITS GENETIC PARAMETERS AND MOST PROBABLE PRODUCING ABILITY IN BLACK AND WHITE COWS

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Abstract

Phenotypic and genetic variability and Most Probably Producing Ability were assessed on the set of production results of 17259 lactations of black and white cows. Cows were the daughters of 62 bull-sires and they were raised on 7 farms belonging to the Agricultural Corporation Belgrade during 17-year period. During the period analyzed the cows produced on average 7370.8 kg milk 3.55% milk fat and 260.05 kg milk fat. The average yield of 4% fat corrected milk was 5537.08 kg. A highly significant effect (P<0.01) of all studied factors (farm, year, season, lactation in order and HF genes ratio) on all analyzed dairy traits was confirmed.

Variance components were estimated by the means of the Restricted Maximum Likelihood (REML) of Statistical Analysis System - SAS, Version 9.1.3. (2012.). For single models, the estimated heritability values for milk yield, fat yield, fat percentage and 4% fat corrected milk were 0.177, 0.141, 0.106 and 0.138 respectively. The estimated repeatability values for mentioned traits were 0.224, 0.254, 0.170 and 0.192.

The results obtained for MPPA show a great variability of studied traits among the cows. The results also indicate great possibilities for further selection work on improving the milk yield traits what can simultaneously be positively reflected on the economy and can save time in making decisions about the removal of low producing individuals from the production.

Keywords: milk yield traits, heritability, repeatability, MPPA, black and white cows[25]

Introduction

Genetic improvement of the most important milk yield traits, milk yield in particular, is an important component of a comprehensive strategy for improving the profitability and sustainability of dairy cows production, from the economic aspect. Determining the significance of genetic and non-genetic factors which essentially affect the variability of dairy traits and the selection of best female and male individuals on the basis of assessed breeding value is necessary in order to assess the program of genetic improvement of dairy cattle and specify the research fields that should be improved (Sarakul et al. 2011).

Development of animal breeding plans requires knowledge of heritability, repeatability, and phenotypic and genetic correlations of the traits included. These parameters are needed to evaluate the breeding plan itself as well as to predict animal breeding values (Konig et

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al. 2005; Hammami et al. 2008; Gorbani et al. 2011). Breeding plans for dairy cattle have to account for repetitive performance of cattle, i.e. potential for each cow with more than one lactation (Sahin et al. 2012).

On the basis of greater number of measurements of one trait in the same animal it is possible to calculate the repeatability coefficient for given trait and thus increase the accuracy of the estimation of breeding value (Stanojević et al. 2013). Repeatability coefficient is used to calculate the Most Probable Producing Ability (MPPA) in cows on the basis of the first production results (Lakshmi et al. 2010; Sharma et al. 2011). The regression seen in the future results towards the present ones or the level up to which the result can be repeated should be known in order to efficiently choose the cows which can be expected to realize higher production during the next lactation. Ranking of cows on the basis of their most probably producing ability is an efficient way to collect the information for removal from the breeding stock.

The aim of this research was to study the effect of different factors on the phenotypic variability of the milk yield traits in black and white cows, and to assess the values of genetic parameters and MPPA of studied traits. Obtained results are important starting point for further research, particularly for the assessment of the bovine breeding value.

Material and Methods

Phenotypic variability and values of heritability and repeatability coefficients and MPPA have been assessed on the data set of production results of 5735 black and white cows. The analyzed animals were raised on 7 farms of Agricultural Corporation Belgrade for the period of 17 years. The cows are the daughters of the bull-sires and all of them had first three standard lactations concluded what makes the total of 17259 lactations.

The values of phenotypic and genetic parameters and MPPA are calculated for the following milk yield traits in standard lactation: milk yield (MY-305), milk fat content (FC-305), milk fat yield (FY-305) and yield of 4% fat corrected milk (4%FCM-305).
In order to determine the mean values and the effects that factors have on the studied traits we have used the following mixed model equation:

$$Y_{ijklmn} = \mu + F_i + G_j + S_k + L_l + H_m + o_n + e_{ijklmn},$$

where: Y_{ijklmn}- is the phenotype expressiveness of studied trait, F_i- fixed effect of the farm where the animal had production (i=1...7), G_j- fixed effect of the calving year (j=1...17), S_k- fixed effect of the calving season (k=1...4), L_l- fixed effect of the lactation in order (l=1...3), H_m- fixed effect of the yield of Holstein-Friesian (m=1 if share HF genes smaller then 50%, m=2 if share HF genes is between 50 and 75%, m=3 if share of HF genes is larger then 75%), o_n- random effect of bull sire (n= 1....62), e_{ijklmn}- random error.

The variance components used for the estimation of heritability and repeatability of each trait were obtained by the SAS (2012) Variance Components Procedure (PROC VARCOMP) by means of the Restricted Maximum Likelihood Method.

For calculating the Most Probable Producing Ability (MPPA) of dairy traits we have used the following mathematical formula (Lush, 1945; Falconer and Mackay, 1996).

$$MPPA = \mu + \frac{nr}{1 + (n-1)r} \; (\overline{Xi} - \mu)$$

where: μ= is the herd average; n = number of lactations; r = repeatability; $\overline{Xi}$ = average of all the lactations of ith animal. The animal, which produces less than average MPPA or below a certain MPPA level is being removed from the herd.

Results and Discussion

Table 1 shows the indicators of phenotype expressiveness and variability of the milk yield traits included in the analysis:

Table 1: Indicators of phenotype expressiveness and variability of milk yield traits in standard lactation

Traits	n	$x\bar{x}$	SD	CV (%)	min	max
MY-305(kg)		7370.8	1761.72	23.9	2558	14705
FC-305(%)	17259	3.55	0.3	8.45	2.04	5.74
FY-305(kg)		260.05	60.71	23.34	88.81	597.48
4%FCM-305 (kg)		6849.08	1586.31	23.16	2376.95	14001.4

n – number of cows**;** xx� mean value; SD - standard deviation; CV – coefficient of variation; min- trait minimal value; max- trait maximal value; MY-305-milk yield; FC-305- milk fat content;   FY-305- milk fat yield; 4%FCM-305- yield of 4% fat corrected milk

The animals raised in the analyzed period produced on average 7370.8 kg milk, with wide variation interval from 2558 to 14705 kg. The values specified for standard deviations and variations coefficients for the studied traits are relatively high what shows that here we have heterogenous population which provides sufficient space for successful selection.

The results reported for milk yield in this study are in conformity with the research conducted by Carlén et al. (2004) and Đedović et al. (2013). Significantly higher values for milk yields in Holstein Friesian cattle populations are reported by a number of authors (Weller and Ezra, 2004; Kadarmideen et al. 2005; Heins et al. 2006; Zink et al. 2012), while lower yields are reported by Đedović et al. (2003), Espinoza et al. (2007), Lateef et al. (2008), Gaidarska (2009), Katok and Yanar (2012) and Bunevski et al. (2013).

The effect of systematic non-genetic factors on the milk yield traits in the first three standard lactations (farm, year, season, lactation in order and yield of HF genes), as well as the mean values and standard deviation studied per mentioned factors are shown in Table 2. The results of F- test confirmed a highly significant effect of all studied factors (P<0.01) on milk yield traits.

Table 2: The effect of systematic non-genetic factors and the mean values and milk yield trait SD

Class	n	Traits			
		MY-305	FC-305	FY-305	4%FCM-305
Overall mean	17259	$\bar{x}\pm$SD	$\bar{x}\pm$SD	$\bar{x}\pm$SD	$\bar{x}\pm$SD
Farm					
1	2814	7834±1920	3.66±0.021	287.27±73.14	7442.6±1852.4
2	2904	7718±1736	3.41±0.033	262.26±59.78	7020.9±1552.4
3	2199	7300±1654	3.63±0.039	261.69±50.86	6845.3±1375.2
4	2310	7237±1620	3.52±0,036	252.38±53,40	6680.5±1402.5
5	2517	7412±1750	3.47±0,025	255.17±54,85	6792.4±1503.3
6	2901	6687±1658	3.56±0,022	238.83±56,20	6227.4±1491.8
7	1614	7393±1639	3.62±0,018	266.69±57,11	6597.6±1503.8
Significance		**	**	**	**
Calving year					
1996	124	5592±979	3,70±0.012	206.83±34,68	5339.7±907.3
1997	223	5871±1155	3,73±0.016	219.5±43,58	5641.3±1108.7
1988	318	6374±1170	3,71±0.019	236.22±42,70	6092.9±1096.3
1999	653	5570±1009	3,64±0.012	210.12±36,37	5459.9±949.9
2000	970	5237±956	3,66±0.017	191.63±34,64	4969.4±893.6
2001	1483	6243±1195	3,69±0.022	230.17±44,76	5950.1±1136.1
2002	1797	7073±1282	3,70±0.025	261.69±44,76	6754.5±1206.5
2003	1723	7171±1335	3,63±0.023	259.46±46,15	6760.5±1197.5
2004	1779	7300±1511	3,51±0.030	255.75±53,13	6756.3±1369.3
2005	1731	7753±1531	3,45±0.037	266.92±58,62	7105.2±1438.3
2006	2170	7834±1598	3,37±0.032	263.57±58,05	7087.3±1467.2
2007	1951	8217±1675	3,42±0.030	280.63±60,06	7496.3±1535.4
2008	1542	8708±1791	3,49±0.026	303.54±64,04	8036.4±1649.2
2009	795	8982±1805	3,53±0.023	317.18±64,68	8350.7±1668.9
Significance		**	**	**	**
Calving season					
1	4043	7401±1698	3.53±0.030	260.17±58.56	6863.1±1525.4
2	4186	7044±1702	3.56±0.035	249.60±59.37	6561.9±1542.7
3	4878	7325±1788	3.55±0.029	259.21±61.12	6818.5±1605.9
4	4152	7722±1783	3.53±0.029	271.45±61.65	7160.8±1607.7
Significance		**	**	**	**
Lactation number					
1	5753	6474±1374	3.57±0,029	230.18±47.28	6042.4±1229.5
2	5753	7568±1658	3.54±0,030	266.75±56.87	7028.5±1482.6
3	5753	8070±1827	3.52±0,029	283.20±64.15	7467.4±1662.4
Significance		**	*	**	**

Different farm management (applied technology of raising, nutrition, protection, hygiene, health care, organization of work and management), and unequal number of daughters per bull - sires resulted in unequal milk yield in standard lactation being in the range of 6687±1658 (farm 6) to 7834±1920 (farm 1). Each year, in which the animals realized production had its own special characteristics expressed through climatic factors, method of preparation and use of feeds, the organization of work on the farms, and they affected overall operation, therefore the phenotype expressiveness of the milk yield traits had oscillations during the studied period. Highest milk production was realized in 2009 (8982±1805), and lowest in 2000 (5237±956) kg.

In standard lactation, the highest quantity of milk was produced by the cows calved in IV season-autumn (7722±1783 kg). Lowest milk yield was produced by the cows calved in II season-spring (7044±1702 kg). Negative effect of high temperatures and air humidity terminates during the first autumn months so the animals liberated from these stress factors utilize more nutritive substances in ratio and thus increase the milk production. The highest yields were realized in third lactation (8070±1827), what is in harmony with the previous research results (Trifunović et al. 2002; Bunevski et al. 2013).

Highly significant effect of systematic factors on phenotype expressiveness of milk yield trait was reported by Lateef et al. (2008). According to them calving season and lactation in order had a significant effect on milk production. Differences in the height of milk yield, milk fat, 4% MKM and milk fat content per calving year were statistically highly significant what is in accordance with the results reported by Katok and Yanar, (2012).

The effect of season was dominantly expressed by the model of nutrition and micro climate in the facilities. The confirmation of these results can be found in the study of Lateef et al. (2008), who also confirmed that Holstein Friesian cows realized highest milk yields in autumn season.

Table 3 shows the calculated variance components (additive, phenotypic and the variance of permanent environment effects), on the basis of which the heritability and repeatability coefficients were estimated. We have obtained relatively low heritability coefficients for all studied traits of milk yield in standard lactation. Heritability for milk yield was 0.177, for milk fat content 0.141, for milk fat yield 0.106, and 0.138 for yield of 4% FCM, respectively.

Table 3: Estimated variance components, heritability (h^2) and repeatability (r) of milk yield traits

Traits	σ_{aa}^2	σ_{pppp}^2	σ_{pp}^2	h^2	r
MY-305(kg)	444843.759	117846.962	2515371.004	0.177	0.224
FC-305(%)	104.822	84.656	745.734	0.141	0.254
FY-305(kg)	330945.033	200321.410	3122604.771	0.106	0.170
4%FCM-305(kg)	287728.893	113738.797	2091102.631	0.138	0.192

σ_{aa}^2 - estimated variance component for additive genetic effect; - σ_{pp}^2 estimated variance component for permanent environmental effects; σ_{pp}^2 - phenotypic variance

One of the biggest problems that researchers face is a proper estimation of the variance

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components. Decrease in the error when calculating the additive and non-additive components of variance contributes to more accurate estimation of genetic parameters. Low heritability values in the said research can be explained by low additive variance and relatively high dominance variance. In populations in which cross-breeding is conducted or improved (as is the case with the studied one) the occurrence of heterosis effect is possible with simultaneous action of other non-additive gene effects what can cause lower values for hereditary coefficients (Đedović et al. 2002). Relatively low values of trait hereditary coefficients in standard lactation can be explained by the increase in the environment variance which is not followed by proportional increase in genetic variance and can result in decrease in the heritability value. The selection intensity should also be taken into account as well as an important effect of systematic factors (Table 2), what was confirmed in this study.

Obtained heritability values were lower than values reported by Lidauer et al. (2003, Elzo et al. (2004), Weller and Ezra (2004), Konig et al. (2005), Kunaka et al. (2005), Hammami et al. (2008), Gorbani et al. (2011) and Kheirabadi et al. (2013). If we compare the estimated heritability values in this study with the results reported by Stanojević et al. (2012) and Đedović et al. (2013) little differences can be seen. The reason for that is that the research was carried out in the populations of black and white cows raised in similar breeding conditions and under similar intensity of applied selection.

Since three successive lactations were compared it was possible to estimate the repeatability coefficients of dairy traits. Since repeatability in a denominator includes more components or sources of variability than the hereditary coefficient, the values of the repeatability coefficients were higher: 0.224, 0.254, 0.170 and 0.192 for milk yield, milk fat content, milk fat yield and yield 4% FCM, respectively. The obtained values for the repeatability coefficients confirm the significant effect of permanent environment factors on the milk yield traits variability.

Reported repeatability values are in accordance with the results of Spasić et al. (2012), except for the milk fat content. For this trait Spasić et al. (2012) determined a considerably lower value of repeatability (0.105). Gorbani et al. (2011) also state similar values of this coefficient for milk yield and milk fat yield and lower value for milk fat content (0.18). Studying the repeatability of the milk yield trait in three different genotypes, Adeoye and Ogundipe, (2011) calculated lower repeatability values for milk yield in whole and standard lactation, while considerably greater values for milk yield (0.54) were determined by Mitsouyoshi et al. (1994).

Repeatability coefficient is used for calculating the most probably producing ability in dairy cows (MPPA) which, on the basis of the initial measurements, enables us to make projections of the animal future production.
The results of estimated value MPPA of investigated dairy traits are shown in Table 4.

Table 4: MPPA maximal and minimum values

Traits	MPPA min	MPPA max
MY-305(kg)	4524	8408
FC-305(%)	3.05	4.01
FY-305(kg)	170.71	284.57
4%FCM-305(kg)	4374	7572

Each cow is separately estimated for all studied traits and the MPPA which shows the extent of superiority or inferiority of a given cow in relation to the herd and what could be expected in the next lactation.

Results obtained for MPPA showed that there is a great variability between the cows for investigated traits. The difference between the lowest and the highest estimated value of MPPA for milk yield was 3884 kg, for milk fat content 0.96%, milk fat yield 113.86 kg and yield 4%MCM 3198 kg.

The cows which were extremely low producing during the first lactation under apparently normal conditions should be removed from the herd. Lakshmi et al. (2010) and Sharma et al. (2011) point out that the greatest value of high repeatability estimation and MPPA is the saving of time in making decisions about removing certain individuals from the herd.
The estimation of genetic parameters and MPPA seems to be significant for establishing the effect of the applied selection in population. Higher values of these coefficients provide a reliable estimation of breeding value and more efficient selection and therefore a successful designing and realization of the plan and program of the improvement in dairy cattle.

Conclusion

After the analysis of the productivity of 5735 cows of black and white breed a statistically highly significant effect of studied factors: farm, year, season, lactation in order and yield of HF genes on the traits: milk yield, milk fat content, milk fat yield and yield of 4% fat corrected milk was obtained. As it was expected, the estimated values of heritability of studied traits were lower than the values of the repeatability coefficients. The results of MPPA show that there exists a great variability among the cows. The contribution of repeatability estimation and MPPA is reflected in the fact that it can save our time in making decisions about removing a certain individual from the production.
The estimation of genetic parameters and MPPA seems to be significant for establishing the effect of the applied selection in population. Higher values of these coefficients provide a reliable estimation of breeding value and more efficient selection and therefore a successful designing and realization of the plan and program of the improvement in dairy cattle.

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Poster presentation

EFFECTS OF AGE AND FORCED MOULTING ON PRODUCTION TRAITS OF LOHMANN BROWN HYBRID LAYING HENS

Milojević M.[1], Đermanović V.[1], Mitrović S.[1], Radoičić Dimitrijević M.[1]

Abstract

The primary purpose of this study was analysis of the results of Lohmann Brown hybrid hens egg production after the implementation of forced moulting and subsequent comparison of these with the results in the first year of egg production of laying hens. Study covers three periods: first – egg production during the first year (19 – 71 weeks of laying hens` age); second – moulting period (72 – 76 weeks of age); third – period following the forced moulting (77 – 102 weeks of age). Particular attention was paid to mortality, laying intensity, egg mass and food consumption during these periods. Finally, effects of age of laying hens on the intensity of laying in different stages of the production cycle (before and after the forced moulting) was determined calculating the coefficients of phenotype correlation (r_p). The experiment covered a total of 44,400 commercial layers kept in a cage system on the family farm "Rakić – Komerc" (Batković, Bijeljina, Republic of Srpska, BiH). 153.74 eggs were produced per housed layer (laying intensity 84.47%) from 19 to 44 weeks of age (26 weeks of egg production); 306.03 eggs were produced per layer (82.49%) from weeks 19 to 71 of age (53 weeks of egg production); while during the second stage of laying, after the moulting, 131.78 eggs per layer (72.43%) were produced during 26 weeks (second phase – production cycle) at which point layers were withdrawn from production at 102 weeks of age. 1,431 individual hens died (3.20%) from 19 to 44 weeks of age, by 71 weeks of age 3,778 layers died (8.47%), during the moulting (5 weeks) 504 layers died (1.14%), and during the second cycle, i.e. from 77 to 102 weeks of age, 1,222 layers died (2.74%). Furthermore, mean positive correlation ($r_p = 0.470$) was identified between the age of layers and laying intensity during the first 26 weeks of egg production and coefficient of phenotype correlation was statistically significant ($P<0.05$). However, in other stages of the production cycle, no phenotype correlation was identified.

Keywords: forced moulting, laying hen, age of laying hens, laying intensity, mortality.

Introduction

Considering there are no domestic hybrid laying hens in Serbia nor in Republic of Srpska (parent flocks of different types and hybrids of chicken are imported), many poultry farms are forced to use laying hens for a protracted period of time (longer than one year), primarily for economic reasons, i.e. following a certain period of forced moulting hens are kept in use for another 6 months or more. Additionally, forced moulting allows for

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deferment of the peak production period to the moment when egg prices are at their maximum, thus making the egg production more profitable.

As food restriction as an element of every moulting process causes considerable stress in laying hens, it should be noted here that EU legislation – Law on animal welfare (Council Directive 98/58 EC) prohibits exposure of animals to hunger (Stevenson, 2012). However, moulting is for economic reasons largely implemented in many other countries, such as for example USA, Brazil, Turkey, Israel, Former Yugoslav Republic of Macedonia. In line with this, Bell (2003) states that approximately 90% of commercial chicken farms in USA implement forced moulting for the purpose of extension of the production period of laying hens, while Lee (1982) concludes on the basis of research undertaken that forced moulting of a commercial flock of hens results in a higher number of eggs per housed layer and in better shell quality.

Apart from cited authors, a notable number of researchers examined and applied different methods of forced moulting in poultry production. Following researchers gave their contribution to this matter: Bell (1965, 2000), Swanson and Bell (1975), Mitrović et al. (1995), Ocak et al. (2004), Garcia (2005), Hassanien (2011), Maslić–Strižak (2012), Molino and Garcia (2012), Rafeeq et al. (2013), Schulte-Drügelte and Thiele (2013), Aygun (2013), Aygun and Yetişir (2014). Common features of all their methods are restrictions of food, water and lighting program, i.e. restrictions of daylight length and light intensity.

Forced moulting of hens producing eggs for human consumption is frequently implemented on the "Rakić – Komerc" (Republic of Srpska, BiH) poultry farm which has a capacity of 200,000 hens in one production sequence. For this reason the main purpose of this study was analysis of the results of Lohmann Brown hybrid hen egg production after the implementation of forced moulting and subsequent comparison of these results with those in the first year of exploitation of laying hens. Basically, research can be divided into three periods: first – egg production during the first year (19 – 71 weeks of layers` age); second – moulting period (72 – 76 weeks of layers` age); third – period following the forced moulting (77 – 102 weeks of layers` age). Particular attention was paid to mortality, laying intensity, egg mass and food consumption during these periods. Finally, effects of age of laying hens on the intensity of laying in different stages of the production cycle (before and after the forced moulting) was determined by calculating the coefficients of phenotype correlation (r_p).

Materials and Methods

The analysis of productive traits of Lohmann Brown laying hens commercial flock was conducted on the poultry farm belonging to "Rakić – Komerc" commercial farm (Batković, Bijeljina, Republic of Srpska, BiH). One of the activities of this farm is breeding chicken flocks for commercial purposes - egg production. 44,400 of Lohmann Brown hybrid chicken - potential layers - were used as initial material for monitoring and analysis of productive traits. First stage of egg production period was 53 weeks long (19 to 71 weeks of age) and the second stage was 26 weeks long (77 to 102 weeks of age). Forced moulting was conducted during five weeks, i.e. for 35 days between these two stages (Table 1).

Table 1. Forced moulting of commercial laying hen flock program

Moulting period (days)	Length of light/day (hours)	Light intensity (W/m^2)	Daily amount of food per layer (g)	Water and vitamins
1 – 7	8	3	120	*ad libitum*
7 – 11	8	3	120	water and vitamins
12 – 13	8	1	no food	no water
14 – 17	8	1	no food	water and vitamins
18	8	1	no food	water
19	8	1	no food	water
20	8	2	120	water
21 – 22	8	2	120	water and vitamins
23 – 32	8	2	120	*ad libitum*
33 – 35	12	3	120	*ad libitum*

During the breeding (egg production) of the commercial hens flock, technology proposed by the breeder of this particular hybrid line of hens was used (www.ltz.de), with some minor modifications. All technological phases (food and water supply, airing, lighting, collection and transport of eggs from the facility to egg sorting area, sorting of eggs and waste removal) were automatically regulated and all irregularities were notified by light and sound signals on the control table placed at the entrance of the production area as well as in entry halls of each facility.

Since the laying intensity and egg mass were very low in week 18, week 19 was considered as the first week of egg production for the purpose of analysis, although this is somewhat early according to technological norms of this particular hybrid line. Basically, this research covers three periods: first – egg production during the first year (19 – 71 weeks of layers` age); second – moulting period (72 – 76 weeks of layer's age); third – period following the forced moulting (77 – 102 weeks of layer's age).

During the given period (before, during and after the moulting), i.e. during the breeding of the commercial flock of layers up to 102 weeks of age, particular attention was paid to mortality, laying intensity, egg mass (class) and food consumption.

Basic data processing was carried out using the common variation statistics methods (Hadživuković, 1991; Latinović, 1996), i.e. elementary variation statistics parameters were calculated (descriptive statistics): arithmetic mean ($\bar{x}$), arithmetic mean error ($S_{\bar{x}}$), standard deviation (S) and variation coefficient (C.V.). Differences established between individual parameters of two analyzed periods of egg laying (before and after the moulting) were tested with t-test applying adequate model, while calculation of the coefficients of phenotype correlation (r_p) between the age of the layer and laying intensity was carried out according to the model for the respective sample.

Results and Discussion

For the purpose of clarity and to facilitate easier discussion of obtained results, the following presentation starts with the general production data of the analyzed Lohmann Brown hybrid commercial flock before, during and after the forced moulting of layers (Table 2). Following this information, mean values and variability of production indicators, as well as significance of differences between mean values of laying intensity and mortality (Table 3), two most important indicators of this type of production along with food consumption, are presented.

From 19 to 44 weeks of age (26 weeks of egg production) 153.74 eggs were produced per housed layer (laying intensity 84.47%); from 19 to 71 weeks of age (53 weeks of egg production) 306.03 eggs were produced (82.49%); while during the second stage of laying, after the moulting, 131.78 eggs per layer were produced during 26 weeks (second phase – production cycle) at which point layers were withdrawn from production at 102 weeks of age (Table 2).

Garcia (2005) reported egg production in the second phase (after moulting) to be 5 – 10% lower that in the first phase (before moulting). Compared to our results, results achieved by the flock analyzed in that study were insignificantly lower in terms of laying intensity, particularly in the second production cycle. The author also observes that the most opportune time to start with moulting is when the flock is around 70 weeks old, which was the case in our research, also (the flock was 71 weeks old).

Contrary to the previous author, Maslić-Strižak (2009) conducted forced moulting of Hy Line hybrid layers at 87 weeks of age (laying intensity was 57.65%), moulting period was 6 weeks long, while the second production phase lasted 37 weeks. Up to 87 weeks of age, food consumption per housed layer was 133 g, 355 eggs were produced, while 4.85% of egg shells were of bad quality. During the second phase, (37 weeks) 170 eggs per laying hen were produced (65.64%), food consumption was 135 g, and there were 0.8% fewer eggs with bad quality eggshell than in the first phase of egg production. These results were partly similar to ours, except that the moulting period was longer, resulting in a higher mortality, so that the deaths during the moulting and the second phase of the production cycle were 6.4% (in comparison to 3.88% in our study). In case of somewhat longer forced moulting, (49 days) of Isa Brown hybrid layers (light, semi light, semi heavy and heavy) initiated at 80 weeks of laying hen's age, Ocak et al. (2004) reported significantly lower laying intensity of hens in all groups (from 40.08% to 45.08%) which is understandable, considering that the layers were used for 80 weeks during the first phase. In comparison to our study where the layers were used for 26 weeks during the second phase, these authors have shortened the second phase down to 21 weeks and still reported lower laying intensity of all weight categories, except of the light category of laying hens which achieved similar level - 72.17% as our hens - 72.43% (Table 2).

Table 2. Basic productivity indicators of commercial flock of laying hens

Indicators	Before moulting	Moulting	After moulting
EPHH (19 – 44 weeks old)	153.74	-	-
EPHH (19 – 71 weeks old)	306.03	-	-
EPHH (77 – 102 weeks old)	-	-	131.78
LI - % (19 – 44 weeks old)	84.47	-	-
LI - % (19 – 71 weeks old)	82.49	-	-
LI - % (77 – 102 weeks old)	-	-	72.43
NDL (19 – 44 weeks old)	1,431	-	-
NDLDM (5 weeks)	-	504	-
NDL (19 – 71 weeks old)	3,778	-	-
NDL (77 – 102 weeks old)	-	-	1,222
%DL (19 – 44 weeks old)	3.20	-	-
%DLDM (5 weeks)	-	1.14	-
%DL (19 – 71 weeks old)	8.47	-	-
%DL (77 – 102 weeks old)	-	-	2.74
FPD, g (19 – 44 weeks old)	125.60	-	-
FPD, g (19 – 71 weeks)	134.50	-	-
FPD, g (77 – 102 weeks)	-	-	121.00
FPE, g (19 – 44 weeks old)	148.69	-	-
FPE, g (19 – 71 weeks old)	163.11	-	-
FPE, g (77 – 102 weeks old)	-	-	167.11

EPHH - Eggs per housed hen; LI - Laying intensity (%); NDL - Number of dead layers; NDLDM - Number of dead layers during moulting; %DL - % of dead layers; %DLDM - % of dead layers during moulting; FPD - Food per day; FPE - Food per egg.

Data in Table 2 demonstrate that a total of 1,431 hens (3.20%) died between 19 and 44 weeks of age, 3,778 (8.47%) by 71st week of age, 504 laying hens (1.14%) died during moulting, and 1,222 layers (2.74%) died during the second cycle, i.e. from 77 to 102 weeks of age.

Considering that hens on the farm are fed *ad libitum* with food restrictions imposed periodically, data referring to food consumption should be considered as partly accurate. Restriction program, i.e. periods of hunger during the moulting is given in Table 1, and daily consumption per laying hen and per egg produced is given in Table 2.

The highest daily food consumption per housed laying hen (134.55 g) was observed in the flock which was bred throughout the entire production cycle (19 to 71 weeks of age) and lowest (121.00 g) in case of hens bred after the moulting up to 102 weeks of age (Table 2). During the second cycle of egg production (77 – 102 weeks of age), food consumption per produced egg, compared to the first year of hen breeding, i.e. before the moulting, was considerably higher – 167.11 g, but it was on the upper limit of technological norms for this particular hybrid of laying hens.

Similar research was conducted by Mitrović et al. (1995) with Isa Brown hybrid hens implementing a similar forced moulting program of 33 days. Breeding period of hens before the moulting was 40 weeks and 26 weeks after the moulting (from 64 to 89 weeks

of age). Results reported by these authors in terms of laying intensity, were considerably lower than ours with laying intensity 78.57% before moulting and 63.71% after the moulting. Mortality of hens before and during the moulting was somewhat lower – 4.76% and 1.11% (both egg production period and moulting period were shorter), while the mortality after the moulting period was considerably higher (3.902%).

Average laying intensity during the first 26 weeks of egg production was 84.47% and during the first 26 weeks after the moulting 72.43% (Table 3). The difference of 12.04% was statistically significant (P<0.001), while the difference in mortality of laying hens during the listed periods of the production cycle (0.01%) was not statistically confirmed (P>0.05).

Table 3. Average values and variability of laying intensity and mortality of laying hens before and after the forced moulting

Age/production weeks	n	x̄	$S_{\bar{x}}$	S	C.V.
Laying intensity per housed laying hen (%)					
19 – 44/1 – 26 (before moulting)	26	84.47	3.77	19.21	22.74
19 – 71/1 – 53 (first phase - years)	53	82.49	1.95	14.17	17.18
77 – 102/1 – 26 (after moulting)	26	72.43	1.96	9.98	13.78
Mortality in relation to the number of housed laying hens (%)					
19 – 44/1 – 26 (before moulting)	26	0.12	0.01	0.05	45.58
19 – 71/1 – 53 (first year)	53	0.16	0.01	0.06	37.11
72 – 76/1 – 5 (during the moulting)	5	0.23	0.02	0.05	24.13
77 – 102/1 – 26 (after moulting)	26	0.11	0	0.04	21.82

Apart from the fact that forced moulting affects the number and percentage of laid eggs in certain phases of the production cycle, moulting and hen's age also have certain effect on the mass, i.e., class of eggs (Table 4).

Table 4. Egg classes according to weight category during the first 26 weeks of egg production (19 – 44 weeks of age) and after moulting (77 – 102 weeks of age)

Week of age/production	No. %	"S" <53 g	"M" 53-63 g	"L" 63-73 g	"XL" >73 g	Total eggs
Before moulting	Eggs %	568.720 8.33	4.262.086 62.43	1.882.858 27.58	113.608 1.66	6.827.272 100.00
After moulting	Eggs %	472.981 8.08	3.592.025 61.35	1.689.621 28.86	99.946 1.71	5.854.573 100.00

It is evident from the data given in Table 4 that the relative share of eggs produced before the moulting (26 weeks), compared to eggs produced after the moulting (also 26 weeks) was higher in case of "S" and "M" classes, and lower in case of "L" and "XL" classes, which indicates that the laying hens produced insignificantly heavier eggs after the moulting. This

is reported by Bell (2000) who also points out that, for commercial reasons, laying hens should moult between 65 and 70 weeks of age and that the second egg production cycle after the moulting should last from 7 to 9 months, i.e. laying hens should be withdrawn from the production at the age of 100 to 110 weeks.

Complementary to effects of the age of layers and implementation of the forced moulting on the productivity of the commercial flock of hens, coefficients of correlation were calculated between these traits (Table 5).

Table 5. Phenotype correlation between the age of laying hens and laying intensity before and after the forced moulting of a commercial flock of hens (%)

Weeks of age/production	r_{xy}	$t_{exp.}$	Correlation strength
19 – 44/1 – 26 (first phase - before moulting)	0.470[*]	2.608	Medium
19 – 71/1 – 53 (first phase – production year)	-0.076[NS]	0.544	None
77 – 102/1 – 26 (second phase - after moulting)	0.004[NS]	0.020	None

NS>0.05; *P<0.05.

Medium phenotype correlation between the age of laying hens and the laying intensity during the first 26 weeks of egg production was determined (r_p = 0.470) and the phenotype correlation coefficient was significant (P<0.05). However, no phenotype correlation was observed between these indicators during other phases of the production cycle (Table 5). These coefficients lead to conclusion that the analyzed flock should not be used for more than 53 weeks (71 weeks of age of laying hens), and not longer than 26 weeks (102 weeks of age) after the moulting.

Similar conclusion was reached by Pandurević (2011) and Zemcov (2015) with a commercial flock of Lohmann Brown hybrid laying hens. Pandurević (2011) also determined medium correlation between the age of laying hens and laying intensity r_p = 0.475[*] (26 weeks of production – 45 weeks of age of layers) and a very week negative correlation r_p = -0.178[NS] (53 weeks of production – 72 weeks of age of layers). In a research involving layers kept in conventional (C) and enriched (E) cages, Zemcov (2015) reported positive medium correlation between the age of layers and laying intensity at 26 weeks of production (44 weeks of age), but the phenotype correlation coefficients ere determined at P<0.01. At 53 weeks of egg production and 71 weeks of age of layers, negative very week (C) and weak (E) correlation was established, but correlation coefficients were not statistically significant (P>0.05).

Finally, we should add that forced moulting is relatively frequently conducted in the poultry management, with the same purpose but using different programs that may vary in terms of length of the moulting period, i.e. in schedules of food, water and light restriction, or some other factor (adding of supplements or low nutritional value food (wheat bran), such as demonstrated in the studies of Hassanien (2011), Molino and Garcia (2012), Schulte-Drüggelte and Thiele (2013), Aygun (2013), Rafeeq et al. (2013).

Conclusion

In certain, particularly non-EU countries, (for example USA, Former Yugoslav Republic of Macedonia, Serbia, Republic of Srpska (BiH), Turkey, Brazil), forced moulting is implemented for economic reasons. By conducting a method of forced moulting after the first production phase which lasts approximately one year, egg production cycle can be prolonged for another six months. This method has economic justification, eliminates the need to obtain a new flock of hens (potential layers), number of eggs produced is increased, quality of eggs is improved, particularly the quality of egg-shell which reduces egg breakage rate. It should be stressed, however, that there exists a difference of opinion regarding this matter among the manufacturers, consumers and researchers, and particularly supporters of animal well being.

There exists a considerable number of different program of forced moulting, although they are practically all based on partial or total restriction of food, water and lighting program within a certain time period lasting from several days up to couple of weeks.

This research conducted on 44,000 commercial laying hens kept in a cage system on the family farm "Rakić – Komerc" (Batković, Bijeljina, Republic of Srpska, BiH), demonstrates that the implementation of forced moulting yielded positive results in terms of prolonging the period of productive life of layers up to 102 weeks of age, increasing the number and quality (mass) of eggs, while the mortality remained within acceptable limits throughout all phases of the production cycle (before, during, and after the moulting). However, it should be noted that phenotype correlation coefficient between the age of layers and monitored indicators lead to conclusion that the analyzed flock should not be used for more than 53 weeks (71 weeks of age of laying hens), and not longer than 26 weeks (102 weeks of age) after the moulting.

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Poster presentation

EFFECT OF PHOTOPERIOD AND FREQUENCY OF EJACULATION ON SPERM TRAITS OF BOARS

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Abstract

A main objective of this study was to assess the effect of photoperiod and interval between two consecutive collections (frequency of ejaculation) on boar sperm traits: volume of ejaculate (ml), sperm concentration $\times 10^6$ sperm/ml), total sperm count $\times 10^9$) and the number of produced doses. The study included 333 collections from seven Large White boars. Photoperiod was analyzed as an effect of decreasing (from early summer to late autumn) and increasing (from early winter to late spring) photoperiod. Interval between two collections included two levels: ≤ 7 and >7 days. Impact assessment was carried out by applying the General Linear Model procedure of SAS 9.3 (2002-2010) statistical package. Volume of ejaculate varied under the effect of photoperiod ($p < 0.0001$) and interval between two collections ($p = 0.0004$). During decreasing photoperiod, sperm concentration was higher ($p < 0.0001$) by $\times 10^6$ sperm/ml. When interval between two collections was ≤ 7 days, total sperm count and number of produced doses was higher by $\times 10^6$ sperm ($p = 0.0005$) and 2.64 doses ($p = 0.0085$). Within both photoperiods and interval between two collections, correlation between volume of ejaculate and sperm concentration varied from -0.66 to -0.46 with $p < 0.001$. The sperm production depends on photoperiod and frequency of ejaculation. Interval between two collections (≤ 7 days) showed better effect on sperm production implying that collecting the boar sperm be performed at least once a week.

Keywords: boars, collection, ejaculation, photoperiod, sperm traits

Introduction

Ejaculate traits vary under the effect of different genetic and paragenetic factors. Various studies on sperm traits showed that these traits could vary under the effect of breed (Wolf and Smital, 2009), season (Kondracki et al., 2009) and interval between two collections (Frangež et al., 2005; Wolf and Smital, 2009).

Boar reproductive traits show distinct seasonal changes (Pinart and Puigmulé, 2013). Regardless optimal microclimate parameters in facilities for boars housing, sperm traits may vary throughout the year. The effect of photoperiod on boar fertility traits is not completely understood. Thus Pinart and Puigmulé (2013) indicate, on one hand, the studies proving that an autumn photoperiod, with shortened daylight, had a stimulative effect on

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fertility in boars while some other studies suggest significant differences existing between breeds and their reaction to light regime. It is well known that photoperiod is a modulator of sperm production in mammals acting as an effect of melatonin regulation mechanism (Knecht et al., 2013).

The intensity of boar exploitation is an important parameter which can impact the fertility of herd and present an essential segment in reproductive monitoring. Prolonging the interval between two collections from two days to six and/or ten days resulted in the increase in sperm concentration by approximately 100×10^3 and/or 150×10^3 spermatozoa per mm^3 (Wolf and Smital, 2009).

The objective of this research paper was to assess the effects of photoperiod and interval between two collections on average manifestation and variability of boar sperm traits.

Material and Methods

The trial was conducted on a repro-farm during 2011 and 2012. The boars were housed in 2×4 m pens with partially barred concrete floor in especially built structures. Microclimate parameters were automatically controlled. The boars were fed with balanced mixture and fresh water was available ad libitum. The ejaculate was collected by a standard manual method introducing a mobile phantom in a boar pen.

We have analyzed 333 ejaculates of seven Large White boars during two years of their exploitation in reproduction (Table 1). Photoperiod was studied as an effect of decreasing (from early summer to late autumn) and increasing (from early winter to late spring) photoperiod. The effect of interval between two collections was studied at two levels (≤ 7 and >7 days).

Table 1. - Number of collections by interval between two collections within photoperiod

Photoperiod	Interval between two collections (days)		Total
	≤ 7	>7	
Decreasing	96	65	161
Increasing	102	70	172
Total	198	135	333

Research included following parameters: volume of ejaculate (ml), sperm concentration ($\times 10^6$ sperm/ml), total count of spermatozoa in $\times 10^9$) and number of produced doses.

The volume of ejaculate was measured by graduated cylinder with the precision of ± 2 ml. Concentration of native sperm was assessed by means of photo-colorimeter. Doses for insemination were standardized to the volume of 100 ml and 2.5 billion spermatozoa per dose. Total number of spermatozoa in ejaculate was obtained by multiplying concentration of sperm by volume of ejaculate.

Impact assessment was carried out by applying the General Linear Model procedure of the SAS 9.3 statistical package (SAS Inst. Inc., 2002-2010), using the following model:

$$y_{ijk} = \mu + P_i + I_j + e_{ijk},$$

where: y_{ijk}- is an analyzed sperm trait, μ- general population average, P_i- effect of photoperiod (i=1,2), I_j- effect of interval between two collections (j=1,2) and e_{ijk}- random error.

The comparison of the Least Square Means (LSMeans) values of the sperm traits was done by means of t-test on three levels of significance ($p<0.05$, $p<0.01$, $p<0.001$). Correlation of studied traits was determined by means of Pearson correlation coefficient and was interpreted on the grounds of rough approximation of the height of correlation according to Petz (2004).

Results and Discussion

Interval between two collections had an effect on average manifestation and variability of studied traits except for the sperm concentration (Table 2). The intervals between two collections lasting 7 days or less resulted in higher volume of ejaculate (by 42.64 ml), higher count of spermatozoa in ejaculate (by $\times10^9$) and higher number of doses per collection (by 2.64) compared to the intervals lasting longer than 7 days. When the intervals exceed 7 days, a productivity of boar per collection decreases at an annual level as well what can be negatively reflected on boar performance increasing the cost of production (less number of doses per collection, less number of collections at an annual level and necessity to raise greater number of boars).

Table 2. - Effect of interval between two collections on sperm traits (LSMeans)

Sperm traits	Interval between two collections (days)		Significance
	≤7	>7	
Volume of ejaculate (ml)	255.52	212.88	0.0004
Total number of spermatozoa ($\times10^9$)	47.14	39.46	0.0005
Sperm concentration ($\times10^6$ sperm/ml)	214.42	205.33	0.3907
Number of produced doses	16.66	14.02	0.0085

The results obtained in this study correspond to the results obtained in the research by Frangež et al. (2005) in which differences in the values of sperm traits between different frequencies of boar exploitation were determined. In the research of Wolf and Smital (2009) a low increase in the volume of ejaculate when the interval between two collections was prolonged from two days to seven days was determined and when it comes to longer intervals the changes were minor. The same authors state that the effect of interval between two collections on the volume of ejaculate was lower in relation to sperm concentration so we got the opposite results from the aforementioned ones. Performed research is in opposition to the research of Savić et al. (2013) in which the effect of interval between two collections on ejaculate volume was not determined.

By comparing different photoperiods a higher volume of ejaculate in increasing photoperiod (Table 3; p<0.0001) was determined. Simultaneously, concentration of sperm was lower (p<0.0001) by $\times 10^6$ sperm/ml.

The research results are to some extent consistent with the results obtained by Sancho et al. (2004) who, studying an increasing photoperiod, determined higher volume of ejaculate, higher sperm concentration and larger number of doses per ejaculate. The results of this research are contrary to the research of Knecht et al. (2013) who, in the period July-December (decreasing interval) determined a volume of ejaculate of 261.16 ml which was 17 ml higher in comparison with the period January-June (increasing interval). They are opposite also to the results obtained by Pokrywka et al. (2014) who determined, during autumn period, highest volume of ejaculate of 280.61 ml. Pinart and Puigmulé (2013) point out a stimulative effect of daylight shortening during autumn on boar reproductive capacity what is only partially confirmed in our research taking into account a greater concentration of sperm during decreasing photoperiod. Petrocelli et al. (2015) determined higher volume of ejaculate and lower sperm concentration in decreasing photoperiod so that our results are entirely inconsistent with the above stated results.

Table 3. - Effect of photoperiod on sperm traits (LSMeans)

Sperm traits	Photoperiod		Significance
	decreasing	increasing	
Volume of ejaculate (ml)	198.39	270.01	<0.0001
Total number of spermatozoa ($\times 10^9$)	44.21	42.39	0.3979
Sperm concentration ($\times 10^6$ sperm/ml)	246.51	173.24	<0.0001
Number of produced doses	15.61	15.08	0.5894

Analyzing the ejaculates in decreasing photoperiod a correlation between studied traits was confirmed (Table 4).

Table 4. - Correlation (r) between sperm traits within decreasing photoperiod

Sperm traits	Volume of ejaculate	Sperm concentration	Number of produced doses
Volume of ejaculate	-	-0.66[***]	0.26[**]
Sperm concentration	-0.54[***]	-	0.32[**]
Number of produced doses	0.46[***]	0.28[*]	-

Above of diagonal showed correlation for interval between two collections ≤7. Below of diagonal showed correlation for interval between two collections >7; Significance: [*]-p<0.05, [**]-p<0.01, [***]-p<0.001

Between the volume of ejaculate and sperm concentration, regardless the duration of interval between two consecutive collections, a negative correlation of a moderate strength was confirmed (r=-0.66 and r=-0.54). Differences existed in the strength of relationship (weak to moderately strong) between volume of ejaculate and number of doses depending on a duration of interval between two consecutive collections. Weak correlation (r=0.32 and r=0.28) was confirmed between the concentration and number of doses.

Table 5. - Correlation (r) between sperm traits within increasing photoperiod

Sperm traits	Volume of ejaculate	Sperm concentration	Number of produced doses
Volume of ejaculate	-	-0.55^{***}	0.20^{ns}
Sperm concentration	-0.46^{***}	-	0.60^{***}
Number of produced doses	0.48^{***}	0.34^{**}	-

Above of diagonal showed correlation for interval between two collections ≤7. Below of diagonal showed correlation for interval between two collections >7; Significance: ^{ns}-p>0.05, **-p<0.01, ***-p<0.001

Similarly to decreasing photoperiod, negative relationship between volume of ejaculate and sperm concentration of moderate strength was confirmed in an increasing photoperiod (Table 5; r=-0.55 and r=-0.46). Moderately strong correlation between volume of ejaculate and number of doses was confirmed in ejaculates of boars whose intervals between consecutive collections exceeded 7 days. Differences existed in the strength of relationship (weak to moderately strong) between sperm concentration and number of doses depending on a duration of interval between two consecutive collections.
Regardless the strength of relationship, a positive correlation was confirmed between the number of doses and other traits which indicate the increase in the number of doses per collection with increasing the volume of ejaculate and sperm concentration.
Wolf (2010), investigating two genotypes, established a negative correlation (-0.48 and -0.50) between the volume of ejaculate and sperm concentration so that our results are similar to these. Similarity can be found also with the research of Oh et al. (2006) who confirmed moderately strong correlation between analyzed traits (volume of ejaculate and sperm concentration, r=-0.48; volume of ejaculate and number of produced doses, r=0.40; sperm concentration and number of produced doses, r=0.41).

Conclusion

The sperm production depends on photoperiod and frequency of ejaculation. Interval between two collections (≤7 days) showed better effect on sperm production implying that collecting the boar sperm be performed at least once a week. If not, costs of production may increase due to a smaller number of doses per collection, smaller number of collections at an annual level and necessity to raise greater number of boars. The correlations between the volume of ejaculates and sperm concentration are negative and of moderate strength. Between the number of doses and other traits (volume of ejaculate and sperm concentration), a weak positive to moderately strong correlation was confirmed.

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Poster presentation

PHENOTYPE VARIABILITY OF REPRODUCTIVE TRAITS OF ALPINE GOAT BREEDS DEPENDING ON BODY WEIGHT AT FIRST CONCEPTION

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Abstract

The aim of this paper was to determine optimal body weight of goat at first conception and its influence on fertility, kid body weight at birth and in the age of 30 days.

Three groups were formed, in third group goats whose body weight was in between 41-45 kg were put (control group), in the second group goats whose body weight was between 36 – 40kg were allocated, and in first group goats weighing between 30 – 35kg were put.

Research results have shown that average body weight during first conception was 36.92 kg which was bellow optimal values which was supposed to be over 40 kg for alpine goat population which is reared in our rearing conditions. Goat body weight at the moment of conception had significant influence on number of kids at birth, as fertility per groups was I-118%; II-140% and III-150%.

Differences between researched groups regarding the kid body weight at birth were not statistically significant (P<0.05), their body weight at birth averaged 3.52 kg, which can be considered satisfactory.

At the age of 30 days kids that belonged to the third group had body weight of 10.23 kg, second group 8.68 kg and first group 8.35 kg, which is averagely for all three groups 8.86 kg.

Determined difference in body weights between kids of III and II group was 1.55kg in favor to third group and it was statistically significant (P>0.05), while difference between III and I group that was 1.88 kg in favor of third group was statistically very significant (P>0.01).

Results have clearly shown that young goats have to have minimum 75 – 80% developed body compared to fully grown goats of specific genotype, which in our conditions is over 40kg of body weight, and that is when one can expect optimal production results.

Keywords: Alpine goat, age, body weight, fertility, kid weight.

Introduction

French alpine goat "Alpine", originates from French Alps from where it spread in to many countries across the globe. During the creation of this breed long term selection on high milk production was used, and that is why Alpine goat belongs to goat breed that has highest milk yield.

French alpine goat is big animal roughly built. Average body weight for does is 60-80 kg, and for bucks 80-100 kg. When it comes to reproductive traits, French alpine goat has

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average fertility rate of 1.8 to 2.0 kids per goat per year. In the past 15 – 20 years alpine goat has become most popular goat breed for milk production in Republic of Serbia.

Goat reproduction is seasonal. Beginning and length of mating season depends on multiple factors such are: geographic region, climate, breed, physiological phase, buck presence, rearing systems and photoperiod (Fatet et al., 2011; Mekić et al., 2015). Goats are very fertile domestic animals with high conception rate of 90% (Duygu, 2010).

Reproductive trait of goats is main productivity and economic viability characteristic at goat farm. Mekić et al., (2016). Reproductive rate is influenced by selection intensity, therefore degree of genetic improvement has high influence too Abegaz et al., (2002). Improvement of reproduction rate increases with increase of selection intensity, therefore genetic advancement of productive traits is also increased Bagnicka et al., (2007). There are numerous inner and outer factors that influence the number of healthy offspring within the type of breed they belong Snyman, (2010).

Doe age is one of inner factors that have significant influence on total efficiency of the heard Van der Westhuizen et al., (2004). Body weight of does at the time of conception is important factor that helps with reproductive efficiency Snyman (2010). Positive correlation between reproduction and body weight at conception time for sheep was determined by Safari and Fogarty (2003), and for goats Constantinov (1989).

Body weight of does during the first conception is important economic factor that influences goat fertility and kid growth after birth (Paul et al., 2014; Mc Gregor, 1984). Reproductive trait is main factor that determines goat productivity regardless of their production orientation, meat, milk, skin or production of sackcloth (mohair), Sivaraj (1991).

Kid body weight at birth and doe body weight are considered important traits, because there is positive correlation between kid body weight at birth and growth intensity Banergee (1989) which has influence on future productive and reproductive life of the animal. Considering that reproductive traits are basis for goat milk, meat, skin, sackcloth production profitability, aim of this paper was to determine influence of body weight at first conception at alpine goat reproductive traits (fertility, kid body weight at birth and at the age of 30 days).

Materials and methods

Reproductive characteristics were monitored at the milk farm that rears alpine goat breed. Depending on achieved body weight at the time of first conception three groups were formed: first group was goats weighing 30-35 kg; second group 36-40 kg and third group 41-45 kg (control group).

After the first mating and successful conception reproductive traits were monitored. Goat fertility after first birth, kid body weight at birth and kid body weight after 30 days were parameters that were monitored.

Data rendering was done using usual statistical methods for this type of research. For testing

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arithmetic means variance analysis method was used. To calculate significance between differences of determined means LSD test was used.

Results and discussion

All reproductive functions in animal body are controlled by complex neuro - endocrine mechanisms on the central neural system – hypothalamus – hypophysis – gonads (ovaries or testicles) axis. Environment and body stimuli are transmitted *via* central neural system to hypothalamus and/or hypophysis and they influence their secretory activity. Therefore as hypothalamus and hypophysis are main endocrine organs reproductive functions are subject to strong outside factors influence (light, smell, sound, stress, diet. etc.).

Young female has to achieve certain minimal age (which is genetically determined for every breed and unit), in order to create conditions for starting and maintaining neuro – endocrine mechanisms, which lead to first ovulation and first estrus, reaching maturity.

In high interest of goat breeders is to mate young goat as early as possible and use it in reproduction for as long as possible. However, one has to have in mind that young animal have to be physically ready for additional weight (during pregnancy).

Young animal has to be physiologically and physically ready for first conception. Goats grow most intensively in their first year when they achieve two thirds of their body weight (in better dietary conditions even more). In the literature it is often mentioned that body weight, body development is better criteria than age to determine puberty. Average body weight of grown alpine goats is between 50 and 80 kg depending on breeding conditions In the research by Maksimović et al. (2015), body weight of grown alpine goat at 22 small farms for 330 goats was averagely 54.96 kg. Results of our research regarding the achieved body weight at the moment of first conception are shown in Table 1.

Table 1. Average values and variability of goat body weight at first conception (kg)

Group	$\overline{x}$	$S_{\overline{x}}$	Sd	CV(%)	Variations	
					Min.	Max.
I	32.94	0.39	1.60	4.86	30	35
II	37.72	0.27	1.34	3.55	36	40
III	42.87	0.77	2.17	5.06	41	50
Average	36.92	0.52	3.70	10.02	30	45

From obtained data we can conclude that body weight for goats during first mating was averagely 36.92 kg (table 1). If average body weight of alpine goat that have finished their growth around 55 kg in our breeding conditions, and considering that does have to have two thirds of grown body weight, minimal weight at first conception should be around 40 kg, therefore our average value of 36.92 kg lower than it should have been. That was the reason for forming three groups based on their body weights at the time of conception.

Difference in body weight between third and first group of 9.93 kg; third and second group of 5.15 kg in favor of third group as well as difference of 5.15 kg between second and first group in favor of first group were statistically very significant (P>0.001).

Goat fertility is very significant trait, it differs for different goat breeds and it is expressed unevenly therefore it is considered important breed trait. Goat fertility determined through

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number of live born kids per goat was shown in Table 2.

Table 2. Alpine goat fertility

No	Parameters	Group			Total average
		I	II	III	
1.	Goats that had kids	17	25	8	50
2.	Total number of kids	20	35	12	67
3.	Fertility %	118	140	150	134

According to literature Drobnic et al. (1998), quotes that alpine goat fertility in Slovenia was 1.64. Crepaldy et al. (1999), determined that alpine goat fertility reared in Italy was 1.60. Kasap et al. (2012) determined that fertility for alpine goats was 1.46 for mature animals in Croatia, while fertility after first birth was 125%.

Determined fertility in our research after first birth was averagely 134% (table 2) which is higher than value determined in Croatia by Kasap et al. (2012). However, we were interested how body weight at the moment of first conception influences the number of kids born. Fertility higher by 10% was achieved for third group compared to second group, and 32% compared to first group (table 2). At the same time higher fertility by 22% was achieved in second group compared to first group.

According to obtained data, best results for number of kids born were achieved at third control group, whose body weight at the moment of conception was in the interval between 40-45 kg, 75-80% of grown animal body weight in our breeding conditions. Age of goat at first conception was averagely 9 months. This research has clearly shown that one has to pay attention that anima has reached puberty and that it has achieved certain physical maturity, that they have certain body weight at the moment of conception which is minimally 75-80% of fully grown animal of specific genotype.

Kid body weight at birth is variable and it mainly depends on goat breed Mekić et.al. (2015). It averagely represents 1/15 of body weight of grown goat Morand-Fehr (1981). Within the breed body weight depends on type of birth, gender, kidding order, development and age of doe, length of pregnancy, diet, kidding season, health condition of doe Laest-Fettback and Peters (1995). Male kids have higher body weight at birth than female by 200-500g Mavrogenis et al. (1984). Results of our research regarding the body weight at birth according to groups was shown in Table 3.

Table 3. Average value and variability of kid body weight at birth (kg)

Group	n	Pol	$\overline{x}$	$S_{\overline{x}}$	Sd	CV(%)	Variations	
							Min.	Max.
I	11	♂	3.44	0.17	0.56	16.28	2.70	4.60
	9	♀	3.58	0.22	0.66	18.43	2.40	4.30
	20	♀+♂	3.50	0.13	0.59	16.86	2.40	4.60
II	13	♂	3.48	0.17	0.63	18.10	2.30	4.60
	22	♀	3.49	0.12	0.56	16.04	2.30	4.60
	35	♀+♂	3.48	0.10	0.57	16.38	2.30	4.60
III	12	♀+♂	3.65	0.17	0.58	16.76	3.00	4.70
Averagely (I+II+III)	67		3.52	0.07	0.57	16.19	2.30	4.70

Difference in body weights at birth between third and second group was 0.17 kg in favor of third group; third and first group was 0.15 kg in favor of third group and it was not statistically significant (P<0.05).

Therefore body weight of does at the moment of conception even though it was different did not have significant influence on kid body weight at birth. This is possible only if goats were adequately fed during the pregnancy time which enabled fetal development and increase of their own body weight.

In our research average body weight of kids at birth was 3.54 kg. Group I– 3.50 kg; group II – 3.48 kg and group III – 3.65 kg. Kid weight at birth for alpine goat breed of 3.92 kg was determined by Urošević et al..(1999). Lower values than ones that we obtained in our research for alpine goat were determined by Maksimović at al (2015), where kid body weight at birth was averagely 2.73 kg. Our results are in compliance with results by De Menezes et al. (2007), where determined body weight at birth for alpine breed was 3.61 kg. Kid body weight of 3.54 kg at birth determined by our research can be considered satisfactory and it shows that doe body weight in the moment of conception did not have significant influence on kid body weight at birth.

Otuma and Osakowe (2008) say that kid body weight at birth is under significant influence of season, type of birth, gender of newborn kids, doe age, therefore values obtained from our research considering that very young goats were used can be considered satisfying. Thirty days old kids body weights were shown in table 4.

Table 4. Average values and variability of 30day old kid body weight

Group	n	Pol	$\overline{x}$	$S_{\overline{x}}$	Sd	CV(%)	Variations	
							Min.	Max.
I	11	♂	8.66	0.55	1.84	21.25	5.60	13.10
	9	♀	7.98	0.16	1.47	18.42	4.80	9.30
	20	♀+♂	8.35	0.37	1.68	20.12	4.80	13.10
II	13	♂	8.80	0.33	1.20	13.64	6.70	10.70
	22	♀	8.62	0.34	1.61	18.68	6.60	11.20
	35	♀+♂	8.68	0.24	1.43	16.47	6.60	11.20
III	12	♀+♂	10.23	0.48	1.65	16.13	8.30	13.50
Averagely (I+II+III)	67		8.86	0.20	1.66	18.73	4.80	13.50

In the age of 30 days difference in body weight between kids has been determined. Difference between III and II group was 1.55 kg in favor of III group which was statistically significant (P<0.05). Between III and I group difference was 1.88 kg in favor of III group which was statistically very significant (P>0.01), while difference between II and I group of 0,33 kg in favor of II group was not statistically significant (P<0,05). Maksimović et al. (2015) quote that body weight of 30 days old alpine goat kids was averagely 8.70 kg which was similar to our results achieved in II group. De Menezes et al. (2007) have achieved weight of 7.35 kg for 30 days old kids which is lower than our results. Kume and Hajno (2010) have also determined lower body weight of alpine goat kids at 30 days of age and it was averagely 6.71 kg. Determined average body weight of kids at the age of 30 days in our research was 8.86 kg and can be considered satisfactory considering that body weight

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of does at the conception have had significant and very significant influence on kid development during the first month.

This results were most probably due to young goats who had higher body weights at the moment of conception and were better prepared for lactation which followed after giving birth.

Conclusion

Based on obtained results about the influence of doe body weight at the moment of first conception, 30 days old alpine goat kid weight following conclusions can be noted:

- Average body weight for does during first conception was 36.92 kg, which was bellow physical maturity for the genotype. Weight range for researched does was within these values 32.94-42.87 kg.
- Young goats that belonged to third (control) group have had average body weight of 42.87 kg and that value can be considered optimal starting weight for usage in reproduction, while does that were in second and first group haven't achieved physical maturity at the moment of conception and differences between groups were statistically highly significant (P>0.001).
- Goat fertility was averagely 134%. For the first group fertility was 118%; II – 140% and III group 150%. Third group had 32% higher fertility rate compared to first group, and compared to second group it was 10%. At the same time does that were in second group had higher fertility rate than does from first group by 22%. Doe body weight at moment of conception had significant influence on the number of kids born and best result of 150% was achieved in third group where goats had good body weight and were properly physically developed.
- Kid body weight at birth was averagely 3.52 kg, and differences between tested groups were not statistically significant (P<0.05).
- At 30 days of age kid body weight was averagely 8.86 kg, in the group comparison I:II:III values were 8.35 : 8.68 : 10.23 kg. Kids of third group have achieved higher body weight than kids of second group by 1.55 kg, which was statistically significant (P>0.05), while difference between third and first group of 1.88 kg in favor of third group was statistically very significant (P>0.01).

Results of this research have shown that goats that are being used in reproduction for the first time after reaching physiological maturity have to achieve proper body weight (physical maturity). Results show that third group of goats that at the moment of conception had body weight over 40 kg had much better fertility and kids were growing much faster up to the age of 30 days.

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Poster presentation

FLUCTUATION OF DAILY PROTEIN AND UREA IN MILK OF DAIRY COWS IN EASTERN CROATIA DUE TO HEAT STRESS

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Abstract

Considering the rapid climate change worldwide in order to reduce financial losses of dairy farmers and enable the sustainable farming, the necessity of implementation of breeding values for heat resistance in breeding strategies, have become more and more pronounced. First step in estimation of breeding values is determination of THI threshold value. Therefore, the objective of this paper was to determine THI threshold value for daily protein and urea content in first parity Holsteins and dairy Simmentals in Eastern Croatia. With that purpose, test-day records with data on ambient temperature and relative humidity in the barns collected in regular milk recording from January 2005 to December 2012 were analyzed. Based on analyzed data it could be concluded that THI ≥ 66 cause significant change in daily protein and urea content in milk of Holstein and Simmental first parity cows. Daily protein contents statistically highly significant decrease due to heat stress condition (THI in 66 – 80) in both, Holsteins and Simmentals, while daily urea content statistically highly significant increase. The THI = 66, as the lowest value at which significant decrease in analyzed traits was determined could be taken as the threshold value for first parity cows in Eastern Croatia.

Keywords: dairy cows, heat stress, threshold, Eastern Croatia

Introduction

The impact of the climate change becomes is more and more expressed worldwide. In accordance to the forecasts (IPCC, 2007), in future dairy cattle will be exposed to the unfavorable climatic conditions in regions that at the time are not characterized as extreme climate. Accordingly, Reiczigel et al., (2009) in Hungary determined increase of heat stress days/year (temperature-humidity index THI > 68) from 5 to 17 in a period of 30 years. Also, Gauly et al., (2013) stated that when the scenarios of global warming are considered, heat stress of high-yielding dairy cows is an increasing concern of milk producers in Europe. Dunn et al., (2014) concluded that the in future the number of days exceeding the

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THI threshold in southern parts of the UK could will increase from on average 1–2 per year to over 20 per year by 2100. In dairy cattle breeding in indoor housing, the optimal microclimate conditions in the barns are necessary for the realization of the productive potential of individual cows. The interrelation between ambient temperature and relative humidity is important from the aspect of animal welfare, reproduction and finally profitability of dairy farm. The combination of high temperature and high relative humidity has the most detrimental effect through inducing heat stress in cows. Under heat stress conditions, lactating cows tend to reduce their dry matter intake (DMI) and milk production (West et al., 1999). Moreover, besides milk production heat stress is associated with changes in milk composition, somatic cell counts (SCC) and mastitis frequencies (Bouraoui et al., 2002.; Collier and Hall, 2012; Correa-Calderon et al., 2004; Ravagnolo et al, 2000.; St-Pierre et al., 2003; West, 2003). Additionally, deteriorate effect on reproductive performances was also observed (Bohmanova et al., 2007; Ravagnolo et al., 2000). The most common measure of heat stress in dairy cows is the temperature-humidity index (THI) that present combination of ambient temperature and relative humidity and is a useful and easy way to assess the risk of heat stress (Kibler, 1964). Du Preez et al., (1990a, b) determined that milk production and feed intake is affected by heat stress when THI values are higher than 72. Bouraoui et al. (2002) put the threshold on 69, while Bernabucci et al., (2010) as well as Collier and Hall, (2012) on 68. Vitali et al., (2009) suggested that the risk of cow's death starts to increase when THI reaches 80. The significant decrease of daily milk traits (yield and contents) was also determined in Croatian environmental conditions with highest decline during summer period in Eastern and Mediterranean Croatia (Gantner et al., 2011). In many dairy-producing areas of the world heat stress condition represent a significant financial burden, for example in the USA between $897 million and $1,500 million per year (St-Pierre et al., 2003). There are many methods to decrease the impact of heat stress including the shading, cooling, nutrition (Kadzere et al., 2002; West, 2003) and selection for resistance on heat stress (Bohmanova, 2006). Ravagnolo et al., (2000) determine deteriorate effect of selection on productivity on cow's resistance to heat stress due to antagonistic relationship between cow's production and heat tolerance. The unfavorable genetic relationship between THI and productive and reproductive traits was found in few studies (Ravagnolo and Misztal, 2002a,b; Freitas et al., 2006; Aguilar et al., 2009). On the other hand, the high yielding Holstein cows in Israel is good example that selection on production could be successful in terms of heat stress (Aharoni et al., 1999). Implementation of breeding values for heat resistance in breeding strategies would certainly reduce financial losses of dairy farmers and enable sustainable farming. Estimation of breeding values requires determination of THI threshold value. Therefore, the objective of this paper was to determine THI threshold value for daily protein and urea content in milk of first parity Holsteins and Simmentals in Eastern Croatia.

Material and Methods

Individual test-day records of first parity Holstein and Simmental dairy cows collected in regular milk recording performed by alternative milk recording method from January 2005 to December 2012 in Eastern Croatia were used for the analysis. Monthly, at each recording, milk yields were measured during the evening or morning milkings. Logical control of milk data was performed according to ICAR standards (2003). Additionally, at each recording, ambient temperature and relative humidity in the barns 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 > 500 days), age at first calving in (< 21 and > 36 months), missing or parity > 1, 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, provided by the Croatian Agricultural Agency, after logical control, consisted of 205,714 test-day records from 24,307 first parity Holsteins, 188,512 test-day records from 30,013 first parity Simmentals and 322,174 records of microclimate parameters.

The THI threshold values for daily protein and urea content in milk were determined by least square analyses of variance for each given THI value (***from 66 to 80)*** in regard to the breed (Holstein, Simmental) using the PROC MIXED procedure in SAS (SAS Institute Inc., 2000). Following mixed model was used:

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

Where y_{ijklm} = estimated daily protein and urea content);

μ = intercept;

b_1, b_2, b_3, b_4 = regression coefficients;

d_i = days in milk (i = 6 to 500 day);

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

A_k = fixed effect of age at calving class k (k = 21 to 36 month),

R_l = fixed effect of region k (l = Croatian counties),

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

e_{ijklm} = residual.

The significance of the differences between the THI classes were tested by Scheffe's method of multiple comparisons. The lowest threshold value at which significant differences in analyzed traits was determined, was taken as the threshold value.

Results and Discussion

Analysis of the microclimate parameters recorded in the barns located in the Eastern Croatia during the summer season (June, July, August) in period 2005 – 2012 are presented in Figure 1 (Holsteins) and Figure 2 (Simmentals).
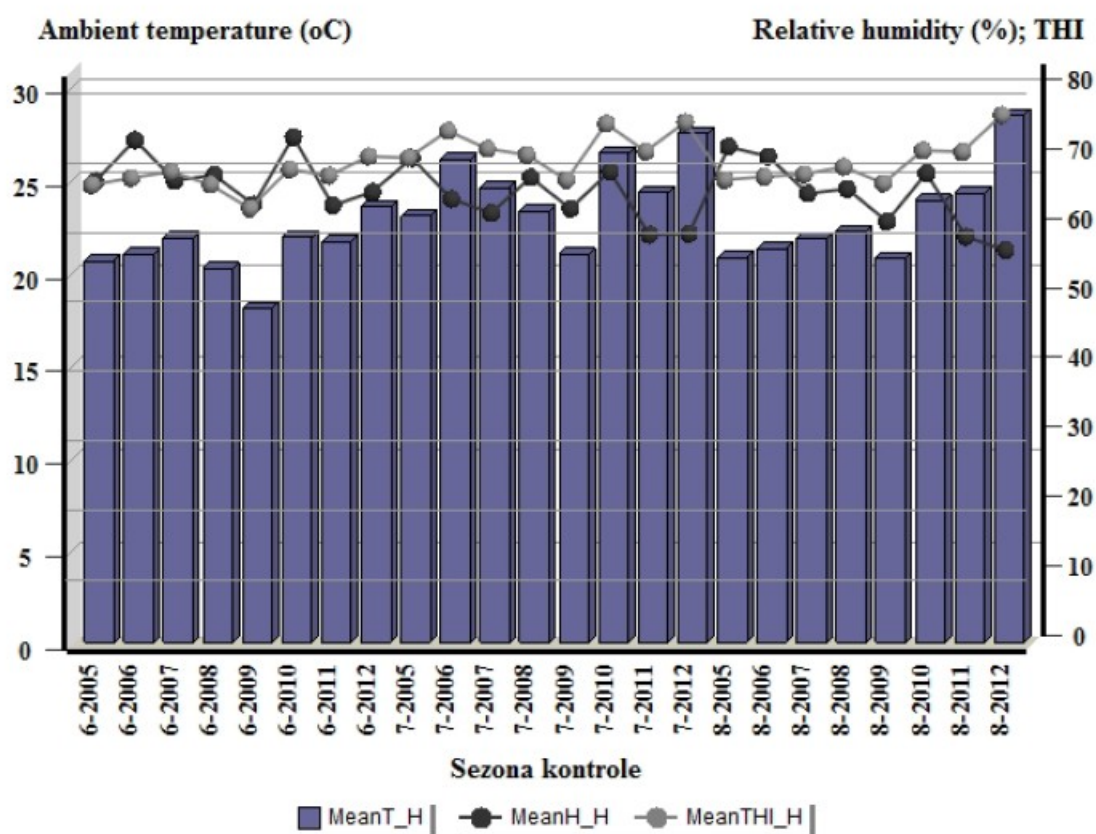
Figure 1. Mean daily ambient temperature, relative humidity and THI measured during milk recording of Holstein cows

During the June, mean ambient temperature in Holstein's barns varied in interval from 19 till 26°C, in July from 21 till 27°C, while August characterized mean ambient temperatures in interval from 21 till 29°C. Taking into account that upper limit of cow's comfort zone is 25°C (Yousef, 1985), even mean values of measured ambient temperatures in Holstein's barns indicate prevalence of heat stress conditions. Maximum measured daily ambient temperatures reached 40°C. Combination of these high temperatures with relatively high relative humidity resulted in heat stresses condition during the entire summer period.

Similar mean values of environmental parameters were also determined in the Simmental's barns, while higher prevalence of days with extreme conditions ambient temperature = 40°C, relative humidity > 96%, as well as THI > 96) was observed.
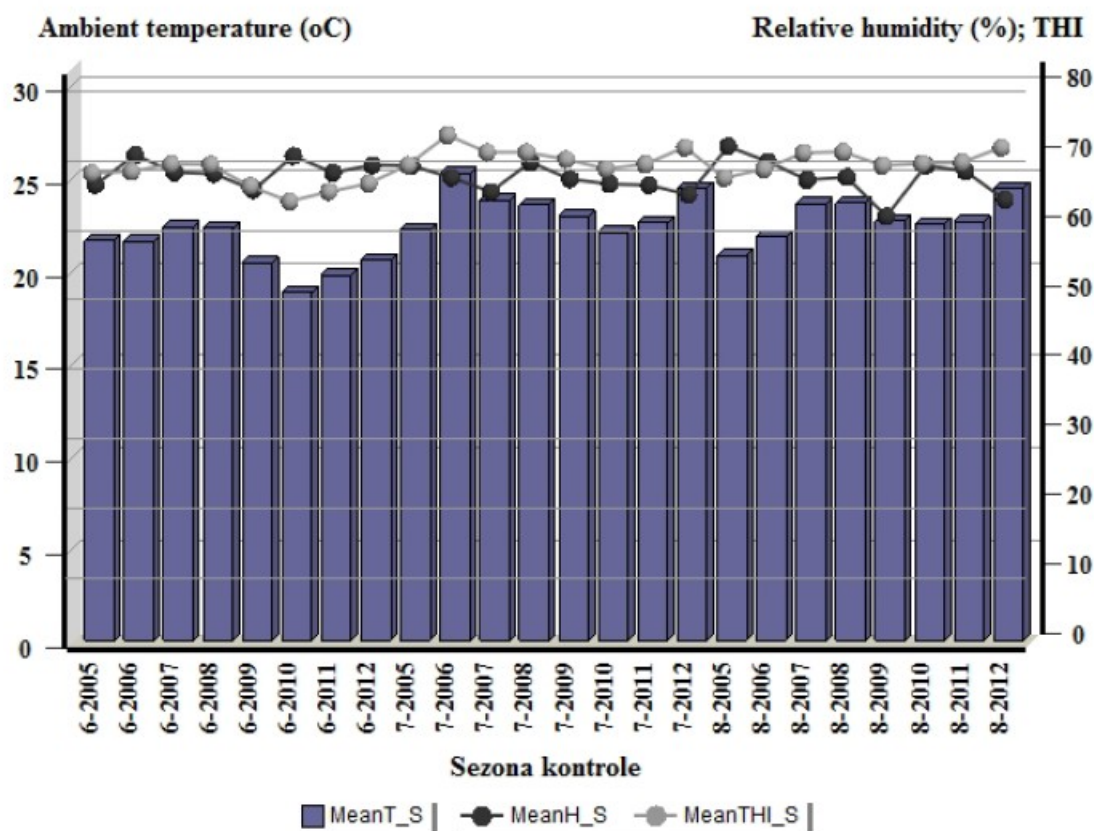
Figure 2 Mean daily ambient temperature, relative humidity and THI measured during milk recording of Simmental cows

Least square means regarding the fixed effect of THI class (0, 1) on daily protein content in accordance to the breed (Holstein, Simmental) are shown in Table 1. Environmental conditions in the barns with THI values in 66 caused statistically highly significant difference in daily protein content in milk of both analyzed breeds.

Table 1 Least square means of daily protein content in milk (%) regarding the given threshold value in accordance to the cattle breed

	Holstein			Simmental		
ThHo	**Ls0**	**Ls1**	**Difference**	**Ls0**	**Ls1**	**Difference**
THI66	3.45 ± 0.002	3.31 ± 0.002	0.135 ± 0.002***	3.47 ± 0.004	3.35 ± 0.004	0.117 ± 0.003***
THI67	3.45 ± 0.001	3.31 ± 0.002	0.134 ± 0.002***	3.47 ± 0.004	3.34 ± 0.004	0.121 ± 0.003***
THI68	3.44 ± 0.003	3.31 ± 0.004	0.130 ± 0.002***	3.46 ± 0.004	3.34 ± 0.004	0.122 ± 0.003***
THI69	3.44 ± 0.005	3.31 ± 0.005	0.132 ± 0.002***	3.46 ± 0.004	3.34 ± 0.005	0.122 ± 0.003***
THI70	3.44 ± 0.002	3.31 ± 0.003	0.131 ± 0.002***	3.46 ± 0.004	3.33 ± 0.005	0.127 ± 0.004***
THI71	3.44 ± 0.000	3.30 ± 0.001	0.135 ± 0.002***	3.46 ± 0.004	3.32 ± 0.005	0.135 ± 0.004***
THI72	3.44 ± 0.000	3.29 ± 0.000	0.143 ± 0.002***	3.46 ± 0.004	3.32 ± 0.005	0.135 ± 0.004***
THI73	3.43 ± 0.002	3.29 ± 0.003	0.147 ± 0.002***	3.45 ± 0.004	3.32 ± 0.005	0.139 ± 0.004***
THI74	3.43 ± 0.002	3.28 ± 0.003	0.152 ± 0.003***	3.45 ± 0.004	3.31 ± 0.006	0.141 ± 0.005***
THI75	3.43 ± 0.001	3.28 ± 0.003	0.154 ± 0.003***	3.45 ± 0.004	3.31 ± 0.006	0.145 ± 0.005***
THI76	3.43 ± 0.003	3.27 ± 0.004	0.153 ± 0.003***	3.45 ± 0.004	3.30 ± 0.006	0.145 ± 0.006***
THI77	3.43 ± 0.002	3.27 ± 0.004	0.158 ± 0.003***	3.45 ± 0.004	3.29 ± 0.007	0.159 ± 0.006***
THI78	3.42 ± 0.003	3.28 ± 0.004	0.147 ± 0.004***	3.45 ± 0.004	3.28 ± 0.007	0.164 ± 0.007***
THI79	3.42 ± 0.002	3.27 ± 0.005	0.148 ± 0.004***	3.45 ± 0.004	3.29 ± 0.008	0.161 ± 0.008***
THI80	3.42 ± 0.003	3.26 ± 0.006	0.163 ± 0.005***	3.45 ± 0.004	3.27 ± 0.009	0.174 ± 0.009***

Higher drop due to heat stress condition in the Eastern Croatia was determined in first parity Holsteins comparing to the Simmentals. The highest decrease in daily protein content was

determined in environmental condition characterized with THI = 80 in amount of 0.163 %/day in Holsteins and 0.174 %/day in Simmental cows.

The decrease of daily protein (2.88 vs. 2.96%) content, as well as decrease of daily protein (0.56 vs. 0.43) yields during heat stress and normal condition that is in summer in regard to spring period was also determined by Bouraoui et al., (2002). The drop in daily protein contents caused by heat stress environments were also determined by Rodriguez et al., (1985), Kadzere et al., (2002) and Lambertz et al., (2014). Knapp and Grummer, (1991) the decrease of protein with increase of maximum daily temperature explained by a decreased dry matter intake and energy intake.

Table 2. Least square means of daily urea content in milk (mg/dl) regarding the given threshold value in accordance to the cattle breed

ThHo	Holstein			Simmental		
	Ls0	Ls1	Difference	Ls0	Ls1	Difference
THI66	22.01±0.065	25.94 ±0.073	-4.161±0.054[***]	20.36 ±0.143	24.82 ±0.159	-4.464±0.090[***]
THI67	22.08±0.065	26.07 ±0.074	-4.188±0.055[***]	20.41 ±0.143	24.95 ±0.160	-4.544±0.093[***]
THI68	22.14±0.065	26.15 ±0.075	-4.251±0.057[***]	20.49 ±0.143	25.06 ±0.163	-4.564±0.097[***]
THI69	22.21±0.065	26.17 ±0.076	-4.260±0.059[***]	20.55 ±0.143	25.27 ±0.166	-4.720±0.102[***]
THI70	22.26±0.065	26.29 ±0.077	-4.121±0.063[***]	20.62 ±0.143	25.45 ±0.169	-4.829±0.107[***]
THI71	22.33±0.065	26.42 ±0.079	-4.028±0.066[***]	20.69 ±0.143	25.85 ±0.174	-5.153±0.114[***]
THI72	22.36±0.065	26.82 ±0.081	-4.097±0.070[***]	20.78 ±0.143	25.99 ±0.179	-5.212±0.121[***]
THI73	22.46±0.065	26.81 ±0.084	-4.115±0.074[***]	20.85 ±0.143	26.00 ±0.185	-5.152±0.129[***]
THI74	22.56±0.065	26.66 ±0.088	-4.146±0.080[***]	20.91 ±0.143	26.10 ±0.191	-5.195±0.137[***]
THI75	22.61±0.065	26.62 ±0.091	-4.058±0.086[***]	20.96 ±0.143	26.33 ±0.200	-5.365±0.149[***]
THI76	22.66±0.065	26.85 ±0.094	-4.212±0.094[***]	21.01 ±0.143	26.46 ±0.210	-5.443±0.162[***]
THI77	22.70±0.065	27.24 ±0.100	-4.273±0.104[***]	21.08 ±0.144	26.32 ±0.225	-5.239±0.181[***]
THI78	22.79±0.066	26.73 ±0.111	-4.362±0.115[***]	21.13 ±0.144	26.33 ±0.241	-5.201±0.200[***]
THI79	22.84±0.066	25.95 ±0.121	-4.454±0.129[***]	21.16 ±0.144	26.49 ±0.258	-5.328±0.221[***]
THI80	22.85±0.066	26.60 ±0.134	-4.648±0.146[***]	21.19 ±0.144	26.93 ±0.287	-5.738±0.254[***]

Analysis of daily urea content in milk regarding the given threshold value separately for each breed is given in the Table 2. Statistically highly significant increase of daily urea content was determined at all tested THI values (66 – 80) in both analyzed breed. Comparing to the Holstein breed, in Simmentals increase was higher at all threshold values. The increase of daily urea content amounted from 4.28 – 4.648 mg/dl in Holstein first parity cows, while in Simmentals increase ranged from 4.464 – 5.738 mg/dl.

Obtained results indicate similar trends in fluctuation of analyzed traits due to heat stress condition in both breeds. Regarding the variation in daily protein content, smaller decrease was determined in Simmentals comparing to the Holsteins, while when daily urea content was analyzed, higher differences were determined in Simmental breed.

Conclusion

Based on analyzed data it could be concluded that THI ≥ 66 cause significant change in daily protein and urea content in milk of Holstein and Simmental first parity cows. Daily protein contents statistically highly significant decrease due to heat stress condition (THI in 66 – 80) in both, Holsteins and Simmentals, while daily urea content statistically highly significant increase. The THI = 66, as the lowest value at which significant decrease in analyzed traits was determined could taken as the threshold value for first parity cows in Eastern Croatia.

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Poster presentation

POSSIBILITIES FOR BREEDING POLLED CATTLE IN CROATIA AND SERBIA

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Abstract

In today's modern production, horned cows may represent a specific security risk. It refers to causing injuries to cattle's own body, or to other cows in its environment. Horned cows can be also dangerous for workers on a farm. If taking into account the animals' wellbeing that is recently emphasized as important, then it can be clearly understood that it is easier and safer to breed polled cattle than horned ones. The aim of this paper is to point out certain advantages of breeding polled cattle and how those advantages can be utilized in terms of increasing profit of milk producers in Croatia and Serbia. This possibility presupposes some genetic background, which is naturally inherited. It is scientifically confirmed that the offspring of both horned parents (homozygous) will be also horned. However, in some other combinations of parents (heterozygous), different occurrences in the offspring are possible. There are many advantages found in breeding of polled cattle, such as higher selling price, fewer abortions and injuries of skin and body, easier transport, more peaceful behavior of animals in a group, etc. It is also important to point out that selection procedures managed so far to counterbalance milk production between polled and horned cattle.

Experts share this idea with positive expectations and believe that breeding of polled cattle in Croatia and Serbia will certainly expand in the coming years. Polled cattle breeding in developed Western countries is well advancing, which can be taken as an example. Possibilities of breeding polled cattle should be facilitated through development of appropriate breeding programs for all interested producers.

Keywords: polled cattle, advantages of breeding, Croatia, Serbia

Introduction

Standing horns are an important feature recognition of Bovidea families with complex morphogenetic place that taken soon after birth (Međugorac et al., 2012). Cattle breeding today significantly altered in comparison to the one that existed a few decades ago (the system of keeping cattle in the barn, the manner and place of milking, monitoring production and health indicators, etc.). Free way of keeping cattle, which is now increasingly applied, requires certain technical adjustments of the equipment, as well as the animals themselves. In doing so, horned cattle is not very suitable for this type of cattle growing and keeping because horned animals can damage the measuring equipment, injure the workers, another animal or itself. It is noticeable that the breeding and selection

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objectives with primary (milk, meat) are increasingly focusing on secondary objectives (constitution, reproduction, health and welfare) (Mijić, 2016).

Although certain polled cattle breeds are known (Angus, Galloway) or certain types (Simmental), in today's modern production the Polled cattle phenotype obtained by forced removal of horns (mechanically or chemically) is used mostly. In such horn removals the welfare of animals is often undermined. That is why this issue has become a long scientific interest, as shown by the researches carried out in Germany 120 years ago (Arenander, 1896).

The aim of this work is to point out to the professional and scientific community in Croatia and Serbia the possibility of phenotype polled cattle growing with all its advantages and disadvantages, and to point out certain financial benefits that those cattle breeders could achieve in their production.

History of polled cattle breeding

Knowing the ethology of cattle and their residence, clearly we can say that the horns of cattle in the past had very important functions and tasks. They were usually reflected in the following:
- They served as a defense against the predators,
- They were weapons for fighting in the hierarchy of the herd,
- It is a part of the body, or an organ which could tie the yoke to plow hitch, carts or similar to that.

It not known precisely since when the polled cattle have been bred. However, the records of polled bovine animals found in Egypt date back to 3000 BC (Blench and MacDonald, 2005), while Kyselý (2010) states that on an archaeological dig in the Czech Republic skeletons of polled cattle were found whose age is estimated at 3800-3500 BC. Furthermore, drawn images on stone were found in a cave in Ireland with polled oxen painted 1,000 years before Christ , while the leaders in Greece and the Roman Empire minted coins with the image of polled cattle (Schmidt, 2015). Based on the above, it is clear that the polled cattle growing has been long known, but such cultivation has not shown more interest by the manufacturers. It was not until the late 19th century when the professional literature mentioned some cattle breeds that are polled and then was the time when the first scientific researches occurred (Arenander, 1896). Genetic polled cattle or individual breeds are now grown in many countries around the world. Thus in Scotland are known polled breeds Aberdeen Angus and Galloway; in Finland, Northern, Eastern and Western Finnish breed; Sweden Fjäll breed; Norway Trønder breeds in northern Russia Pechora strain of Kholmogri breed and so on. Also, some other horned cattle breeds, such as Charolais, the Limousin, a Shorthorn, a Pinzgauer, a Brown Swiss, a German Holstein-and, Gelbvieh and Simmental, there are certain breeding lines that have no horns. Although in Germany with Simmental Polled lines are used mostly as a meat type, they are increasingly starting to use as a milk type (Grupp, 2002; Höfer, 2003).

Growing and polled inheritance in cattle
Ramljak et al., (2011) think that in cattle, as well as in other *Bovidea*, the development and morphology of horns are the indicators of the development degree of polymorphism. The best example of this is the European cattle (*Bos taurus*), where some are completely polled

cattle breeds (such as Angus 's) , then breed with very short horns (such as Buša) , and breeds with very long horns lyre-shaped (like the Pannonian podolac). Although 80 years ago the polled cattle dominant inheritance has been scientifically proven (White and Ibsen, 1936), a causal mutation scanning (mapping position) for 23 years (Georges et al., 1993), still it remains unknown, the molecular nature of polled cattle inheritance. Scientific studies suggest that a key gene for the appearance of horned cattle is gene P (S – locus). Besides this gene, there are some others that also have an impact on the appearance of horns in cattle (humped oxen, cattle without horns or cattle with small horn -like growths). Here we should mention the three most important: H, P and S. H allele is responsible for the construction of real horns on the front bones and is always present both within horned, so also with hornless cattle as homozygous (HH). Allele P (P - locus) is crucial for the emergence of hornlessness in cattle and it is on the first bovine chromosome (BTA1). It manifests in two ways (alleles). P allele is dominant over H, or if at least one allele P present, cattle will not have normal horns. However, it is possible to create small pink and loose horns. For hornlessness occurrence a P allele could be also responsible. The possible combinations of P-locus can be seen in Table 1.

Table 1: Possible combinations of P - locus for the emergence of hornlessness in cattle (LFL, 2016)

Mark - locus	Interpretation
PP	Homozygous (completely) polled cattle
Pp	Heterozygous (mixed genetically) polled cattle
P	Phenotypically pure polled cattle, but the genotype (PP or Pp) is not exactly known
pp	Horned cattle

The offspring of parents where both were with horns is assumed to be homozygous (PP). However, if both parents were polled but not homozygous too, their offspring is indicated with P and they can have different combinations (PP or Pp). If one parent is with horns and the other one hornless (PP, Pp, P and PS), the offspring is purely polled (hornless) with a status of (Pp). The cattle, with no conventional horns can have pure hornlessness or a transitional form of pink growths or something like bumps on the frontal bone. The responsibility for the appearance of pink loose growths is in an S allele (SCURS = pink loose or wobbly growths) (Schmidt, 2015). Table 2 shows some combinations of polled heredity in cattle.

Table 2. Polled heredity in cattle regarding the P allele (Schmidt, 2015)

Horns status in 1st parent - genotype (phenotype)	Horns status in 2nd parent - genotype (phenotype)	Horns status in offspring - genotype	Horns status in offspring - phenotype
PP (polled)	PP (polled)	100% PP	all polled
PP (polled)	Pp (polled)	50% PP 50% Pp	all polled all polled
PP (polled)	pp (horned)	100% Pp	polled
Pp (polled)	Pp (polled)	25% PP 50% Pp 25%pp	polled horned
Pp (polled)	pp (horned)	50% Pp 50% pp	polled horned

For practical breeding work on polledness of the cattle, first we must know the inheritance of P - locus. Animals with small horns (PS allele) are not a problem, since they have Polled P allele. Figure 1 shows one possible cross combinations of polled bull (Pp) and a horned cow (pp). In 50% of cases their offspring will be heterozygous polled (Pp), while 50% of the offspring will be horned (pp) (Stückler, 2015).
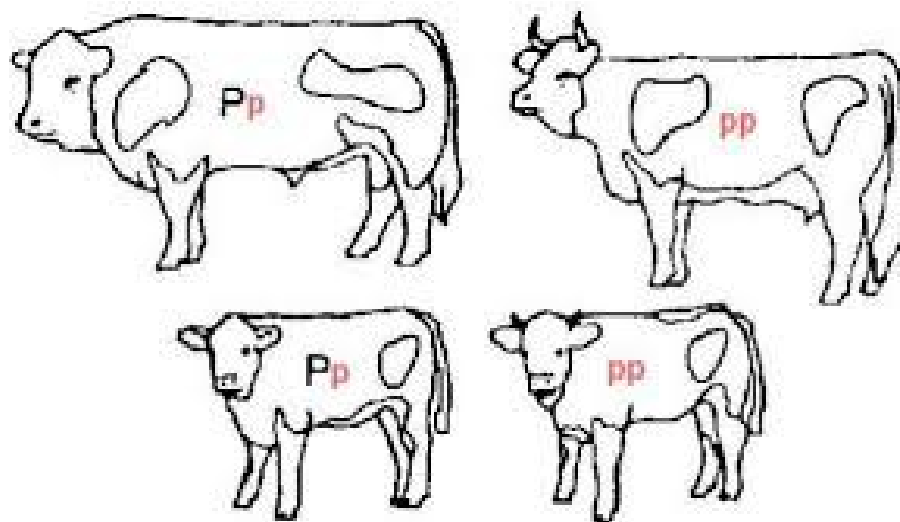
Picture 1. Example of the crossing heterozygous hornless bull (Pp) x horned cow (pp). (Grupp, 2002)

Benefits of polled cattle breeding
Based on professional and scientific analysis, there can be a number of advantages for polled cattle breeding (Lončar and Mijić, 2016). The most common general benefits of this type of cattle breeding are: high prices of the head at the same production in comparison to the horned cattle, a small number of animal injuries (reduced the number of abortions, injuries of the skin and the body), less risk of injury to workers in the environment of the cattle, easier transport of animals and calmer behavior of animals in the group. In addition to these most common, there are some additional benefits such as: no additional expenditure of labor for maintenance, no additional financial costs for the job, and increased well-being, avoiding stress and pain in animals. It should be noted that due to the forced removal of horns and increased public pressure and the media to such procedures. It is evident that polled cattle has a number of important arguments for the use in production (Burren et al., 2015). A result of research (Table 3) implemented in Bavaria on 50 dairy cows (25 polled and 25 horned) can go in favor of it, which aim was to compare the production features polled and horned dairy cows.

Table 3. Comparison of production output for 2011/2012 between natural polled and genetically horned dairy cows (LWT Bayern EV, 2015)

Group of cows	Milk (kg)	Fat (%)	Proteins (%)
Naturally polled	9.764	3,94	3,47
Horned	9.870	3,94	3,45
Divergence	-106	+0,00	+0,02

It is evident that the milk production of horned cattle was slightly higher (106 kg), but was not statistically significant. The content of milk fat and protein in milk were almost equal. This is certainly an important information for the promotion of breeding polled cattle.

Some provinces in Germany have already recognized these advantages and have launched specific projects to improve breeding polled cattle or breeding of certain breeds, lines and families. Thus, in Bavaria researches on polled cattle have been conducted from 1974 (LFL 2016), and in the province of North Rhine-Westphalia of 2012. (Holstein). Relevant ministries of the provinces launched certain procedures according to the Ministry of Agriculture of the European Union, in order to include the polled cattle breeds into the financial support schemes due to the benefits applied.

Conclusion

Based on the data in this study, it can be concluded that the cultivation of naturally polled cattle should be promoted, except for those breeds where this is a breed characteristic. In recent years the number of farms growing polled cattle is increasing. Farmers who still growing horned cattle justify themselves that they do not want to take the maintenance jobs and expenses that arise in those proceedings. It is expected in years to some in Croatia and Serbia to have an expansion of polled cattle breeding production. By using the appropriate farming strategies we should seek to expand the polled cattle gene population to the interested manufacturers and thus ensure the provision of high-quality features heritage that carry the gene to their offspring.

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Poster presentation

PHENOTYPIC VARIABILITY AND EFFECTS OF FARMS, CLASSIFIERS AND LACTATION STAGE ON LINEAR TYPE TRAITS SCORES OF PRIMIPAROUS HOLSTEIN-FRIESIAN COWS

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Abstract

In this study we aimed to evaluate average values and phenotypic variability of linear type traits scores and the effects of farms, classifiers and lactation stage on linear type traits scores of primiparous Holstein-Friesian cows in Vojvodina.

Evaluation of linear type traits was carried out in 2012, including 1,217 primiparous cows from 22 dairy farms in the Vojvodina province. Ten classifiers used linear classification system which included 18 type traits. The statistical analysis was carried out using ANOVA in Statistica software, version 12.

The coefficient of variation for all type traits ranged from 17.9% for top line to 32.5% for foot angle. From the observed fixed factors farm had a highly significant effect on the variability of all type traits. Classifiers fixed factor had statistically significant effect on the following traits: front teats placement, udder depth and suspensory ligament. Lactation stage fixed factor had a highly significant effect on the fore attachment and suspensory ligament.

Keywords: phenotypic variability, fixed factors, linear type traits scoring, primiparous Holstein-Friesian cows

Introduction

One of the important factors for successful milk production is a group of secondary traits: health, longevity, type traits. Modern studies in dairy production has placed more emphasis on conformation (type) traits and body development of dairy cows. Conformation analysis using type traits scoring is objective and unbiased method to determine production ability of dairy cows, and it is used for evaluation of dairy cows overall functionality. Linear type traits are the basis of all modern type classification systems, and constitute the foundation of all systems for describing the dairy cow (Janković, 2012). Being numeric, linear type

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scoring allows interpretation of biological relationship between type traits; also, the importance of linear scoring system is that the genetic variability of type traits can be estimated. Furthermore, we can make important conclusions regarding type traits, especially for the relationship between milk and milk components production and type traits, as well as relationship between the reproductive parameters and type traits.

The first author in Serbia who investigated the linear type scoring systems of the Holstein-Friesian, using a classification scale from 1 to 9 on PK "Beograd" farms was Latinović (1985). Scores for type traits ranged from 4.59 for teats placement to 5.79 for the rear udder height. Linear type scoring of the primiparous cows was introduced as a obligatory selection procedure in 1988 on PK "Beograd" farms (Stojić et al., 2002). The program has encompassed evaluation of 14 traits (6 body traits and 8 udder traits). A linear type traits was recorded on the scale from 1 to 9. Živanović (2002) estimated the variability of the linear type scores and milk production of 2,976 primiparous cows reared on PK "Beograd" farms. Average values of linear scores ranged from 5.28 to 7.15 for body traits and from 5.06 to 7.02 for udder traits. Jankovic et al. (2012) also estimated the variability of the linear type traits and milk production of 221 primiparous cows on PIK "Bečej" farm. Average values of linear scores ranged from 4.96 to 6.78.

Main breeding program for Holstein-Friesian (2010) cattle has introduced linear type traits scoring as a obligatory procedure into genetic improvement programs in Vojvodina Province. It is recommended to perform linear type scoring during the first lactation, between 60–150 days in milk, due to the capacity and activities of the udder during this period, and because of the assumptions that the genetic basis evaluation is reliable as well as the effect of environmental factors are insignificant in this period (Pantelić, 2004).

The aim of this study was to estimate average values and variability of linear type traits scores as well as to estimate the effect of farms, classifiers and lactation stage on the variability of linear type traits scores of primiparous Holstein-Friesian cows.

Material and Methods

Data were provided by main breeding organizations of Vojvodina belonging to 1217 primiparous cows, reared on 22 farms in Vojvodina, which was linearly scored in 2012. Linear type classification system used by 10 trained classifiers included 18 traits grouped in 4 functional units - body, dairy character, feet and legs and udder. Each individual linear type traits was scored on a 9-point numerical scale. Scores include biological extremes and describe the level of traits expression. Basic analysis of average scores and variability of linear type traits were calculated using standard statistical procedures in the software Statistica 12 (StatSoft, 2015).

Statistical analysis of effects of farms, classifiers and lactation stage on linear type traits scores was also carried out using the software Statistica 12 (StatSoft, 2015). The effect of each of the factors was examined individually using univariate ANOVA. The farm had 22 factor levels in comparison, classifiers 10, and lactation stage 3 levels (stage I: 0-59 days, stage II 60-150 days, stage III 151-305 days in milk). Then, also in the software Statistica 12, was carried out analysis of the effect of all three factors on linear type scores by

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multivariate analysis of variance with GLM (general linear model) procedure. The analysis has been done according to the following model:

$$Y_{ijk} = \mu + F_i + O_j + L_k + e_{ijk},\qquad \text{where:}$$

Y_{ijk} - vector of observations (linear type score);
μ - overall mean (average value of linear type score for individual trait);
F_i - effect of ith level of factor F (fixed effects of farm);
O_j - effect of jth level of factor O (fixed effects of classifier);
L_k - effect of kth level of factor L (fixed effects of lactation stage);
e_{ijk} - random error.

Results and Discussion

The aim of this study was to evaluate average values and variability of linear type traits scores (Table 1), as well as to evaluate the effects of the farms, classifiers and lactation stage (Tables 2 and 3) on linear type traits scores of primiparous Holstein-Friesian cows in Vojvodina.

Table 1 shows the average values and variability of linear type traits scores of primiparous Holstein-Friesian in Vojvodina. For the body traits the average linear scores ranged from 5.59 for stature to 6.42 for the rump width. The results for stature, chest width and body depth are similar to those in the study of Nemcova et al. (2011). Campos et al. (2012) reported similar results for the chest width and rump width, while the scores for top line differ significantly. Average linear types score of primiparous Holstein-Friesian cows in Vojvodina for top line, rump angle and rump width are close to the ideal scores for these traits (7, 5, 5). This is important because the pelvis size and structure are related to female reproduction and affects calving difficulty.

Table 1. Average and ideal value of linear type scores and variability of linear type scores of primiparous Holstein-Friesian cows in Vojvodina

Type traits	N	$\overline{X}$	Average value HF	Ideal scores HF	SE	SD	CV (%)	Min	Max
1-Stature (STA)	1217	5,59	5	7	0,06	1,76	31,49	1,00	9,00
1-Top line (TL)	1217	6,41	5	7	0,04	1,15	17,94	2,00	9,00
1-Chest width (CW)	1217	5,72	5	7	0,05	1,50	26,20	2,00	9,00
1-Body depth (BD)	1217	5,87	5	7	0,05	1,41	24,04	2,00	9,00
1-Rump angle (RA)	1217	5,91	5	5	0,05	1,40	23,73	2,00	9,00
1-Rump width (RW)	1217	6,42	5	9	0,04	1,23	19,22	2,00	9,00
2-Angularity (ANG)	1217	6,33	5	9	0,06	1,72	27,19	2,00	9,00
3-Rear legs rear view (RLR)	1217	5,86	5	8	0,05	1,44	24,66	1,00	9,00
3-Rear legs side view (RLS)	1217	5,12	5	5	0,04	1,33	25,96	2,00	9,00
3-Foot angle (FA)	1217	4,93	5	7	0,05	1,60	32,51	1,00	9,00
4-Fore udder attachment (FUA)	1217	5,41	5	9	0,06	1,65	30,60	2,00	9,00
4-Front teat placement (FTP)	1217	4,91	5	5	0,05	1,49	30,28	1,00	9,00
4-Front teat length(FTL)	1217	5,01	5	5	0,04	1,25	25,04	1,00	9,00

4-Udder depth (UD)	1217	5,88	5	5	0,05	1,53	25,96	2,00	9,00
4-Rear udder height (RUH)	1217	5,97	4	9	0,05	1,41	23,68	1,00	9,00
4-Udder cleft (UC)	1217	5,74	5	9	0,05	1,54	26,92	1,00	9,00
4-Rear teat placement (RTP)	1217	5,92	5	5	0,05	1,63	27,49	2,00	9,00
4-Rear teat length (RTL)	1217	4,51	5	5	0,05	1,40	31,00	1,00	9,00

1-body traits; 2-dairy character; 3-feet and legs; 4-udder.

Angularity is the trait in the linear classification system that we evaluate in the category of dairy character/dairy form. Good dairy character means well developed and broad chest with open ribs and more angularity. Such a cow is with a visible frame indicating disease resistance and high milk production. The average score of angularity was 6.33, which is closer to the mean (5) than to the ideal score in Holstein-Friesian (9) according to ICAR (*International Committee for Animal Recording*, 2005) nomenclature. The average score is higher than the one reported by Berry et al. (2004), Guliński et al. (2005), Němcova et al. (2011), and closer to the scores reported by Campos et al. (2012).

Feet and legs average scores were similar to those reported by Pantelić et al. (2011) for rear legs - rear view and rear legs - side view and Jankovic et al. (2012) for rear legs - side view. Foot angle score is very similar to the score reported by Duru et al. (2012). Average scores for rear leg – side view and foot angle are near to the ideal scores for those traits (5, 7). This is important because the correct feet and legs provide good mobility and welfare. The Holstein breeding programs worldwide emphasize the importance of the feet and legs traits due to the improvement of cows disease resistance, as well as the correlation of these traits with longevity.

Average linear scores for udder traits ranged between 4.51 (front teat length) to 5.97 (rear udder height). Average scores for front teat placement and front teat length, udder depth, as well as the rear teat placement and rear teat length are near to the average scores for the primiparous Holstein-Friesian (5), which are also ideal scores in the evaluation of this traits in the common ICAR classification. Average values of linear score for fore udder attachment, rear udder height and suspensory ligament-udder cleft, are also much more near average (5) than the ICAR ideal scores for these traits (9). In their research, for almost all the traits, except for the rear teat placement, Pantelić et al. (2010) reported different scores, as well as Kruszinski et al. (2013) (except for the suspensory ligament-udder cleft). Average scores for udder depth and rear teat length similar to those obtained in this study reported Berri et al. (2004), while very similar scores for all udder traits reported Nemcova et al. (2011) and Duru et al. (2012).

The coefficient of variation for all type traits ranged from 17.9 for top line to 32.5 for foot angle (Table 1). Pantelić et al. (2011) reported a lower coefficient of variability (from 6.90 for dairy character to 24.7 for stature), while Duru et al. (2012) reported very similar coefficient of variation for several type traits, although the interval for the coefficient of variation was much wider (from 2.60 for stature to 38.9 for suspensory ligament).

The results obtained by unifactorial analysis of variance (Table 2) showed that the effect of the farm and the classifier was highly statistically significant for all type traits scores, while the lactation stages effect was highly statistically significant on linear scores for the stature, top line, rump width, angularity, rear legs-side view, front teat placement, udder depth, rear udder height, the strength of the suspensory ligament/udder cleft, and rear teat placement and rear teat length.

GLM analysis of the effects of all three factors on linear scores showed different results for the effects of classifier and lactation stage on linear type traits scores (Table 3). Fixed factor of farm had a highly significant effect on the all traits scores. The classifier fixed factor had highly statistically significant effect on the scores for stature, rump angle, and rear teat length, while the classifier factor had statistically significant effect on the scores for following traits: front teat placement, udder depth and suspensory ligament. Lactation stage fixed factor had statistically significant effect for scores for top line and rear teat placement, while had highly statistically significant effect on fore udder attachment and the suspensory ligament.

Lukas et al. (1984) reported a statistically significant effect of farm on linear scores of most traits (stature, body strength, dairy character, rump angle, rear legs rear view, heel depth, rear udder width and teat placement), while the classifier factor had no statistically significant effect only on the scores of fore udder attachment, suspensory ligament and the teat length. Lactation stage factor had a statistically significant effect on the dairy character, fore udder attachment and udder depth.

Pantelić et al. (2010) reported a highly statistically significant effect of farm fixed factor on all linear type traits scores, while Esteves et al. (2004) reported that lactation stage factor had a statistically significant effect on all udder traits except on the fore udder attachment, udder texture and front teat length.

Table 2. Effects of observed factors on linear type scores using unifactorial ANOVA

| Traits | One-way ANOVA | | | | | | | | | | | | | | |
| | Farm | | | | | Classifier | | | | | Lactation stage | | | | |
	df	SS	MS	F	P	df	SS	MS	F	P	df	SS	MS	F	P
STA	21	1186,89	59,34	32,99	0,000**	9	592,57	65,84	26,81	0,000**	2	219,24	109,62	38,323	0,000**
TL	21	267,18	13,36	12,80	0,000**	9	151,85	16,87	14,52	0,000**	2	22,09	11,04	8,49	0,000**
CW	21	305,36	15,27	7,85	0,000**	9	241,50	26,83	13,46	0,000**	2	6,01	3,00	1,338	0,263
BD	21	289,84	14,49	8,52	0,000**	9	203,89	22,65	12,74	0,000**	2	5,48	2,74	1,376	0,253
RA	21	200,98	10,05	5,65	0,000**	9	106,77	11,86	6,36	0,000**	2	2,25	1,13	0,572	0,564
RW	21	344,56	17,23	14,85	0,000**	9	207,46	23,05	17,70	0,000**	2	31,50	15,75	10,56	0,000**
ANG	21	1001,69	50,08	26,73	0,000**	9	821,70	91,30	44,40	0,000**	2	65,96	32,98	11,381	0,000**
RLR	21	443,31	22,17	13,66	0,000**	9	270,19	30,02	16,68	0,000**	2	7,21	3,60	1,729	0,178
RLS	21	297,83	14,89	10,18	0,000**	9	186,97	20,77	13,22	0,000**	2	16,62	8,31	4,740	0,008**
FA	21	381,52	19,08	8,72	0,000**	9	66,74	7,42	2,94	0,000**	2	15,73	7,86	3,074	0,047*
FUA	21	290,75	14,54	5,90	0,000**	9	170,77	18,97	7,38	0,000**	2	8,06	4,03	1,474	0,229
FTP	21	474,80	23,74	13,85	0,000**	9	149,06	16,56	8,02	0,000**	2	24,12	12,06	5,504	0,004**
FTL	21	155,97	7,80	5,45	0,000**	9	109,42	12,16	8,30	0,000**	2	6,81	3,41	2,170	0,115
UD	21	383,40	19,17	9,88	0,000**	9	329,08	36,56	18,48	0,000**	2	37,94	18,97	8,271	0,000**
RUH	21	314,42	15,72	9,37	0,000**	9	176,81	19,65	10,83	0,000**	2	26,88	13,44	6,819	0,001**
UC	21	375,93	18,80	9,37	0,000**	9	221,08	24,56	11,39	0,000**	2	98,73	49,37	21,660	0,000**
RTP	21	294,67	14,73	6,21	0,000**	9	148,22	16,47	6,57	0,000**	2	69,94	34,97	13,567	0,000**
RTL	21	752,17	37,61	33,37	0,000**	9	453,54	50,39	34,64	0,000**	2	104,37	52,18	28,369	0,000**

P<0.01 - ** statistically highly significant effect; P<0.05 - * statistically significant effect

Table 3. Effects of observed factors on linear type scores using unifactorial ANOVA

Traits															
			Farm					Classifier					Lactation stage		
	df	SS	MS	F	P	df	SS	MS	F	P	df	SS	MS	F	P
STA	21	536,48	41,27	23,11	0,000**	9	13,51	6,75	3,78	0,023*	2	5,06	2,53	1,42	0,243
TL	21	106,66	8,20	7,91	0,000**	9	0,80	0,40	0,39	0,681	2	8,09	4,04	3,90	0,021*
CW	21	71,75	5,52	2,85	0,000**	9	7,12	3,56	1,84	0,160	2	6,98	3,49	1,80	0,166
BD	21	92,61	7,12	4,19	0,000**	9	6,50	3,25	1,91	0,148	2	2,51	1,26	0,74	0,478
RA	21	112,34	8,64	4,90	0,000**	9	13,43	6,72	3,81	0,023*	2	6,56	3,28	1,86	0,157
RW	21	139,76	10,75	9,30	0,000**	9	5,51	2,76	2,38	0,093	2	2,20	1,10	0,95	0,387
ANG	21	187,00	14,38	7,68	0,000**	9	7,20	3,60	1,92	0,147	2	1,57	0,78	0,42	0,658
RLR	21	174,66	13,44	8,27	0,000**	9	0,46	0,23	0,14	0,869	2	3,64	1,82	1,12	0,327
RLS	21	111,11	8,55	5,84	0,000**	9	1,91	0,96	0,65	0,521	2	4,99	2,50	1,71	0,182
FA	21	310,16	23,86	10,96	0,000**	9	9,14	4,57	2,10	0,123	2	8,57	4,29	1,97	0,140
FUA	21	133,50	10,27	4,22	0,000**	9	4,47	2,23	0,92	0,400	2	29,20	14,60	5,99	0,003**
FTP	21	311,75	23,98	14,06	0,000**	9	14,59	7,29	4,28	0,014**	2	0,19	0,09	0,05	0,947
FTL	21	46,69	3,59	2,50	0,002**	9	1,29	0,65	0,45	0,638	2	0,20	0,10	0,07	0,933
UD	21	79,49	6,11	3,19	0,000**	9	19,35	9,67	5,05	0,007**	2	9,84	4,92	2,57	0,077
RUH	21	135,45	10,42	6,19	0,000**	9	0,58	0,29	0,17	0,842	2	2,20	1,10	0,65	0,520
UC	21	139,79	10,75	5,47	0,000**	9	21,63	10,81	5,50	0,004**	2	19,91	9,96	5,06	0,007**
RTP	21	124,61	9,59	4,06	0,000**	9	2,47	1,24	0,52	0,593	2	15,05	7,52	3,19	0,042*
RTL	21	275,26	21,17	18,86	0,000**	9	7,72	3,86	3,44	0,033*	2	0,39	0,20	0,17	0,840

P<0.01 - ** statistically highly significant effect; P<0.05 - * statistically significant effect

The effect of classifier factor on linear type traits scores were investigated Veerkamp et al. (2002). They conclude that the classifier may have a significant effect on the variability of type traits scores, especially when the trait is relatively new in the classification system. There was a significant effect of the classifiers on the rump width, foot angle and central ligament. Battagin et al. (2013) found that the classifier factor had effects on the type traits with the highest coefficient of variation; within five traits with a coefficient of variation of more than 30% (stature, foot angle, front udder attachment, front teat placement and rear teat length) classifier factor had a statistically significant effect on the scores for stature and rear teat length, and highly statistically significant effect on the scores for front teat placement.

Conclusion

One of the main reasons for the recording and using type traits information is helping breeders to rear long-lasting, profitable and functional cows. The cow with ideal type traits scores is medium to high, with a broad chest, expressed angularity, good rump structure, sound feet and legs, fine udder texture with high and strong attachments. The aim is that such cow remains in the herd as long as possible with a regular calving and high milk production. In order to get the desired effect of linear type evaluation, it is essential that linear assessments are completed to the recommended international standards to achieve and maintain a uniform level of classification, to get a sound picture of the cow and its production and calving abilities.

Based on the average linear type traits scores of primiparous Holstein-Friesian in Vojvodina, we conclude that the selection should continue to put emphasis on improving the dairy character traits and strength of the udder attachments (front udder attachment, rear udder height, and suspensory ligament). The dairy character is an indicator of the physical abilities of dairy cows for milk production, while the udder with a solid front and height rear attachments, as well as a strong suspensory ligament, less prone to udder diseases because strong attachments protect udder against bacterial contamination.

It was found that the effect of the farm was highly statistically significant at all type traits scores, while the effect of classifiers, as well as the lactation stage, was highly significant on udder traits scores. Given the fact that classifier had a statistically significant effect only on the udder traits, it should be examined are there any classifiers*lactation stage interaction, and whether are classifiers indirectly affected by lactation stage of primiparous Holstein cows.

Thanks to the linear evaluation, type traits are gaining in its economic importance. The visual assessment and identification of the type traits constitute the preliminary indicator of milk production, longevity and reproductive ability of cows, which is important from the aspect of economic efficiency of milk production. Linear evaluation also provide interpretation of biological relationships between type traits and other traits that are important for the productivity and longevity of dairy cows. In order to increase the productive life of cows, it is very important to determine type traits which are related to milk traits, health and fertility, in further research, and that the type traits are adequately represented in the breeding programs for dairy cattle.

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Poster presentation

BREEDING IN COW-CALF SYSTEM IN BOSNIA AND HERZEGOVINA, PROS AND CONTRAS

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Abstract

Cattle breeding in the cow-calf system becomes more significant nowadays, not only because of increasingly common interest of consumers for such a kind of meat. This type of farming has intensified in the last 10 years in Bosnia and Herzegovina which was largely boosted by federal agricultural subsidies for this manner of breeding cattle and large unused pastural capacities. The cow-calf system is certainly economically beneficial and acceptable for animal health.

However, that system of cattle breeding involves many risks, and not only the weather conditions (disasters). To avoid these risks for animals and the environment, or at least reduce them, methods of farm management that differ from conventional farming in the barns must be taken into account. Here it is especially necessary to take care of the winter and summer pasturing, where in addition to a permanent summer grazing, winter pasturing time should be provided for animals.

Success is possible but only if this system of cattle breeding is observed through the prism of the system with high demands and not only by the principle, that it is sufficient to provide only the land surface and a sufficient number of animals. The Cow-calf system is different both in the preparation and the performance compared to the conventional way of cattle breeding.

The property has to be very well organized in order to achieve sufficient income in breeding herds, in the given relations in meat prices on the market.

Production characteristics of animals, depending on the breed, should reach the level of calving of 98%, calving interval of 370 days, while losses in the herd must not exceed 5%. Male calves should achieve daily gains of 1,200 g, and female calves daily gains of 1000 g. The observed production of meat should be followed by very good marketing. There will be no success without good marketing in spite of good efforts on the farm.
Only under these conditions it is possible to breed healthy animals all year around on the pasture in the cow-calf breeding system. The cow-calf breeding system is a low cost system that promotes animal health

Keywords: cow, calf, production system, cattle

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Introduction

Cow-calf breeding system in Bosnia and Herzegovina as well as in the former Yugoslavia is regarded as a separate and under-accepted way of producing beef. This way of farming is still lagging behind the traditional intensive fattening when it comes to the production of beef. Cow-calf breeding system is the most widespread form of beef production in the world. Over last 10 years this way of breeding cattle has slowly spread in Bosnia and Herzegovina, ant it is becoming increasingly popular especially in organic farming. The system cow-calf does not require large investments and at the same time it is possible that the animals stay a large part of their time on pasture without major investments in expensive feed supplements for animals.

The challenge for farmers is the transition to organic production, first of all, the use of organic animal feed, meeting the prescribed required space in the barn and on the pasture, a stopping of the application of mineral fertilizers and synthetic pesticides and medications whose use is allowed only restrictively.

On the economic side of the cow-calf system, it is very important to have a high level of reproductions, as well as the high daily gains of calves. It is necessary to keep the feeding and barn cost as low as possible, for sufficiently high prices for calves and young bulls.
Cow-calf system can be organized as either extensive or intensive. The transit from conventional intensive farm to organic farming is economically difficult because of the cost of reconstruction of facilities and the possible reduction of the number of animals in the herds.

Year-Round Grazing System is possible in areas where the climate conditions permit it, where attention should be paid to the autumn and winter calving avoidance, because the calves still do not have a high tolerance for low temperatures.

In the EU-28, between 2007 and 2014, the number of non-dairy cows decreased by 4 %, from 12.5 to 12.0 million head (Figure 3). This trend was especially striking in the EU-15, where the decrease reached 6 %. Most of the meat bovine herd is located in four EU Member States: France (34.4 %), Spain (15.2 %), the United Kingdom (12.8 %) and Ireland (8.7 %) according to the EUROSTAT 2016.

The European cow-calf production systems are similar to those in North America in the sense that pastures form the feed basis. In the EU (except Ireland) winter housing of the cows and their progeny is more common than in North America. When it comes to beef finishing systems, there are distinct differences between the countries considered and other important beef producers (Deblitz and Dhuyvetter, 2013).

Cow-calf breeding system in Bosnia and Herzegovina
The production of meat and milk, together with cereals, represent the most essential agricultural production and each state is trying to achieve production that will turn off the dependence on imports of food as a basic human need. In Bosnia and Herzegovina, a significant progress has been made in milk production, which cannot be said for meat production, especially if we know that B&H imports nearly 75% of (beef) meat. This situation is not characteristic only for B&H. Bearing in mind that Bosnia and Herzegovina

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has a large proportion of pastures, 930,000 ha (36.7%), out of which a big percentage is not utilized. Here we find a big opportunity for the beef cattle breeders in B&H to be more focused on the sources of animal feed from under-used pastures and meadows in cow-calf production systems. Breeding cattle in herds beside meat production has its own significance for the conservation of biological variability. Cattle presence on the pasture in fact represents a form of eco system cleaner and it prevents the degeneration of grassland vegetation to the forest community. This type of cattle breeding has spread in B&H in the past 10 years, partly with help of subsidies in agriculture, especially in animal breeding for herds with cow-calf breeding system, which ranged from 225 to 300 EUR per head depending on the year. This trend of financial support for the system cow-calf is recognized as a positive one throughout the European Union. According to Offermanna, 2009, "direct payments play an important role in the financial viability of organic farms in both Western and Eastern European countries".

Historically, cow-calf production is a by-product of land ownership and does not represent the primary source of income for most operations (Troxel et al., 2007). Cow-calf breeding system implies growing in the pasture whereby the cows in the pasture nurture a calf or a young bull that by the end of the grazing season stays on the farm, goes to the market, or less frequently remains on the farm for further fattening. The breeding uses combined and meat breeds of cattle.

The selection of the breed is a very important for a successful breeding in the cow-calf system. The selection of breeds should be linked to manufacturing requirements, conditions of keeping as well as the quality of nutrition. Troxel et al., 2007, state that in the USA breeds or breed types that increased in value from the year of 2000 to the year of 2005 were Angus × Hereford, Angus, Angus × Charolais, and Brahman. The sale price of Angus × Hereford, Angus, and Angus × Charolais increased.

The basis for the economic success of the cow-calf breeding system is a good production technology. Due to low profit margins, weaknesses in the technology segment are clearly reflected in the performance of production and operating profits. The main point to observe is the number of calf per suckler cow and per year. Raised (brought up) calves are the only profit on the farm, excluding the sale of the old cows and possibly lower number of heifers. Table 1 shows the indicators of production (Hampel G, 2009).

Table 1: Production indicators in cow-calf herds

	Measure	Value
Level of calving	Pieces/Cow	0,98
Sold calves (< 6 months)	Pieces/Cow	0,75
Losses during calf rearing	%	5
Calving interval	days	370
Number of heads	grazing livestock unit/ha	0,74
Period in barn	days	120-180
daily gains	g/day	♂-1200; ♀-1000

By choice of the breed the current market conditions should be taken into account. The following breeds have been proven as a good choice: Angus, Hereford, Simmental, Limousin, Pinzgauer, Salers. For those who want to do fattening on the farm the following breeds are recommended as well: Charolais, Simmental, Limousin, Pinzgauer, Gelbvieh or Blonde d'Aquitaine with the remark to pay attention to possible problems

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while calving. For extensive breeding and year-round grazing breeds of Galloway, Highland, Salers, Devon are suitable.

In the conditions of Bosnia and Herzegovina accompanying measures are insufficient:

- Improvement of agricultural structures and management on farms
  The improvement of agricultural structures and management involves the collection of data on the properties that would be measurable and comparable and that is primarily related to: the number of units sold or culled calves per cow and year, calving interval, daily gain, income from the sale, animal health.

- The continuous quality of the value chain – Continuous quality production
  This is the problem that is often encountered by farmers in the field because they are neither aware nor well informed how important it is to market a continuous quality production. This point is closely linked with insufficient education.

- Education and advisory service
  Insufficient education and advising farmers can be felt in the practice. This situation is the product of dual inaction. On one side, farmers have a lack of interest for new and different, and on the other hand, there is a lack of Government engagement with an advisory service to assist farmers who express the need. Of course both are associated with the lack of money. The farmer is not ready to further invest in the professionals for extension services, while state/Government on the other hand is not prepared to take complete advisory role and also does not have money. One of the possibilities may be to organize private extension services whose funding was covered in a ratio of 50/50%, (win-win situation), between farmers and the Government through subsidies in the agricultural sector.

In order to increase the profitability of farming in herds it is necessary to make use of and improve the existing potentials on the farm itself, and to introduce management on the farm. Improved management on the farm means keeping complete documentation in the area of fertility, loss at calving and raising cattle, diseases, strict selection of animals that have problems with gravidity, routine checks in gravidity and increased monitoring of the herd during calving season. There is a space for improvement in the field of nutrition and corrective mating. All this will not be enough for successful breeding in cow-calf herds if the owner does not dedicate proper attention to the marketing of its products. This is especially the case in a situation where the market is flooded with cheap meat whose quality cannot be compared with the quality of meat from cow-calf breeding herds.

The most important measure of success is the number of calves reared per suckler cow and year. This value varies greatly. Number of calves born per suckler cow gives information about the fertility of animals. The differences between individual farms is in the range of 0.4 to 1.45 calves per suckler cow and year. Values of more than one calf per suckler cow may be explained by the existence of twins and shorter between-calves period. Values less than one calf indicates reproductive problems or errors in the management of the farm (for example, incorrect diet, inadequate climatic conditions or poor detection of estrus in animals where they carried out artificial insemination). The strategic approach for the cow-

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calf farms must be paying special attention to the development and raising of calves. The corresponding revenues from the sale of calves are very important; therefore each cow must have a minimum of one calf per year.

Despite the low heritability for reproductive traits, the weaning rate is the trait that has the greatest impact on profit and should be taken into account in the implementation of breeding programs for low input family-based beef cattle operations (LaskeI at al., 2012). Another important factor especially from an economic point of view is grazing. The goal of the efficient use of pastures with growth in height of 4 to 7 cm. Then grazing has a high level of energy (> 6.3 MJ NEL / kg DM) and thus it is comparable to the level of concentrated feeds. Energy savings through the elimination of additional harvest and preservation of nutrients is a huge potential to earn and save money. Use of grazing alongside positive effect in animal feed in herds, positively reflect on overall quality of life of farmers and their families, because during a grazing period, there are no works in the barn, which opens a space for work in other fields or free time for family.
Beck at al., 2016, state that "using rotational grazing, stockpiled bermudagrass, and complementary cool-season annual grasses can drastically reduce winter feed requirements and simultaneously increase carrying capacity and net return".

Conclusion

The economic situation is for many cow-calf farms in Bosnia and Herzegovina very difficult. Production and marketing structures can only change conditionally and in a long-term run. Prices of beef in the world market are relatively stable with a slight growth trend, but this positive trend has almost no effect on the domestic farmers because the world market for meat exports for the local breeder is not available due to prohibition of meat exports to most international markets. Therefore, they are currently forced to seek new models for marketing of their products in the domestic market, and these are, first of all, ecological products. Demand for organic products is growing, but one should be careful of the trap that if all farmers produce ecological food, which is more expensive, the same product must be sold for significantly lower prices. It is necessary to find a new way of marketing a product of the cow-calf breeding system, which will be recognized through constant quality and therefore preferred by consumers through willingness to pay more for a product that guarantees quality.

The cow-calf breeding system is cost-effective, but only provided that the farmer/breeder adheres to clear breeding norms by using all the available information and extension service, both in the area of cultivation/growing/breeding and in the area of marketing activity. Any other way of farming in the cow-calf breeding system is not cost effective and will inevitably bring problems to farmers which can ultimately lead to a shutdown of production and a serious existential problem for the farmers in Bosnia and Herzegovina.
Economy is very dependent on the prices obtained for calves and young cattle, (and that is the problem in Bosnia and Herzegovina). In order to achieve the high price, it is necessary to pay attention to the following: market conditions, a unique product (seasonal calving, herd size), as well as the fact that these products require a higher price. However, "marketing options for smaller producers are limited because of their relative size" (Schmitz et al., 2003).

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Poster presentation

STUDY OF OXIDANT-ANTIOXIDANT STATUS AFTER MELATONIN TREATMENT OF RAMS OF NORTHEAST BULGARIAN FINE FLEECE BREED

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Abstract

Regarding the main sperm characteristics, the application of melatonin result in a very diverse range of data. Under the influence of the hormone, in some cases semen parameters improved, but in other cases no comparable improvement was observed. The reason for this multidirectional effect of melatonin is unclear. In some cases this may be due to breed, individual or other factors. In other cases, melatonin might have had enhanced antioxidant effect. The aim of this study was to determine the influence of the hormone melatonin on the oxidant-antioxidant status in a brood of *Ovis aries* species.

The experiments were conducted on three groups of breeding rams with northeast Bulgarian fine fleece through treatment with melatonin (implants were used and melatonin *per os*). The experiment started two months prior to the insemination period. Changes in oxidant-antioxidant status were determined by measuring total antioxidant activity (Kovačević et al.) Blood samples were taken on day 1, 30 and 70, respectively.

After analyzing the results, we found no relationship between overall antioxidant activity in blood of the rams where melatonin was administered *per os*. In rams with melatonin implants the activity of principal antioxidant enzymes showed a slight tendency to a progressive increase. Both in THE tested and control group of animals, the oxidant-antioxidant status was determined largely by individual peculiarities of each animal.

Keywords: ram, antioxidant enzymes, melatonin

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Introduction

Most studies related to melatonin and pineal gland in the first 40 years after the discovery of the hormone (Lerner et al., 1958) relate to its properties to regulate the reproduction of photoperiod-dependent animals (Reiter, 1973; Reiter, 1980). Recently, however, other effects on the organism have been established. For example, studies have been conducted to investigate its impact on many functions such as controlling various processes, including glucose and lipid metabolism (Cardoso Alonso-Vale et al., 2008, Zhernakova and Rybnikova, 2008) and protection from oxidative stress **(**Reiter et al., 2000 **)** as it acts as universal cleaner oxygen (ROS) and nitrogen (RNS) radicals (Acuna-Castroviejo et al., 2002., Tan et al. 2012)**.** Mechanism of action of melatonin is complex and there are multiple effects since it can pass easily cellular plasma membrane and exert action in all cells of the body (Pandi-Perumal et al. 2006).

Implants containing melatonin CE has been used recently to shift the insemination period in anestrus photosensitive animals. This provides for high plasma concentrations of melatonin for 24 hours every day, without suppressing the secretion of endogenous pineal hormone overnight. The implants contained 18 mg melatonin and are designed to maintain high plasma concentrations of melatonin for at least 60 days, although most of them continue releasing hormone for more than 100 days**.** Releases from the implant maintain daily hormone melatonin plasma concentrations > 100 pg / ml in sheep (Zuniga et al., 2002, Forcada et al., 1995).

Regarding the main sperm characteristics, the application of melatonin result in a very diverse range of data. Under the influence of the hormone, in some cases semen parameters improved (Ortiz et al, 2011), but in other cases no comparable improvement was observed. The reason for this multidirectional effect of melatonin is unclear. In some cases this may be due to breed, individual or other factors. In other cases, melatonin might have had enhanced antioxidant effect.

The aim of this study was to determine the influence of the hormone melatonin on the oxidant-antioxidant status in a brood of *Ovis aries* species.

Material and Methods

The experiments were conducted with 3 groups of 4 rams of Severoiztochnobalgarska fine fleece breed. The groups were selected by age and body condition. First was the control group and the second group was injected with melatonin implants (Melovine "Ceva Sante Animale, France" was used – the content of melatonin was18 mg). These were placed in three implants of animal skin behind the ear. The third group had its diet supplemented with melatonin in the evening (Natrol-Melatonin) at a dose of 5 mg per ram. The experiment started two months before the event campaign. Changes in oxidant-antioxidant status were determined by measuring total antioxidant activity (AOA) in blood samples taken on day 0, 30 and 70, respectively, from the start of the experiment. The principle behind this method is based on the fact that the standardized solution of Fe-EDTA complex reacts with hydrogen peroxide by the Fenton reaction leading to the formation of hydroxyl radicals (OH •). These reactive oxygen species degrade benzoate, leading to the release of TBARS. Antioxidants of blood sample cause suppression of TBARS production. This reaction can be measured spectrophotometrically at 532 nm against adsorption of deionized water. AOA results are

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calculated using the formula:

$$AOA = (C_{UA}) (K - A)/(K - UA)$$

where

K = absorbance of control ($K_1 - K_0$)
A = absorbance of sample ($A_1 - A_0$)
UA = absorbance of uric acid solution ($UA_1 - UA_0$)
C_{UA} = concentration of uric acid (in mmol/l).

and measured in mmol / l   (Kovacevic et al.)

Results and Discussion

In graphs 1,2 and 3, respetively, values of the measured AOA in the blood of the rams of the treated groups and the control group are presented. These data were measured in the blood of animals taken at three different dates - at the beginning of the experiment, in the middle and at the end, respectively, on May 13, June 18 and July 25th.
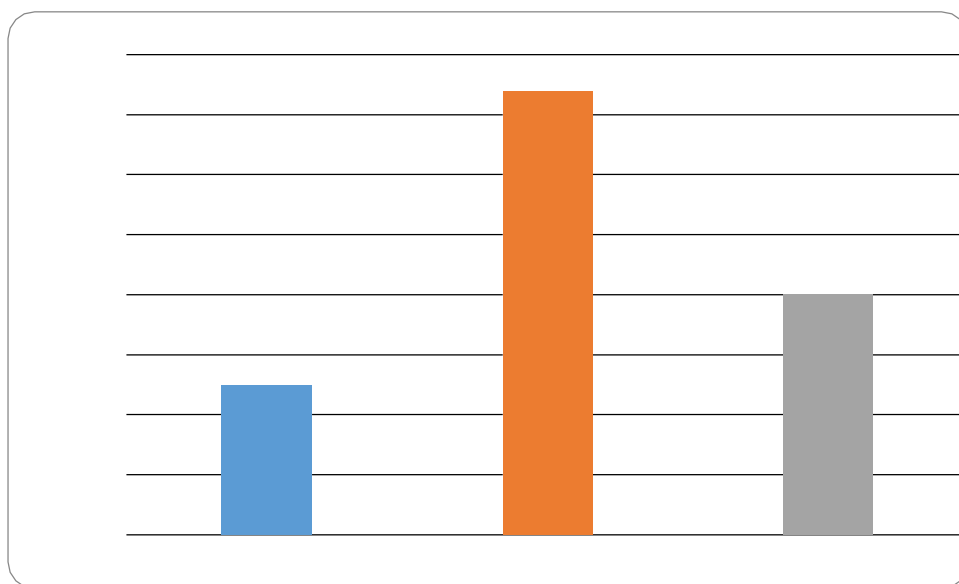
Graph 1. Values of AOA measured in blood from three groups of brood received at the start of the period under scrutiny
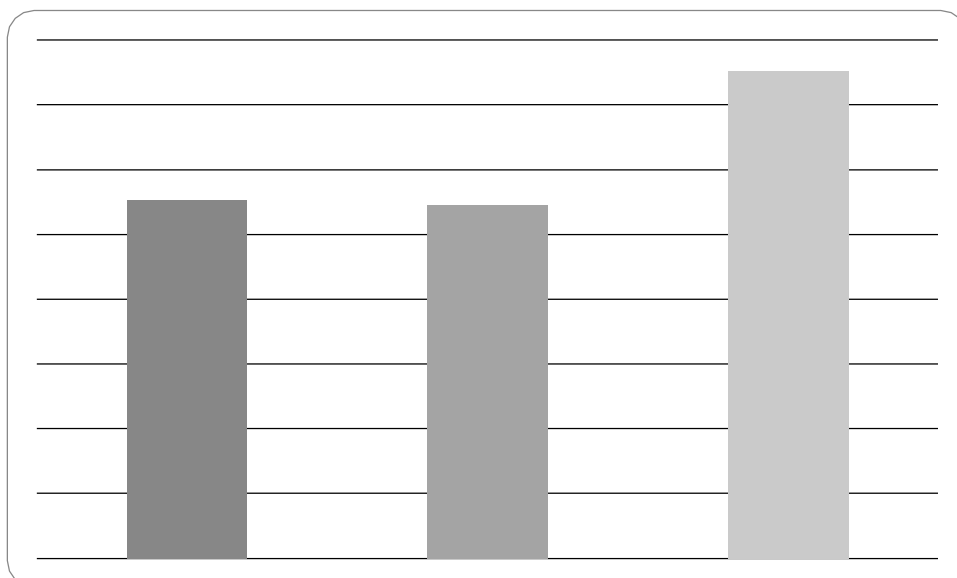
Graph 2. Values of AOA measured in blood from three groups of brood received in the middle of the period under scrutiny
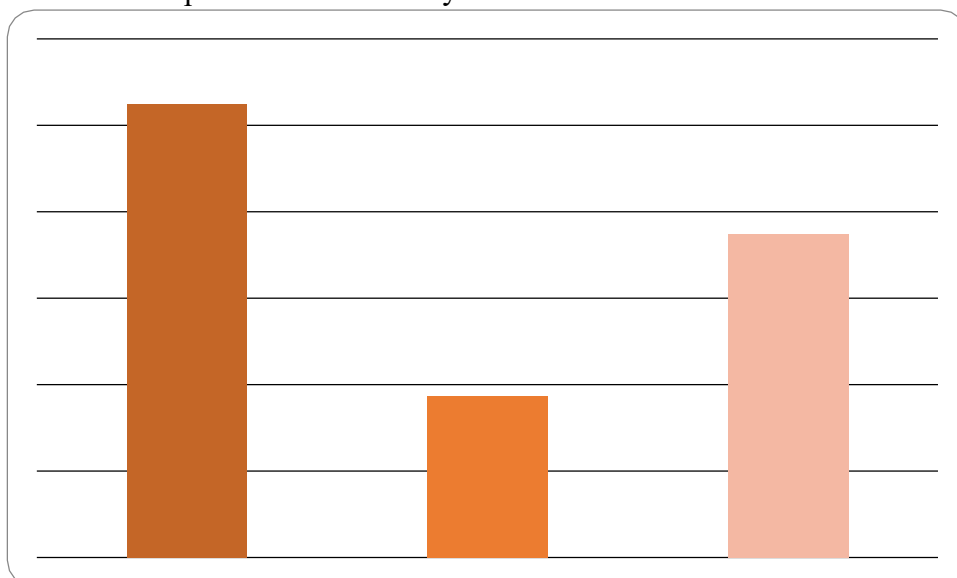
Graph 1. The AOA values measured in the blood of three groups of brood obtained at the end of the period under scrutiny

The figures above showed that the group of experimental animals which were treated with melatonin through the application of melatonin implants had their AOA values increased, albeit not significantly, as the experiment progressed. In other two groups of rams such a correlation was not observed.

After analyzing the results, we did not find a correlation between the total antioxidant activity in the blood of studied rams and melatonin administered *per os*, whereas the rams treated with melatonin implants showed a slight tendency to progressively increase the activity of main antioxidant enzymes. Both in the tested and control group of animals, the oxidant-antioxidant status is determined largely by individual peculiarities of each animal.

The first evidence of the ability of melatonin to dispose of highly toxic hydroxyl radical was published by Tan et al., 1993. Later, Gitto et al., 2001, demonstrated synergistic effect between melatonin and some antioxidants. It was found that *in vitro* medium containing end products of lipid peroxidation such as indices of free radicals, melatonin increased the antioxidant protective action of vitamin E, vitamin C and GSH against these free radicals.

Stimulating effect of melatonin in relation to the main antioxidant enzymes such as superoxide dismutases (SOD), both MnSOD and CuZnSOD, catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRd) and glucose-6-phosphate dehydrogenase (G6PD) (Reiter et al., 2000) was also found.

In order to bring to bear the antioxidant properties in the blood of an organism, however, melatonin is to be set in doses which are to be applied in the form of implants. When administered *per os* its quantity in the blood is insufficient to exhibit antioxidant properties.

Conclusion

In conclusion we may say that for melatonin to have its antioxidant effect, it has to be applied to the animal organism in the form of implants. When melatonin is administered *per os* its antioxidant effect is very weak. At the same time, we should keep in mind the individual characteristics of each organism. The activity of antioxidant enzymes in the blood of brood of *Ovis aries* species depends mainly on the individual peculiarities of the animals and slightly less so in case of treatment with melatonin implants.

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Poster presentation

DESCRIPTION OF PRODUCTION SYSTEM AND MORPHOLOGICAL CHARACTERISTICS OF FOUR LOCAL CATTLE POPULATIONS OF ALBANIA AND KOSOVO

Rexhaj N.[1], Papa L.[1], Kume K.[1]

Abstract

The Busha Cattle has existed in the Balkans since Neolithic times. It is adapted to harsh environmental conditions and spread out with numerous strains, but in small fragmented relict populations throughout the Balkans. Subpopulations of Busha type cattle that spread out in most of Balkans countries are the result of borders divisions only. Actually these trans-boundary breeds are in high danger of extinction. Two types of local cattle are found in Albania and Kosovo: Illyrian Dwarf Cattle and Busha Cattle, both of brachycerous type but different in body conformation, live weight, head size, horn shape, coat color and milk production. The local breeds of cattle in Albania and Kosovo are still present in remote areas. They have not been crossed with exotic breeds because of geographic isolation and harsh conditions. Conservation of this genetic diversity is challenging because these animal populations are small and rapidly declining mainly because of migration. Due to lack of information and data, it is difficult to run sustainable conservation program. Consequently, to compile a sustainable breeding strategy, for implementation of a national or cross border (Albania and Kosovo) conservation program, the identification, phenotypic characterization, monitoring and evidencing the value of these populations for present-time and future farming constitute the first action for their conservation. From the surveys carried until now the Busha type populations in Albania and Kosovo are kept in small-scale family farms. They are managed in natural conditions, with extensive grazing. They are distinguished for their maternal traits and easy calving. Four Busha type populations, Back Rjoll (Velipoje) and Kukes both in Albania and two others from highlands of Gjakova and Sharri of Kosovo were monitored for rearing system, productive performances and morphological characteristics according to Food and Agriculture Organization's Guidelines (2012). Populations of two countries are similar especially Kukes herd to that of Gjakova and Sharri herds in terms of body size, frontal width 19.6 cm, ears and horns length respectively 17.62 and 20.10 cm, pelvic width. Back Rjoll herd is smaller in body size and comparable in other measured traits. Milk yield is lower in Albanian populations 700-1400 kg than in Kosovo populations 1800-2300 kg. Discriminate analysis was used to elaborate morphometric measurements of 81 adult animals. Two distinguished groups are formed: in the first one the cattle population of Back Rjoll and Gjakova highland; in the second group the population of Kukesi and Sharri highland. Referring to the geographical position to which these populations belong, we note that there are similarities between the distances of cattle populations estimated using morphometric indicators and geographical distance between regions. Consequently, the hypothesis is it may rise in the case of Busha cows, the process of evolution of the morphometric traits and selection have been associated with the presence of isolation in distance, which as a rule is main conditions that affect the establishing of isolated genetic niches.

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Introduction

The strategy for rural development of mountain region will be based mainly on traditional systems in Albania and Kosovo. The local breeds of animals are the most suitable for these systems and constitute a strong supporting factor for the production of local food and the development of agrotourism in these areas. Local breeds have remarkable special characteristics like resistance to prevailing diseases, fertility, maternal ability, longevity, adaptation to the environment and unique attributes of their final products (Garcia, 1980). Restriction of the livestock industry to a few specialized breeds has happened in both countries because of increasing demands for livestock products and farming of more profitable breeds. This practice has reduced the use of local breeds and put their survival in danger (Oldenbroek, 1999). Based on the information gathered up to now the local breeds of cattle in Albania and Kosovo are still present in remote areas . They have survived until now because of geographic isolation and harsh conditions that are not suitable for exotic breeds. But the number of these populations is rapidly declining putting them in danger of extinction. One of the main factors of this process is the intensity of migration of people from rural areas to urban regions. (Kume and Papa. 2013). To overcome this problem, the Food and Agriculture Organization (2007) recommended establishing conservation programs for the maintenance and sustainable development of animal genetic resources focusing on the many adaptive breeds that survive well in the low external input agriculture typical of developing countries. . The first actions for sustainable conservation are the identification, phenotypic characterization, monitoring and evidencing the value of these cattle populations for current and future economic value and socio-cultural importance.

 Local cattle of Busha type in Albania and Kosovo have been breed for centuries in this areas (Illyrian cattle) and according to craniological and genetic characteristics belongs to short horn brachyceros type. Different authors have described this native cattle as an animal of small body sized, big head, broad forehead, long face big and bulging eyes, medium size ears, short horns and thick in basal part, front and above inward bent and some times back. (Kume and Papa. 2013). According to Samimi V. 1950, cattle of Brachycerous type is shown different not only for body size but also as to economic values. Because of geographic isolation local cattle of Busha type in both countries has not been subject of crossbreeding with exotic breeds and during the last 100 years, the average values of different morpho-biometric traits have not undergone essential changes.. For both countries the conservation of this genetic diversity is challenging, because these animals are in small rapidly declining subpopulations. Nerveless the farmers of mountain regions still like this cow for its suitability and limited requirements for food and veterinary drugs. Description of morphological traits and their rearing system are necessary for compiling a sustainable breeding strategy that will help to run a national or crossborder conservation program.
Material and Methods

Description of the study area

The study areas were: The local cattle populations of Back Rjoll village in Velipoja commune of Scutari region and Bardhok and Morine villages of Kukes region in Albania . In Kosovo two local cattle population located in Gjilan, (Sharri highland) and Gjakova region. The sampled areas in Albania were well defined in north west and north east of the country; in Kosovo the sampled area were in west and south east of the country.

Data collection

The study was conducted from May 2015 to April 2016. The number of cattle's sampled for phenotypic characterization was 81of which 73 were females and 8 were males. Regions-wise 14, 33, 24, 10 cattle's were sampled from Back Rrjoll, Kukes, Gjakove, Sharri Highland.

A questionnaire was compiled to gather data to describe the system of rearing of local cattle in remount areas of Albania and Kosovo. The questioner was compiled according to FAO guidelines for Phenotypic Characterization of Animal Genetic Resources (2012).

Linear body measurement including body length, heart girth, pelvic width, ear length, horn length, frontal width, were taken using measuring tape while wither height was measured using a 1.5 m ruler. The measures were done in adult animals only. The linear body measurement were done according to FAO guidelines 2012.

Data analyses

The means of morphometric variables were done for animals not divided by sex. Quantitative traits for body measurements were used to calculate correlation coefficient of Pirsons (r). Discriminate analyses model was used to evaluate the level of local differentiation of cattle populations.

Results and Discussion

Figure 1. Sampling areas in Kosovo and Albania

Characterization of quantitative traits
The average values and standard deviations of body measurements for both sexes in different regions of Albania and Kosovo are presented in Table 1.

Table 1. Average values of morphometric traits

Population	Back Rjoll	Kukes	Gjakova	Sharri Highland.
Number of animals	14	33	24	10
Traits	Average ±SD			
Wither height	104.4 ± 2.76^a	114.5 ± 1.12^{bc}	110.2 ± 4.19^c	116.0 ± 2.95^b
Heart girth	143.3 ± 6.78^a	157.5 ± 4.59^b	157.72 ± 9.37^b	162.7 ± 4.42^c
Body length	113.4 ± 3.95^a	112.55 ± 1.74^a	115.2 ± 2.03^a	121.4 ± 5.9^b
Frontal width	19.4 ± 1.01^a	19.85 ± 0.64^a	19.3 ± 0.99^a	19.5 ± 1.06^a
Horn length	19.9 ± 1.44^{ab}	20.75 ± 2.32^b	19.9 ± 0.94^{ab}	18.5 ± 2.82^a
Ears length	16.4 ± 0.94^a	18.0 ± 0.55^b	17.2 ± 0.89^{ab}	18.0 ± 0.75^b
Pelvic width	38.3 ± 1.98^a	40.5 ± 0.93^b	38.4 ± 1.38^a	40.7 ± 1.48^b

The Albanian population of Back Rjoll has smaller body size in comparison to other herds included in the study. Small differences are observed for other measured traits between

herds. The body size of Sharri population seems to be slightly higher specially in body length and heart girth.

The description of qualitative and phenotypic traits of monitored populations are given respectively in tables 2, 3 and 4.

Table 2. Qualitative traits

Population	Coat Color	Head	Horns
Backrrjoll - Albania	Reddish	Very developed orbit of eyes	Short, thin and tip and back bent horns
Kukes - Albania	Reddish, brown or brown opened to grey.	Very developed orbit of eyes	Short, thin and tip and back bent horns
Malesia Gjakoves - Kosovo	intensive red, sometimes dark red	Small, narrow, mug is dark colored with some white or black hair around	lyre shaped horns
Malesia e Sharrit - Kosovo	intensive red, sometimes yellowish, dark red, tiger	Small, narrow, mug is dark colored with some white hair around	lyre shaped horns

Table 3. Phenotypic traits of Albanian local cattle populations

Phenotypic traits/regions	Back Rjoll		Kukes	
Sex of animal	Male	Female	Male	Female
Birth weight	22 kg	18 kg	18kg	13 kg
Body weight – adult animal	230-260 kg	110-135 kg	200-250 kg	105-115 kg
Milk production	First Lactation	900-1000 kg	First Lactation 700-800 kg	
	Third Lactation	1000-1400 kg	Third Lactation 1000-1200 kg	
Average length of lactation	280 -300 days		280 -300 days	
Age at first calving	800 – 850 days		800 – 850 days	
Weight at first calving	105-115 kg		105-115 kg	
% of fertility	85-90		80-85	

Table 4. Phenotypic traits of Kosovo local cattle populations

Phenotypic traits/regions	Sharri highland		Gjakova highland	
Sex of animal	Male	Female	Male	Female
Birth weight	22 kg	16 kg	24g	17 kg
Body weight – adult animal	280-320 kg	145-180 kg	300-340 kg	155-180 kg
Milk production	First Lactation kg	1000-1200	First Lactation 900-1200 kg Third Lactation 1800-2300 kg	
	Third Lactation kg	1500-1700		
Average length of lactation	240-280 days		240-280 days	
Age at first calving	600 – 700 days		600-700 days	
Weight at first calving	105-115 kg		115-120 kg	
% of fertility	80-85		85-90	

Milk production is low in both countries with slightly higher level for animals farmed in Kosovo. Similar results are reported by Bytyqi and Mehmeti (2013).

Production system
Describing the production environment may also be important as a means of identifying potential development opportunities. Both populations of Albania and Kosovo are reared in extensive system. Cows are managed under the natural condition, almost in wild state. During all the day they are grazing. Animals have developed the reflection to coming back, staying in sheltering place (shack), roughly build shed, during all the night. Back Rjoll cows are only kept to produce suckling calves. Cows are not milked and calves suck milk of their mothers during all the lactation. The bull is kept together with cows in herd during all the time. The calves are sold for meat at age of 16-18 months old and average live weight is 180-220 kg (m) and 150-200(f). The population of Kukes is reared for milk and meat productions processed in farm for family consumption. The Kosovo populations are higher milk productive and are kept mainly for this purpose.

The main features of this local cattle are the excellent maternal traits, easy calving and very adapted to harsh conditions. The data about their system of rearing and farming purposes is given in table 5.

Table 5. System of rearing and farming purposes

Population	Main use	Reared as	Housing	Pasture	Feed	Feed during grazing	Place of slaughter
Backrrjoll - Albania	Milk Meat	Dairy	Loose	✓ ✓	Forage	Only grazing	On farm
Kukes - Albania	Meat Milk	Dairy, suckler	Loose	✓	Forage	Only grazing	On farm
Malesia Gjakoves - Kosovo	Milk	Dairy, suckler	Loose	✓	Forage +	Only grazing	On farm
Malesia e Sharrit - Kosovo	Meat Milk	Dairy suckler	Loose	✓	Forage +	Only grazing	On farm

Morphometric measures were used to evaluate the distribution of animals local cattle population of four regions. The results of discriminate analyses are presented in figure 2.

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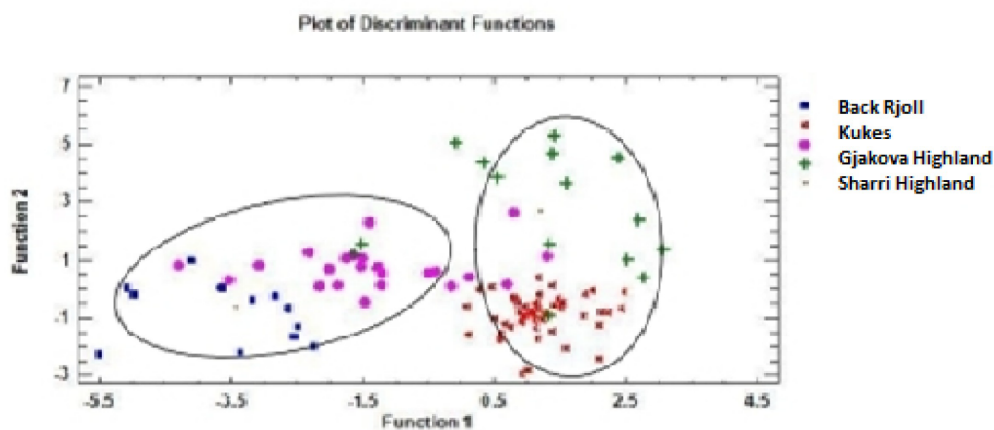
Figure 2. The distribution of local cattle populations of Busha type.

The distribution of individuals of four local cattle populations of Busha type in the plain of two first discriminate functions shows that two groups are formed: Back Rjoll herd of Albania and Gjakova herd of Kosovo one group and the other one where are grouped the herds of Kukes and highland of Sharri. Referring to the geographical position to which these populations belong, we note that there are similarities between the distances of cattle populations estimated using morphometric indicators and geographical distance

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between regions. Consequently the hypothesis it may rise is that in the case of local cattle of Busha type farmed in north of Albania and south of Kosovo, the process of evolution of the morphometric traits and selection have been associated with the presence of isolation in distance. This result needs further verification. More animals must be included in the study to have more reliable results.

Conclusion

The obtained results are preliminary because the populations number and their size are limited. The data of monitored herds shows that different types of Busha cattle have been developed historically. There are modifications in coat color or in size and production. The main purposes of farming are for milk and meat and vice versa according to the region. The milk yield is relatively low, largely because of harsh conditions of management. The animals are reared in typical extensive system and their feeding is based on grazing all round the year. The high level of fertility of all herds is an indication of how well the local cattle of Busha type is fitted to the local environment that promises for continuous farming. The results of discriminate analysis shows that in the case of local cattle of Busha type farmed in north of Albania and south of Kosovo, the process of evolution of the morphometric traits and selection have been associated with the presence of isolation in distance. This result needs further verifications. t its further far and an pro shows that this feature

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Poster presentation

COMPARISON OF GROWTH INDICATORS OF LAYERS' REPLACERS OF THREE VARIETIES OF HYBRID HY-LINE KEPT IN CONVENCIONAL CAGES

Mavromati E.[1], Sena L.[2]

Abstract

The Layers' hybrid Hy-Line is currently being used at many poultry farms in Albania with very good indicators of performance during their growth as well as during the lying period. Although it has been years since this hybrid was introduced in our country, there are no comprehensive studies on the indicators of growth until the start of egg production.

For this reason, the growth parameters of three varieties of Layers' hybrid Hy-Line: Hy Line W98 (V1), and Brown (V2) are studied and compared at a farm in Durrës/Albania, as well as the CV-22 (V3) for the 0-10 week period. The chicks were kept in the same house with conventional cages. Feed formulas applied in the feeding of the three varieties, were the same. The experimental period was split into two phases, respectively: 0-6 weeks and 6-10 weeks. Body weight, weight gain, feed consumption and FCR were monitored, while the performance index was calculated. The results were processed statistically (descriptive analysis method and ANOVA) and for the comparisons the tTest was used.

The results at the end of the study demonstrated that during this period the body weight of the chicken of hybrid Hy-line W98 and Hy-Line CV-22 varieties had a slight advantage over breed's standard, while the chicks of Hy Line Brown did not meet the standard for 80 gr. While the Hy-line W98 and Hy-Line CV-22 chicks gained respectively 42.7 gr and 27.6 gr more than the standard, the Hy Line Brown ones resulted to be 78.4g less/under the standard. The V1 chicks consumed 4.7% less feed per unit of weight, while the V2 ones consumed 9.5% more feed.

As a conclusion to be inferred from the results of our study, the Hy-line W98 variety demonstrated a better performance than the standard, also when compared to the two other varieties.

Keywords: hybrid, variety, standard, replacers, performance

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Introduction

The Layers' hybrid Hy-Line is currently being used at many poultry farms in Albania. It is adaptable to intensive growth and has a very high vitality. It stands for very good indicators of performance during their growth period, as well as during the laying period. The current practice of many of the country's poultry farms, the chicks keep from the age of 1 day, usually in battery cages.

So far in commercial herds, producing eggs for Hy-Line hybrid has given priority to the study of performance indicators during laying period and the records and the results were compared with the standard of the hybrid. Although it has been years that this hybrid was introduced in our country, there are no comprehensive studies on indicators of growth until the start of the production. The indicators of performance during the period of growth such as the intensity of the growth, uniformity, the vitality the feed consumption rate, depends on a number of factors internal and external (breeding and feeding).

Genetic potential of Hy-Line 98, CV22 and Brown can be achieved by using successful breeding practices and management (Commercial management guide Hy-line Brown, 2011). Birds of this hybrid can be adapted as a holding ground, as well as in the cages in battery.

This study aimed to to monitor and evaluate the performance of the birds in the first 10 weeks of growth of the corresponding three varieties: Hy-Line W98, CV22 and Hy-Line Brown, the comparison between them and the relevant standards.

Material and Methods

Indicators of growth were monitored and studied in three flocks of birds of Hy-Line hybrid, belonging to Hy Line 98 varieties, Hy-Line Brown and CV-22 for the period of 0-10 weeks. During the growth period the birds were kept in battery cages. The birds were in the same housing with conventional battery cages. Birds of every variety (which marked the V1, V2 and V3) were placed in a row, each battery to 10 thousand within the same house leaders with the same conditions of breeding and feeding. Initially (in the first week) birds were placed on the first three floors of batteries. In the second week they were divided into the four floors and starting from the third week of growth until the end of the five floors battery was occupied by birds.

The indicators that were numbered:

- The number and weight of the birds put on one-day rise for each batch.

- Dynamics of growth of birds from the age of 1 day up to 10 weeks of age. Recording to the performance of live weight was achieved through individual weighting that took place once a week at the same hour, before eating the feed to determine the number of leaders of the flock. Every week were weighed 180 heads of chicken randomly for each battery (monitoring equal number of heads on each floor). Weights obtained for each variety of hybrid faced with those standards, according to the respective guides.

- The amount of feed consumed throughout the period has been the standard norms of each hybrid variety. Food rations used were the same for each of the hybrid variety. It's applied feeding phase: 0-6 weeks and 6-10 weeks.

Table 1. Components of feed rations by phases of growth for the three varieties of hybrid.

Age in weeks	Crude Protein (%)	Metabolized Energy (Kcal/kg)	Ca (%)	Phosphorus (%)	Lysine (%)	Methionine+ Cystine (%)
0-6 weeks	20	2900	1.05	0.76	1.10	0.82
6-10 weeks	18	2880	1.00	0.71	0.91	0.74

- The Feed Conversion Rate per 1 kg weight gain (FCR) .
- The Performance Index (M.S. Jahan , M. Asaduzzaman and A.K. Sarkar , 2006) was calculated as follows :

$$IP = \frac{\text{The live weight (kg) X 100}}{FCR}$$

In which:
IP=Performance Index
FCR= Feed Conversion Rate

Data processing: The results were processed statistically (descriptive analysis method and ANOVA) while the tTest was applied for the comparisons.

Results and Discussion

1) Body weight

The table below presents the body weights for the three varieties and has become the standard of comparison with the corresponding hybrid variety.

Table 2. Live weight of birds (g) according to the weeks for the three variety and the comparison with the standard

Weeks	Hy- line W 98 (V1)		Hy-Line Brown (V2)		Hy-Line CV-22 (V3)	
	M±SD	Standard	M±SD	Standard	M±SD	Standard
1	70.10±3.47	65	68.41±3.93	70	69.77±3.19	65
2	127.00±6.27	110	133.04±10.14	120	128.26±5.89	110
3	201.18±14.04	180	199.53±15.18	200	205.13±16.34	180
4	288.14±16.19	260	278.81±18.26	280	289.24±18.46	260
5	383.21±33.41	350	374.49±28.61	390	363.93±23.24	350
6	493.20±23.48	450	491.80±39.30	500	414.00±23.24	450
7	589.28±27.85	550	600.20±54.49	620	582.60±33.59	550
8	672.40±38.01	650	738.40±62.06	750	690.20±42.50	650
9	784.4±49.20	750	829±60.25	860	781.20±40.76	750
10	897.80±49.88	850	890±58.97	970	882.40±41.05	850

For hybrid Hy -line W98 (V1) and Hy - Line CV - 22 (V3) had a satisfactory performance weight, even up to the 10th week of bird weight is higher than the standard of the hybrid (Commercial Management Guide hy-line-98, 2008-2010 & Performance Standards Manual Hy line CV-22, 2012) .

Regarding weights for hybrid Hy - Line Brown for 10 weeks of monitoring, there is a lag in comparison with standard of the hybrid (Commercial Management Guide Hy-line Brown 2011) . However, the differences with the standard hybrid in the following weeks, are small, except of week 10 (80 gr. less) .

In the first week not verified differences between V1 and V3 (they have similar values). Noted that in the fourth week there is a lag in V2, fifth week although V3 is inferior to the two other groups, there is still over the standard hybrid value. The differences are statistically significant (P≤0.05) between the three groups (tCrit = 1.65). The same trend is observed in two successive weeks. By week 7, 8 and 9 says an advantage of V2, however as the variety has a lower value than the standard.

Weight gain

Based on the weights obtained for each week in each batch can calculate average weight gain and make a comparison with average weekly additions of standard weight gain corresponding to the hybrid.

Table 3. Weekly body weight gain (g)

Weeks	Hy- Line 98		Hy-Line Brown		Hy-Line CV-22	
	Weight Gain The study group	Weight gain of standard	Weight Gain The study group	Weight gain of standard	Weight Gain The study group	Weight gain of standard
2	56.90	45	64.63	50	58.49	55
3	74.18	70	66.49	80	76.87	70
4	86.96	80	79.28	80	84.11	80
5	95.07	90	95.68	110	74.69	90
6	110.00	100	117.31	110	117.47	100
7	96.08	100	108.4	120	101.2	100
8	83.12	100	138.2	130	107.6	100
9	112	100	90.6	110	91	100
10	113.4	100	61	110	101.2	100

From the table above it is clear that the two varieties W98 and CV22 have almost similar values for the first 10 weeks of the study. Brown variety has higher value than the standard, only in the second, six and eight week of their grown.

In the period of 1-10 weeks, was realized a weight gain of 827.7g (standard 785g), or 42.7g (5.44%) more than the standard for Hy-Line variety W98(V1). Hy-Line variety Brown (V2) was conducted an weight gain of 821.59g (standard 900g), or 78.41g (8.71%) less than the standard. For variety Hy Line CV-22 (V3) was held an weight gain of 812.63g (standard 785g), or 27.63g (3.52%) more than the standard.

So, in summary we can say that the birds of the V1 and V3 are conducted weight gain slightly higher than the standard, while the opposite occurs with V2, which should be characterized by a better weight gain. So, in the latter are not fully exploited the possibilities. During growth period, V1 performed a body weight gain of 15.07g. higher or 1.85% more than V3.

Feed conversion rate for 1 kg weight gain

For the three herds we have calculated the feed conversion rate. During the study they used two different feeds: 0-6 and 6-10 weeks .
The table 4 presents the feed conversion rate for the three varieties of this hybrid in comparison with standards

We analyze this indicator for Hy-line hybrid W98 (V1) as compared with the standard. During the period of growth in most of the time, the feed conversion rate is better in study birds by saving 60g feed per unit weight, or 4.7 %.

Table 4. Feed conversion rate for the three varieties

Weeks	Hy –Line W 98 (V1)		Hy-Line Brown (V2)		Hy-Line Cv-22 (V3)	
	Study group	Standard	Study group	Standard	Study group	Standard
2	2.09	2.64	1.84	2.38	2.03	2.16
3	1.98	2.10	2.21	1.84	1.91	2.10
4	2.33	2.54	2.56	2.54	2.41	2.54
5	2.87	3.03	2.85	2.48	3.66	3.03
6	2.74	3.01	2.57	2.74	2.56	3.01
7	3.35	3.22	2.97	2.68	3.18	3.22
8	4.13	3.43	2.48	2.64	3.19	3.43
9	3.25	3.64	4.02	3.31	4.00	3.64
10	3.33	3.78	6.20	3.44	3.74	3.78
Average	3.08	3.24	3.10	2.83	3.25	3.24

For the second flock-V2, for the period of 1-10 weeks is used 9.54 % more feed that the standard for the unit of live weight. This is because on the one hand is used more feed for bird (270g) and on the other there were lower weight gain than the norm. Third flock-V3, the difference with the standard is negligible for this indication .

Index of performance

The Index of performance is closely related to two indicators - live weight and FCR. So, as much higher to be the live weight and as lower as the feed conversion rate to be, the greater will be the value of the performance index. This indicator is efficient for the first weeks of life where the intensity of growth is higher. Then the growth rates are slower and the feed conversion rate will be worsening.

Table 5. The Index of Performance (%) for the three varieties per week

Weeks	Hy –Line W 98 (V1)		Hy-Line Brown (V2)		Hy-Line Cv-22 (V3)	
	The study group	The Standard	The study group	The Standard	The study group	The Standard
1						
2	6.08	4.17	7.23	5.04	6.32	5.09
3	10.16	8.57	9.03	6.52	10.74	8.57
4	12.37	10.24	10.89	11.02	12.00	10.24
5	13.35	11.55	13.14	15.73	9.94	11.55
6	18.00	14.95	19.14	18.25	18.80	14.95
7	17.59	17.08	20.21	23.13	18.32	17.08
8	16.28	18.95	29.77	28.41	21.64	18.95
9	24.14	20.60	20.62	25.98	19.53	20.60
10	26.96	22.49	14.35	28.20	23.59	22.49
Average	16.10±6.57	14.29±6.08	16.04±7.08	18.03±9.06	15.65±6.00	14.39±5.90

In the V1 flock in 10 weeks of grow shows a performance index higher than the standard rate (with the exception of the eighth week). Average again turns as a higher value (1.81%). V2 flock, there is a lower performance than the standard (1.99%). The third flock - V3, consistently over 10 weeks there was a better performance than the standard, except for weeks 5 and 9 (with minor differences). Even as the average for this period there was a performance index 1.26% higher than the standard.

If we make a comparison between the three varieties we see that V1 has a slight superiority over the two other flocks (0.06% more than V2 and of 0.45% more than V3). But the differences are statistically unproven between three flocks for $P \leq 0.5$.

Conclusion

The Birds of Hy-line variety W98 carried weight and weight gain, higher than the standard and when compared to two other varieties. They used more efficiently the feed by saving 160g feed meals per unit weight and therefore expressed a better performance of the standard and the tendency for better performance than the other two varieties.

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Oral presentation

INFLUENCE OF GOSSYPOL ON ELECTROPHORETIC MOBILITY OF MILK PROTEINS IN DAIRY COWS EXPOSED TO COTTONSEED DIET

Popovski Z.T.[1], Eastridge M.[2], Gjorgievski S.[3], Nestorovski T.[1], Svetozarević M.[4], Wick M.[2]

Abstract

Gossypol is a polyphenolic compound present in cottonseed. It appears in both free and bound forms. In the intact whole seed, gossypol is mostly found as free gossypol. Whole cottonseed is a popular feed for dairy producers. Gossypol is harmful for young ruminants, but mature ones are not affected. When cottonseed is processed, gossypol binds to proteins, possibly to the epsilon-amino group of lysine. The association between gossypol intake and lactation performance in dairy cows is an important issue for dairy producers. The aim of the study was to analyze the influence of gossypol on electrophoretic mobility of milk proteins in dairy cows fed with cottonseed. The treatment diet of ten Jersey cows was performed in 24 days with three subsequent analyses on each of 12 days. The haplotypes of each cow was determined regarding the polymorphism of the following 5 genes encoding milk proteins: α-casein, β-casein, κ-casein, α-lactalbumin and β-lactoglobulin. In ten individuals we found eight different haplotypes for milk proteins. SDS-PAGE and urea PAGE were used as comparative electrophoretic techniques. In SDS-PAGE the milk fractions were divided based on molecular size, while in urea PAGE the milk fractions were divided mostly on the electrical charge. No differences in the mobility features of milk proteins, based on molecular weight or electrical charge, appeared to be influenced by gossypol, suggesting that the association of gossypol with proteins is neither covalent nor strong enough to resist ionic disruptions from the buffer conditions used.

Keywords: gossypol, milk proteins, electrophoresis, mobility

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Introduction

Gossypol is phenolic aldehyde derived from the cottonseed plant (genus *Gossypium*, family *Malvaceae*) and acts as an inhibitor for several dehydrogenase enzymes. It is a yellow pigment that appears in two forms: free and bound. The free form is toxic, whereas gossypol that binds to proteins is in the "bound" or non-toxic form (1).

Whole cottonseed is a popular feed for dairy producers (2). Food and animal agricultural industries must manage cotton-derivative product levels to avoid toxicity. For example, only ruminant microflora can digest gossypol, but only to a certain level, and cottonseed oil must be refined (3). Non-ruminant animals such as pigs have long been known to be susceptible to gossypol toxicity. Ruminants such as cattle and sheep can tolerate higher levels of free gossypol because gossypol binds to proteins in the rumen. Young calves and lambs are quite susceptible to gossypol toxicity (1). A research team at Texas A&M University, has genetically engineered cotton plants that contain very little gossypol in the seed, but still contain the compound in the stems and leaves. This provides protection against pests and diseases, while allowing the seed to be used for oil and meal for human consumption. The plants are modified by RNA interference, shutting down the genes for gossypol production in the seed, while leaving them unaffected in the rest of the plant. The resulting gossypol-free cottonseed is then suitable as a high-quality protein source suitable for consumption not only by cattle, but also by humans, pigs, chickens, or turkeys, making the plant additionally valuable as a food crop. Protein makes up 23% of the cottonseed (4). In 1975, while working with a 700-cow dairy herd in Alabama, gossypol toxicity developed when large amounts of cottonseed meal were fed as the single source of protein to achieve high levels of milk production. Approximately 25 head of mature cows exhibited classical gossypol toxicity symptoms and died. These symptoms are depression, loss of appetite, anorexia and red blood cell fragility (1).

The association between gossypol intake and lactation performance in dairy cows is an important issue for dairy producers. Mena et al. (4) reported that cows consuming more FG in the diet had higher concentrations of PG and produced more milk. They also observed no negative effect of increasing dietary gossypol by adding cracked Pima cottonseed to the diet on yields of milk and milk components (5). The problem with gossypol is the toxic effect that seems to be cumulative. The longer the cows are on a ration, that contains gossypol, the more likely they are to have toxicity problems (1).

Milk proteins are divided into two fractions: milk serum and caseins (5). The major protein components of milk serum are ɑ-lactalbumin (ɑ-La) and β-lactoglobulin (β-Lg) (6). In the casein fraction, five proteins have been intensively studied: a_{S1}-casein (a_{S1}-Cn), a_{S2}-casein (a_{S2}-Cn), β-casein (β-Cn), g-casein (g-Cn) and k-casein (k-Cn). Electrophoretic analyses of milk proteins have demonstrated the existence of different variants for these proteins, all of them determined by codominant alleles of the closely linked autosomal genes.

Currently, there is no data related to gossypol influence on milk protein fractions and their electrophoretic mobility. Therefore, the aim of this study was to analyze the possible effect of gossypol on the mobility of milk proteins in milk samples from the dairy cows exposed to cottonseed diet via electrophoretic profiling during the diet indicating the covalent binding of gossypol to these proteins.

Material and Methods

Diets and experimental design

Ten mid-lactation Jersey cows from The Ohio State University Waterman Dairy Farm were selected for a 12-week trial (Animal Use Protocol 2011A00000120). Three diets were fed in this experiment, a control diet with no cottonseed product, a diet containing whole linted cottonseed (WLCS), and a diet containing cracked Pima cottonseed (CrP). Cows were randomly assigned to one of the three diets and fed in a Switchback experimental design. The control diet consisted of 50% (DM basis) grain and no cottonseed product; the CrP diet consisted of 35% (DM basis) grain and 15% (DM basis) CrP; and the WLCS diet consisted of 35% (DM basis) grain and 15% (DM basis) WLCS. All three diets were composed of 15% [dry matter (DM) basis] alfalfa balage and 35% (DM basis) corn silage, and fed ad libitum with < 5% refusals. Ingredient composition for all three diets is listed in Table 1 and chemical composition provided in Table 2.

Table 1. Ingredient composition (% of DM) of diets.

Item	Control	WLCS[1]	CrP[1]
Legume balage	15.0	15.0	15.0
Corn silage	35.0	35.0	35.0
Dry shelled corn	16.7	23.8	23.8
Soybeans, roasted	14.3	-----	-----
Soybean meal, 48% CP	-----	4.76	4.76
Amino Plus[2]	1.91	4.41	4.41
Cottonseed	-----	15.0	15.0
Wheat middlings	15.2	-----	-----
Feed grade limestone	0.95	0.95	0.95
Magnesium oxide	0.07	0.09	0.09
Potassium sulfate	0.19	0.24	0.24
TM salt[3]	0.50	0.50	0.50
Copper sulfate	0.01	0.01	0.01
Selenium 90[4]	0.12	0.12	0.12
Vitamin A premix	0.02	0.02	0.02
Vitamin D premix	0.04	0.04	0.04
Vitamin E premix	0.06	0.06	0.06

[1]WLCS = whole linted cottonseed and CrP = cracked Pima cottonseed
[2]AMINOPLUS®, Ag Processing Inc.©, Omaha, NE
[3]Trace Mineral Salt, Morton Salt, Chicago, IL
[4]Selenium 90, Vita-Vet Laboratories, Converse, IN

Table 2. Chemical composition of diets.

	Control	WLCS[1]	CrP[1]
DM, %	59.1	58.6	59.0
----------------------------DM basis----------------------------			
CP, %	15.1	15.1	15.4
NDF, %	31.0	29.1	27.0
Ash, %	7.24	7.12	7.07
K, %	1.26	1.24	1.20
P, %	0.41	0.32	0.33
Ca, %	0.77	0.85	0.79
Mg, %	0.28	0.27	0.27

[1]WLCS = whole linted cottonseed and CrP = Cracked Pima Cottonseed

Cows were housed in a tie stall barn with separated feed bunks, and feed intake was measured daily. The corn silage and balage DM was determined weekly and the as-fed total mixed ration (TMR) was adjusted accordingly. The trial lasted for three periods of four weeks each.

In this study SDS-PAGE and urea-PAGE were used to determine the influence of gossypol on the electrophoretic mobility of milk fractions. SDS-PAGE was performed Ten microliters of total fresh milk (~ 300 μg of milk
reducing buffer (8 M urea/2 M thiourea, 75 mM DTT, 50 mM Tris, 3% SDS, and 0.004% brome phenol blue, pH 6.8) were loaded on the gel. SDS-PAGE was performed according to Laemmli method (7) modified by Svetozarevic et al. (6). Briefly, samples were loaded onto a 14 cm × 12cm × 1 mm polyacrylamide running gel consisting of a 12.5% resolving gel [30:0.8, acrylamide/(N,N′-methylene bis-acrylamide)] and a 3% stacking gel containing 1% SDS. Electrophoretic separation was carried out at a constant voltage of 10 V/cm-[1]. Gels were stained with Coomassie Brilliant Blue G-250 and distained with 10% acetic acid. urea-PAGE was done using the procedure modified by Svetozarevic (8). Milk proteins were analyzed on gel with the dimensions of 16 cm x 12 cm x 1 mm cm using 10% acrylamide in 6 M urea. Each milk sample was mixed with a buffer composed of 20% sucrose, 10% 2-mercaptoethanol, 10% acetic acid and 0.01 bromophenol blue. Protein concentrations were adjusted to 2 µg/µl and 20 µl of samples were loaded on the gel. During the electrophoresis the electric power of 300 Volts was used for 5 hours. After the electrophoresis, the proteins were stained with 0.04% Coomassie Brilliant Blue R-250 in 40% ethanol and 5% acetic acid, and then distained in 10% acetic acid.

Results and Discussion

Whole cottonseed provides a unique blend of energy, protein and fiber in the diet of dairy cattle. It is usually one of the most competitive sources of fat and protein to dairy cattle and commonly used in lactating rations. The effect of gossypol on the lactation performance, health, reproduction and its concentration in plasma of dairy cows was a target of some previous studies (9), but there is no data about its influence to electrophoretic mobility of milk fractions.

The optimization of conditions for SDS and Urea-PAGE electrophoresis on milk proteins from dairy cows was done changing the voltage, duration and applied sample. We found that the most conclusive results are reached using 12.5% SDS-PAGE and 10% urea-PAGE.

The representative SDS-PAGE and urea-PAGE electropherograms are shown on Fig. 1.
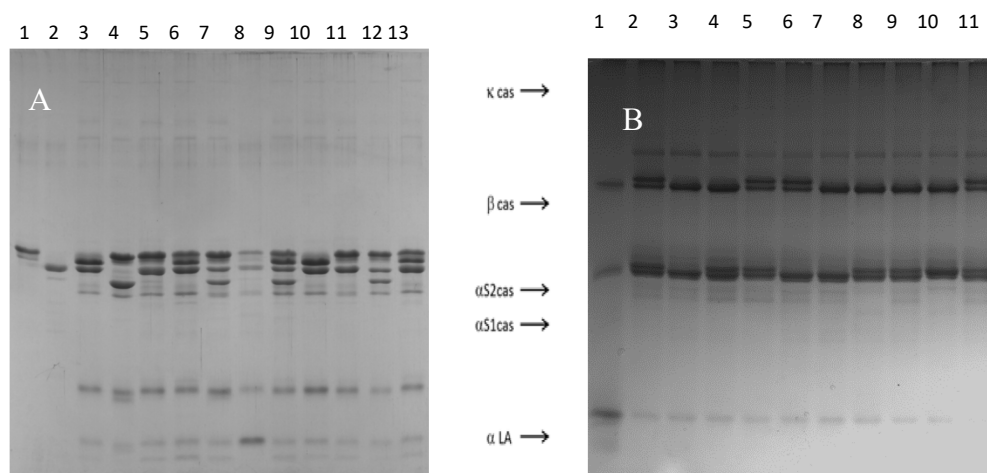
Fig 1. Electropherograms of analyzed milk samples.

A) 12.5% SDS-PAGE #1 TR1106 PIMA 4263, #2 TR1106 PIMA 4284, #3 TR1106 PIMA 42, #4 casein #5 TR1106 WLCS 3045, #6 TR1106 WLCS 4446, #7 TR1106 WLCS 4636, #8 Mix milk standards, #9 TR1106 CONTROL4650, #10 TR1106 CONTROL4202, #11 TR1106 CONTROL4505, #12 TR1106 CONTROL4768, #13 TR1106 CONTROL4419.

B) 10% Native – PAGE. # 1 Mix milk standards, #2 TR1106 PIMA 4263, #3 TR1106 PIMA 4284, #4 TR1106 WLCS 3045, #5 TR1106 WLCS 4446, #6 TR1106 WLCS 4636, #7 TR1106 CONTROL4650, #8 TR1106 CONTROL4202, #9 TR1106 CONTROL4505, #10 TR1106 CONTROL4768, #11 TR1106 CONTROL4419.

Based on the electrophoretic patterns on milk samples we identified six different protein profiles based on polymorphisms in milk proteins. In that sense the same protein pattern have the following samples #3 TR1106 PIMA 4263 and #10 TR1106 CONTROL4202; #5 TR1106 WLCS 3045 and #11 TR1106 CONTROL4505; #6 TR1106 WLCS 4446 and #13 TR1106 CONTROL4419, #7 TR1106 WLCS 4636 and #12 TR1106 CONTROL4768, while samples #4 TR1106 PIMA 4284 and #9 TR1106 CONTROL4650 have a separate profiles on SDS-PAGE. The same distribution of protein profiles are confirmed with Native-PAGE.

The idea of this samples diversity was to analyze the influence of gossypol on the electrophoretic mobility, biochemical and coagulation features of different polymorphic milk proteins.

In order to show a possible influence on gossypol on milk proteins in exposed dairy cows compared with the cows from the control group, in the Fig. 2 and 3 are given separate patterns from each cow before, in the middle and at the end of the diet using SDS and urea PAGE.

MILK SAMPLES FROM COWS EXPOSED TO GOSSYPOL

CONTROL GROUP OF COWS
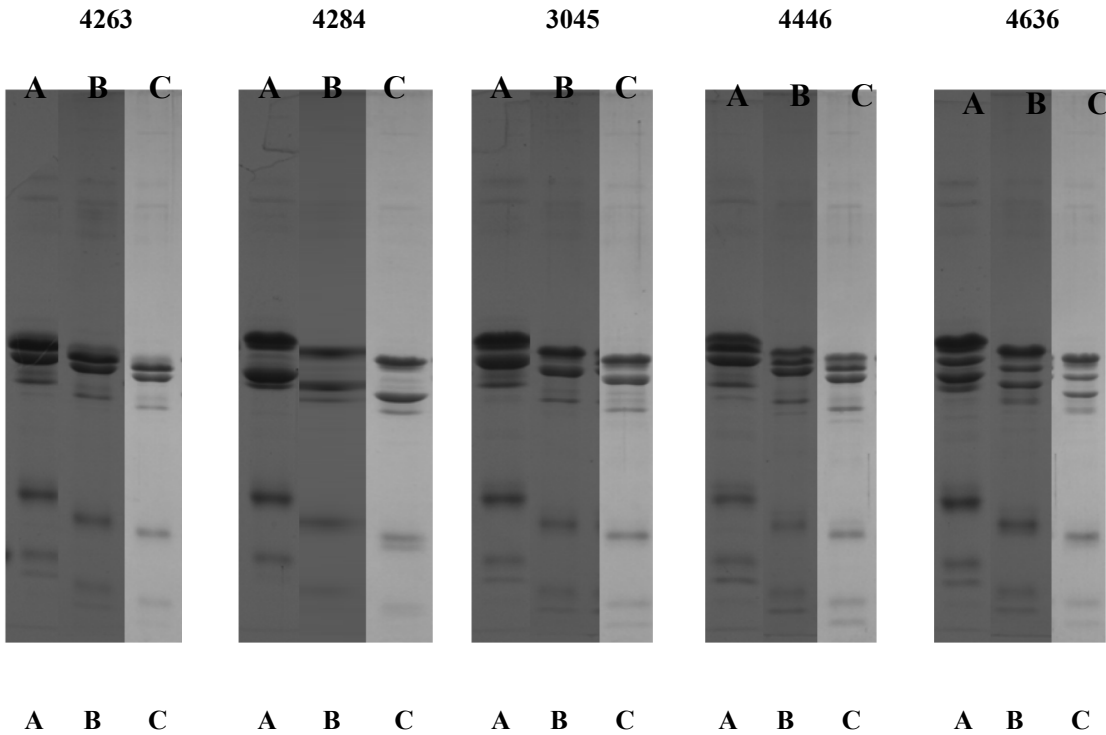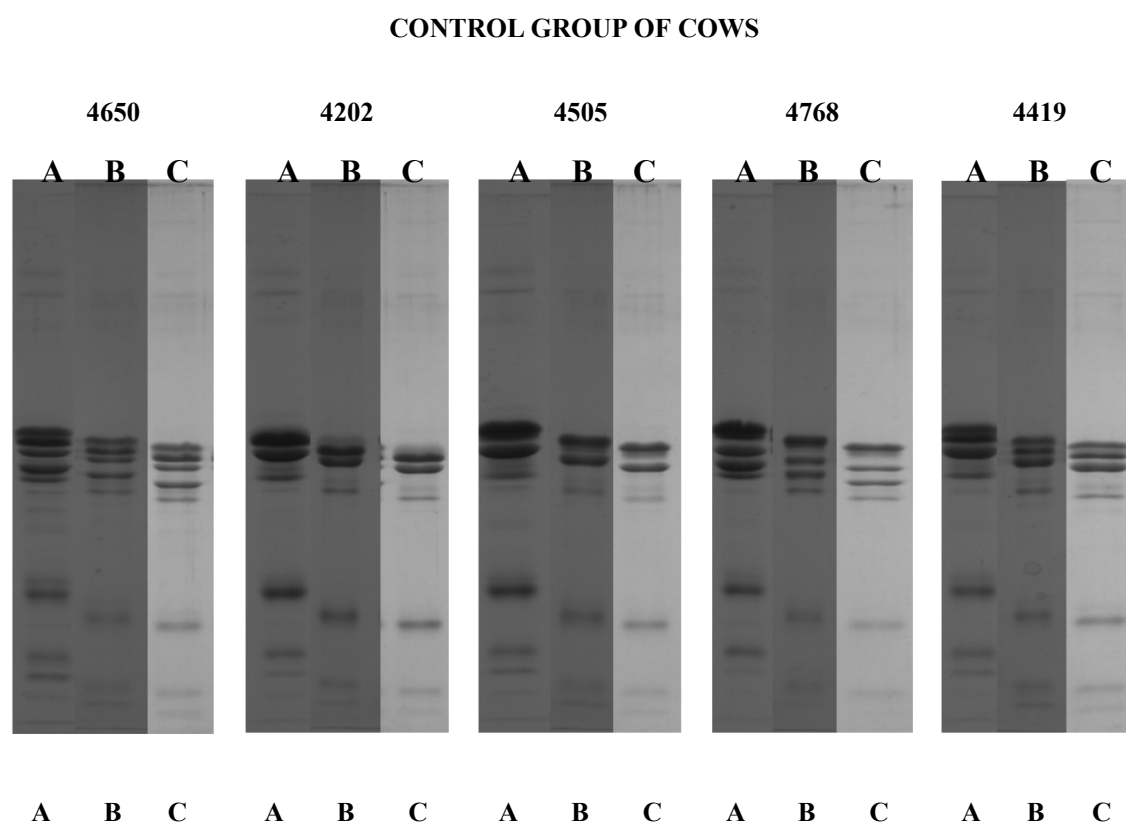
Fig 2. Protein patterns of milk samples on SDS-PAGE gel. A) before the diet, B) during the diet C) at the end of the diet.

MILK SAMPLES FROM COWS EXPOSED TO GOSSYPOL

CONTROL GROUP OF COWS
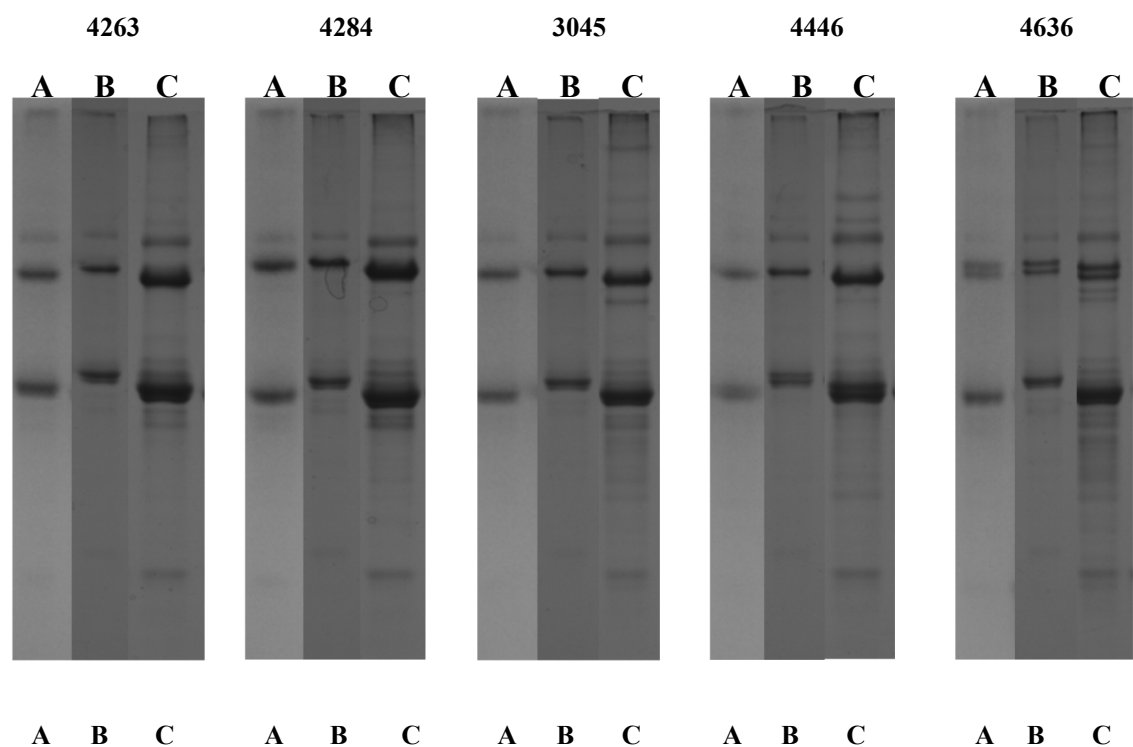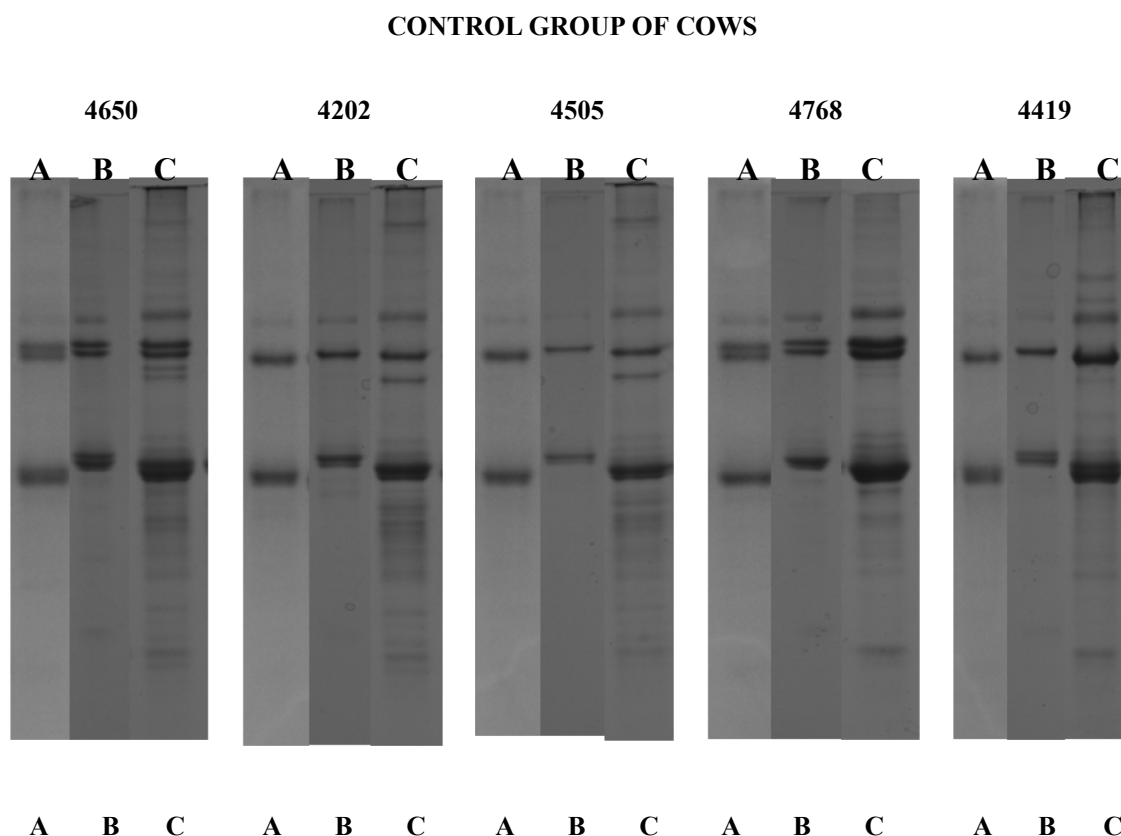
Fig 3. Protein patterns of milk samples on Native-PAGE gel. A) before the diet, B) during the diet C) at the end of the diet.

The separate patterns of each milk sample before, during and at the end of the cottonseed diet on SDS and Urea-PAGE showed that there are no visible differences in mobility of milk proteins nevertheless of their previously determined polymorphisms among them. Molecular weight or electrical charge, appeared to be influenced by gossypol, suggesting that the association of gossypol with proteins is neither covalent or strong enough to resist ionic disruptions from the conditions used.

Conclusion

This study showed that milk proteins in dairy cows exposed to cottonseed diet containing high amount of gossypol dot manifest any change in the electrophoretic mobility of polymorphic forms of milk proteins.

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Oral presentation

PREVALENCE AND CONSEQUENCES OF SUBACUTE RUMINAL ACIDOSIS IN POLISH DAIRY HERDS

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Abstract

Subacute ruminal acidosis (SARA) is one of the most important metabolic disorders in intensive dairy herds and affects rumen fermentation, animal welfare, productivity and farm profitability. Several studies have investigated the etiology and pathophysiology of this metabolic disease; however SARA syndrome is still not well defined. The aim of the present study was to estimate the prevalence of SARA in Polish high yielding dairy herds. Also, the relationship between the chemical composition of the diet, feed particle size, ruminal pH and the occurrence of this metabolic disease and fermentation profile were determined.

The study was conducted in 13 commercial dairy herds located in Western and South part of Poland. 305 dairy cows on the basis of ruminal fluid pH into three groups as health, risk and acidotic cows were divided. Moreover, a herd was considered SARA-positive, if at least 25% of the rumen fluid samples indicated acidotic. Herds with at least 33% of the sampled cows with rumen fluid pH 5.8-5.6 were classified as SARA-risk. TMR samples were collected for analyses by wet chemistry. The particle size of TMR samples was evaluated using The New Penn State Separator.

Rumen fluid were sampled 3-6 hours after the morning feeding by *rumenocentesis* and pH, concentrations of LPS, VFA, $N-NH_3$, total counts of bacteria and *Holotricha* and *Entodiniomorpha* were analyzed. The results obtained were analyzed using the SAS computer software SAS 9.4 (2014). According to those criteria, 21.6 % (66) of cows were classified as acidotic (pH<5.6) and 62% (8) of herds were classified as SARA – positive. In the present study, the ruminal pH decreasing was positive correlated with reducing dietary $peNDF_{>1.18mm}$ to starch ration. A decrease in the $peNDF_{>1.18mm}$ to starch ratio in the diet tended to linearly increase propionate, valerate and the sum of VFA.

The ratio of peNDF>1.18 mm to starch may be a better indicator of SARA than peNDF>1.18 mm and potentially useful marker tool of diet quality proposed to help nutritionist and farmers in diet formulation. For high-yielding dairy cows, the recommended ratio should be higher than 1.00.

Keywords: SARA, Poland, rumenocentesis, rumen fermentation

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Introduction

Subacute ruminal acidosis (SARA) is one of the most important metabolic disorders in intensive dairy herds that occurs during early and mid-lactation and affects rumen fermentation, animal welfare, productivity and farm profitability (Colman et al., 2013). Nutrient density and energy of the diets have to be increased by feeding high concentrates, including rapidly fermentable carbohydrates. However, these diets may cause a depression of ruminal pH due to the accumulation of volatile fatty acids (VFA) and cause occurrence of SARA (Plaizier et al., 2009). Several studies have investigated the etiology and pathophysiology of this metabolic disease, however SARA syndrome is still not well defined (Danscher et al., 2015). SARA is traditionally characterized by a low rumen pH, i.e. below 5.8 for 3-5 hours over a period of 24 hours (Enemark, 2008). However, this definition of SARA may be less representative for field cases, because continuous measurement of ruminal pH is a practical and economical challenge under field condition (Danscher et al., 2015). There is no general agreement on the pH threshold that defines SARA; rumen pH may not even be highly correlated with the disease symptoms (Bramley et al., 2008). According to Nordlund and Garrett (1994), SARA can often affect the fermentation profile by an increase in the total concentration of VFA and acetic to propionic acid ratio and that has been shifted towards propionic and butyric acids. Morgante et al. (2007) suggested that valeric acid could be a potential biomarker of SARA, but more studies are required to detect and define the role of this acid.

The incidence of SARA is difficult to determine, however, many results showed that the chemical composition of diet and its components are important to established rumen metabolism and function and indirectly prevent this metabolic disease (Zebeli et al., 2008; Li et al., 2014). The optimal balance between physically effective NDF (peNDF) and rapidly degradable carbohydrates in the diet is difficult to achieve, but it is crucial for maintaining proper rumen fermentation and metabolic status, enhancing the longevity of dairy cows.

The aim of the present study was to estimate the prevalence of SARA in Polish high yielding dairy herds. Also, the relationship between the chemical composition of the diet, feed particle size, ruminal pH and the occurrence of this metabolic disease and fermentation profile were determined.

Material and Methods

All procedures were approved by Local Ethical Committee no. 10 in Poznań, Poland (32/2014).
Farms

The study was conducted from November 2014 to June 2015 in 13 commercial dairy herds of the Polish Holstein-Friesian breed, located in Western and South part of Poland. All of these herds had more than 100 lactating dairy cows, housed in free stalls with cubicles, under milk performance evaluation conducted by the Polish Federation of Cattle Breeders and Dairy Farmers and a dry off period about 50-60d. The herd size was on average 515 cows, ranging from 210 to 760 animals.

Animals

In total, out of all the farms 305 dairy cows were selected and divided according to the classification of Nordlund and Garrett (1994) on the basis of pH ruminal fluid divided into three groups as healthy (pH>5.81, n=154), risk (pH 5.8-5.6, n=29) and acidotic cows (pH<5.6, n=30). Moreover, a herd was considered SARA-positive, if at least 25% of the rumen fluid samples indicated acidotic (pH<5.6). Herds with at least 33% of the sampled cows with rumen fluid pH 5.8-5.6 were classified as SARA-risk. The rest of the herds were classified as SARA-negative (Garrett et al., 1997; Oetzel, 2004). All the selected animals were between 40 to 150 days in milk (DIM), number of lactation (primiparous and multiparous) and clinical healthy (free of *mastitis, metritis*, hoof disease) were selected. The health status of the cows was estimated based on their recent medical history and through a detailed clinical examination, carried out always by the same veterinarian.

Chemical composition of diet and particle size

TMR samples were collected for analyses by wet chemistry for dry matter (DM, method no. 6496), crude protein (CP, method no. 976.05), neutral detergent fiber (NDF, method no. 942.05) and starch (method no. 64.785) according to AOAC (2007). The particle size of TMR samples was evaluated using The New Penn State Separator (Nasco, USA), with 3 sieves having holes with a different diameter (19, 8, 1.18 mm) and a solid pan according to the technique described by Mertens (1997). The content of each sieve and the solid pan was weighed and recorded.

Rumen fluid samples

Rumen fluid samples were collected from the ventral sack of the rumen of dairy cows by *rumenocentesis* using needles (2x120 mm) and 30 ml syringes. *Rumenocentesis* was chosen as the most adopted technique, providing the most accurate results (Enemark, 2004). The rumen fluid samples (30 ml) were collected 3-6 hours after the morning feeding and according to the methodology presented by Krause and Oetzel (2006). The rumen fluid pH was measured using a CP-104 pH-meter (Elmetron, Poland). The rumen fluid samples were divided into 2 portions. The first portion was transferred into a 5ml plastic probe for analysis of rumen ammonia according to the methods described by Novozamsky et al. (1974). The second portion was transferred into a 5 ml plastic probe deproteinized with 0.5 ml of 85% formic acid and analyzed for VFA according to (Barszcz et al., 2011).

Statistical analysis

The obtained results were analyzed using the SAS 9.4 software (2014). Detailed means were analyzed using t-test and the PROC GLM procedure. The PROC MEANS and PROC UNIVARIATE procedures were also used. Statistical significance was declared at P≤0.05 and trends were considered when 0.05<P≤0.1. The standard error of the mean (SEM) was adopted as a measure of error.

Results and Discussion

Prevalence of SARA in Polish dairy herds

The results of the screening 13 high-yielding dairy herds revealed the presence of SARA (more than 25% of cows with pH <5.6 per herd) in 62%. Unexpectedly, 21.6% of cows were classified as acidotic (pH<5.6). In a similar study in Denmark 25% of cows had pH <5.8 including the risk of SARA occurrence (Enemark et al., 2004). Grant and Mertens (1992) suggested that cellulolytic activity is reduced at pH of rumen fluid of 6.2. Also, Zebeli et al. (2010) found that total tract NDF digestibility increased linearly with increased ruminal pH. The prevalence of SARA, both at herd and cow-levels was similar to that in other countries. In Ireland 25% (3 out of 12), in Italy 33% (3 out of 10), in Greece 33% (4 out of 12) and in Germany 42% (11 out of 26) of herds were SARA-positive (O'Grady et al., 2008; Morgante et al., 2007; Kitkas et al., 2013; Kleen et al., 2013). Moreover, in the Netherlands 14% (27 of 197), in German 20% (63 of 315) of dairy cows were classified as SARA-positive (Kleen et al., 2009; Kleen et al., 2013).

Chemical composition and particle size of the diet

Mertens et al. (1997) suggested that feed particles longer than 1.18 mm are more effective in simulating chewing activity and therefore increasing the secretion of saliva and ruminal buffering capacity compared with smaller particles. In practice, a reduced forage particle size improved the uniformity of TMR resulting in less sorting behavior, which may reduce the risk of ruminal disorders. Zebeli et al. (2012) suggested that 31.2% $peNDF_{>1.18\ mm}$ is enough to prevent SARA. But we have a limitation which differentiates in measuring methods of $peNDF_{>1.18\ mm}$. These limitations are different mixing procedures used to prepare TMR and the relationship between the physical effectiveness of feeds and the cows digestive responses and all of these limitations should be standardized. Moreover, Li et al. (2014) suggest that diets containing excess fiber and low starch may reduce the feed intake and reduce the energy efficiency due to microbial synthesis. Zebeli et al. (2010) showed that increasing peNDF to 31.2% significantly increased ruminal pH which asymptotic plateau was achieved at 6.27 value. However, the effect of peNDF is due to chewing and ruminating activities, meal size, rumen motility and shifts in the site of grain digestion (Allen, 1997). The concept of physically effective fiber proposed by Mertens (1997) was considered more efficient in decreasing the risk of SARA than only dietary fiber level. However, Enemark et al. (2008) concluded that using effective NDF model has been too simple, failing to account for other components of the diet. In our study, in SARA-positive herds the levels of $peNDF_{>1.18mm}$ in the diet were lower (from 25.6 to 28.7) than recommended and $peNDF_{>1.18mm}$ to starch ratio was lower than 1.00. Moreover, in SARA-negative herds the levels of $peNDF_{>1.18mm}$ in the diet were from 28.8% to 33.4% and $peNDF_{>1.18mm}$ to starch ratio from 1.01 to 1.29. There was no linear relationship between $peNDF_{>1.18mm}$ and rumen fluid pH and other rumen fermentation indices (pH, VFA, $N-NH_3$) in contrast to $peNDF_{>1.18mm}$ to starch ratio. It seems that feeding ration with an optimal balance between the amounts of peNDF and rumen fermentable carbohydrates is critical to prevent SARA and improves productivity traits of high-yielding dairy cows. The optimal balance between peNDF and rapid degradable carbohydrates (e.g. starch) in the diet is difficult to achieve, but it is crucial not only for maintaining proper rumen metabolism, but also for maintaining a stable metabolic health status and enhancing the productivity of dairy cows. Already, Zebeli et al. (2012) showed that the ratio of the dietary $peNDF_{>1.18}$ to rapid

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degradable grain is highly correlated with the ruminal pH value (r^2= 0.41) and a diet with a ratio of 1.45 could prevent the occurrence of SARA (pH>6.2) in high-yielding dairy cows. According to the same authors, in practical terms, this ratio may be difficult to reach in cases when high-grain diets are based on cereals rich in rumen-degradable starch, such as barley or wheat. The majority of the summarized results of these scientific studies show that peNDF$_{>1.18mm}$ to starch ratio could be better potential indicator of well-balanced diet, which may useful in prevention of SARA occurrence and its value should not be lower than 1.0   for high producing dairy cows.

Rumen fermentation profile

The ruminal bacteria digest complex and highly fermentable carbohydrates in the rumen and produce VFA, microbial protein and vitamins for the host. The ruminal fluid pH and its daily fluctuation characteristics are important factors in the regulation of microbial community and its activity connected with the occurrence of SARA. The rate and extent of nutrients (starch, protein, and fiber) degradation in the rumen mainly depends on the complex enzymatic system (carbohydrates and protease) of ruminal microbiota and the substrates entering the rumen (Bach et al., 2005). In the current study, decreased the peNDF$_{>1.18mm}$ to starch ratio in the diet linearly increased propionate, valerate, the sum of VFA and N-NH$_3$. Also the same results but in opposite direction with higher value of C$_2$:C$_3$ ratio were observed. Moreover, according to herds' status of SARA, in acidotic farms higher concentrations of acetate, propionate, n-butyrate, n-valerate and the sum of VFA were observed. Contrary to our results, Martin et al. (2006) reported that rumen fermentation in SARA-positive cows were oriented toward butyrate than propionate production as the rumen pH decreased.

Conclusion

Subacute ruminal acidosis is a common and serious health and production problem in many high producing Polish dairy herds. The investigation demonstrated that 21.6% of cows were acidotic (pH<5.6) and the occurrence of SARA at the level of 62% of the herds, which may reflects increased use of starch in Polish dairy farms. The ratio of peNDF$_{>1.18mm}$ to starch may be better indicator of SARA than peNDF$_{>1.18mm}$ and potentially useful marker tool of diet quality proposed to help nutritionist and farmers in diet formulation. For high-yielding dairy cows the recommended ratio should be higher than 1.00.

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Oral presentation

PASTURE IN ANIMAL NUTRITION

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Abstract

The use of grazing as a major part of ruminant ration, and potentials of inclusion of grazed forage in diets for nonruminants, especially in terms of the fact that pasture is important and necessary part of diets for nonruminants in organic system production is considered in the paper. Pasture is the most economic source of nutrients in the ruminant nutrition and with appropriate utilization the forage has the highest quality and nutritive value. For a profitable dairy and meat production, cattle, sheep and goats should be grazed on the good quality pasture, and with high intakes they can meet a major part of their nutritional requirements. The supplementary nutrition for grazed ruminants, is necessary for meeting nutritional needs especially of high-production animals. Pasture can make a significant part of nonruminant diets – pig and poultry, depending on capabilities and efficiency of utilization. An appropriate strategy for using pasture as part of animal rations and practical grazing management system should provide optimization of forage yield, quality, persistence and high intakes of pastured animals. The adequate technology of pasture use is required for providing economically and environmentally viable production.

Keywords: grazing, dairy cattle, beef, sheep, goat, pig, poultry

Introduction

Grazed grass is the forage of high nutritive value for the lactating dairy cow. Intensively managed pasture-based dairy systems can reduce input costs and increase net returns, when compared to conventional confinement dairy systems (Stojanović et al., 2015). A major factor limiting milk production from grazed pasture is low intake. When compared to confinement based systems, cattle consuming even excellent quality pastures typically consume as much as 20% less feed dry matter per day as animals fed similar quality forage in confinement facilities (Stojanović et al., 2014).

Using pasture as a main part of beef ration in growing season, should be economically most efficient beef production. Supplementing energy in the form of grain to grazing cattle has been reported to increase daily gain compared with forage alone but has not always been consistent, especially when high quality forages are grazed (Allen et al., 1996).

Pastures generally contain appreciable quantities of legumes and provide herbage that is low in fiber and high in protein may reach nutritional requirements of sheep. Major

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constraints to pasture consumption can be related to the amounts and spatial distribution of the components of the pasture biomass, the chemical and physical properties of the herbage, environmental attributes associated with climate, disease and sheep behavior (Freer and Dove, 2002).

Changes in forage availability throughout the year influence diet selection of grazing goats (Kababya et al., 1998), since they adapt their choice according to what is available in the pasture. On average, goats chose a diet composed of 70% grasses, 25% forbs (from various botanical families) and only 5% legumes. For goats grazing either natural or cultivated pastures, generally the supply of concentrate increases energy intake and milk production (Landau et al., 1993).

The extent to which pasture and roughage can contribute to the nutritional needs of the swine will depend on voluntary feed intake and ability to utilize the fibrous components. Adult sows have a more developed intestinal system than younger pigs and are better able to digest fibrous diets. Forage is likely to be utilized to a limited extent in growing pigs (Adamović et al., 2005).

Roughage is mainly used as a necessarily supplement in organic egg and broiler production, and nutrient intake from grasslands usually is not included in the consideration of the nutritional needs of poultry. The main factors that affect forage utilization by birds are vegetation, botanical composition of available pasture, height of pasture, preferences of birds for forage species and mixtures, shade structures, climatic conditions, flock size (Antell and Ciszuk, 2006).

Grazed forage in animal diets

Dairy and beef cattle

The use of pasture for dairy cows is considered as lower-cost feeding system because grazed forage is the cheapest source of nutrients. Milk production per cow is usually lower on grazing-based than on confinement-based farms (Stojanović et al., 2009). Milk production of grazing cows is limited by the inability to consume enough pasture DM to meet the nutrient requirements for higher milk production, or an imbalance of rumen fermentable carbohydrate and rumen degradable protein-RDP (Stojanović et al., 2007). Low pasture DMI is identified as a major factor limiting milk production of high producing cows in a grazing system (Grubić et al., 2003). Pasture DMI of high yielding dairy cows might reach 3.25-3.5% of BW, with no pasture quantity and quality restrictions. The intakes of DM and NEL are lower on the pasture-only diet compared with cows fed total mixed ration (TMR), (Stojanović et al., 2015).

Many pasture factors affect DMI, including pregrazing pasture mass (kgDM/ha) and pasture allowance - PA (amount of pasture offered per cow, kg DM/cow/day). Over a range of PA from 20 to 70 kg DM/cow/day, pasture DMI increased 0.19 kg/kg of increased PA (Bargo et al., 2003). A practical recommendation is to provide a PA of 2 times the expected pasture DMI or 25 kg DM/cow/day when cows are fed supplements (Stojanović et al., 2016).

Supplementary feeding of grazing cows decreases pasture DMI while increases total DMI (Stojanović et al., 2014). Bargo et al. (2003) reported that decreasing of pasture DMI was 13%, when pasture was supplemented with 1.8 to 10.4 kgDM of concentrate, while the increasing of total DMI was 24%. Milk production of high producing grazing dairy cows in early lactation increases linearly as the amount of concentrate increases from 1.8 to 10 kg DM/day with milk response of 1 kg milk/kg concentrate, whereas in late lactation, milk response is lower. To avoid metabolic and health problems such as subclinical acidosis, it is not recommended to supplement more than about 10 kg DM/day or >50% of DMI. The high fiber intake (pasture NDF >50% DM) may allow for feeding high amounts of concentrate (Bargo et al., 2003).

Grazed forage can be the main feed of beef cattle and much of the requirement in energy and protein may be provided by pasture. Compared with rotational grazing, continuous grazing on permanent pasture produced approximately 30% less gain per ha in beef calves, and 17 % less gain per ha in Holstein steers (Adamović et al., 2005).

Beef cattle grazing well managed, grass/clover pastures without supplementing may gain approximately 1.0 kg/day. Angus and Angus/Maine-Anjou cross cattle (steers and heifers, twelve months old, 416 kg average BW) in period between May and October on permanent pasture (*Phleum pratense+Dactylis glomerata*, *Trifolium pratense* and *Trifolium repens* with ratio 60:20:20) with target pre-grazing height of 20-25 cm and target post-grazing height of 10-15 cm, gained 900 g/day, without supplement feeding (Jannasch et al., 2012). The dressed weight, dressed percent and lean yield percent of pasture-fed beef were 241.2kg, 51.5%, 59.7%. The cost of production per kg of live weight gain was 38.5% on pasture compared to feedlot.

Angus grazed steers (average BW 327 kg) in finishing phase (July-October) fed corn grain at 1% of BW/day as supplement to pasture, gained an additional 42 kg compared with nongrain-fed steers. Thus, steers were fed approximately 6.7 kg of grain/added kg of gain (Allen et al., 1996).

Beef cattle raised on pasture has a lower intramuscular lipid content than that generally found in meat from cattle fed concentrate diets. Pasture-based feeding system in comparison with a forage + concentrate feeding system resulted in an increased percentage of PUFA n-3 fatty acids in the longissimus muscle lipids of bulls of both Simmental (2.22 versus 0.46%) and Holstein (1.61 versus 0.34%), (Nuernberg et al., 2005).

Sheep and goats

While grazing, sheep have access to a wide variety of plants, which includes grasses, legumes and forbs. Quality pasture generally contain appreciable quantities of legumes and provide herbage that is low in fiber and high in protein (Đorđević et al., 2009). Nutritive value of herbage is variable because of variation in plant genetics, the rate and degree of plant development, environmental conditions under which it is grown and management practices (Božičković et al., 2014). A young growing pasture may support weight gains in excess of 300 g/day in young lambs, whereas a mature pasture may fail to maintain the weight of an adult sheep.

The critical herbage DM content in respect of intake constraint is between 125 and 145 g/kg, and according to some studies up to 190 g/kg (Freer and Dove, 2002). Morris et al. (1994) found that lactating ewes grazing swards with surface height in the optimum range (4.5–12 cm), reached peak intakes of 2.6–3.0 kg OM (38–46 g/kg LW) in week 4 of lactation, approximately 20% higher than the intake in the first week of lactation. Decrease in protein availability results in decreasing of sheep herbage intake. Data from a range of studies indicate that constraint on intake often prevails when the herbage contains less than 100-120 g CP/kg digestible OM.

Creep-feeding of grazed lambs (*Lolium perenne*) with concentrates offered at 250 g/lamb/day from age 5 to 14 weeks (weaning), increased live weight at weaning 3.9-4.4 kg, associated with feed conversion ratios of 4.4 to 6.3. The response to creep grazing ranged from 2.2 to 3.6 kg at weaning (Grennan, 1999). A sward height of 6 to 9 cm is required to maximize lamb growth. Growth rates are better on the grass/clover sward with average growth rates on the grass swards ranging from 70 to 80% of those on the grass/clover swards. The highest growth rate is achieved at an herbage allowance of about 5 kg of DM /lamb/day. Concentrate supplementation of weaned lambs on pasture (at 250 to 550 g/lamb/day) increased live weight for 6.5 and 10.4% with feed conversion ratios of 6.7 and 8.6 kg/kg.

More than any other species, goats are able to select, among the available feedstuffs, parts of plants with the highest protein content and the highest digestibility, choosing feed on the basis of prehension ease, sensorial characteristics and post-ingestive effects.

An earlier utilization at grass height of 6–8 cm increased milk yield of goats compared with a later utilization at grass height of 10–15 cm. A DM content of pasture ranging from 18 to 26% favors intake. A decrease in pasture DM availability, from 2000–3000 kg/ha during spring to 700–1500 kg/ha during autumn, reduced DM intake from 0.73–0.92 kg/day per head to 0.49–0.59 kg/day per head (Fedele et al., 1996).

Intake of grazed forage is not able to satisfy energy and protein requirements of grazing goats all year around. Higher differences between requirements and supply corresponding to the last 2 months of pregnancy until early lactation. In this period, the use of forages and concentrates with a medium to high level of CP is important. In general, a high level of energy supplement (cereal-based concentrates) usually reduces the grazing activity of goats. Goats fed low concentrate amounts spent more time grazing than those fed high concentrate amounts (75 and 59% of total observation time, respectively), (Landau et al., 1993).

For goats grazing either natural or cultivated pastures, generally the supply of concentrate increases energy intake and milk production. The effects of increasing amounts of concentrate supplementation on herbage intake vary with pasture forage quality. In goats grazing mixed vegetation, when concentrate supply increased from 0.33 to 0.66 g/day, diet total intake (forage and concentrate) increased for the low-quality forage, while it did not change for the high-quality forage (Min et al., 2005). Increasing the quantity of concentrate 0-1050 g/day, decreased protein intake from grass by about 30%, and fiber intake by 26%, herbage intake decreased from 53.1 g/kgMW to 14.8 g/kgMW (Claps et al., 1994).

Nonruminants – swine and poultry

Use of forages can lower costs of grain and protein supplementation in swine nutrition (Stojanović et al., 2004). Pastures containing a high percentage of legumes are generally the most practical for pigs. Animals fed regular dietary mixtures ad libitum have very low forage intakes (< 5% of daily DM intake). When the regular feed is restricted, higher intakes of roughage can be achieved (up to 15%).

Pigs absorb more nutrients from forages after an adaptation period of at least 2 months, with nearly all of the fiber digestion taking place in the large intestine, where only 30–40% of the hemicellulose and cellulose is digestible (Adamović et al., 2005).

The extent to which pasture can contribute to the nutritional needs of the pigs depends on voluntary feed intake and ability to utilize the fibrous components. Adult sows have a more developed intestinal system than younger pigs and are better able to digest fibrous diets. If high-quality pasture is available, gestating sows are able to obtain 30–45% of daily energy intake from this source. Sows are capable of ingesting around 1.2 kg of herbage DM per day. Vestergaard et al. (1995) reported that digestibility of grass DM for sows were 50-62%, and for growing pigs 40-58%, and of CP 36-39% and 11-17%, while ME content was 8.3-10.1 and 6.3-9.6 MJ/kg DM, respectively.

Strudsholm and Hermansen (2005) reported that the pastured growing-finishing pigs were not able to compensate for a limited access (approximately 80% of ad libitum intake) to a regular diet by an increase in herbage intake of 18%. Compared with grazed pigs fed *ad libitum*, restricted feeding resulted in a significantly lower daily gain (107g) and a lower feed consumption (6.3 MJ ME/kg gain). Nilzen et al. (2001) found that reduced levels of concentrate feeding and *ad libitum* intake of roughage reduces daily gain in growing pigs, resulting in increased lean meat content but reduced intramuscular fat and tenderness. Pigs on pasture have higher levels of polyunsaturated fatty acids and vitamin E in the muscle than indoor pigs.

Optimum stocking rates depend on soil types, plant species and climate conditions. The stocking rate of about 10–15 gestating sows per hectare (double if irrigated) or about 25–30 growing pigs (double if irrigated) will make good use of the pasture crop without excessive damage. Sows should be fed approximately 1–1.5 kg of an appropriate dietary mixture daily while on pasture. Grower-finisher pigs should have free access to the dietary mixture at the same time they are grazing a pasture crop (Blair, 2007).

Poultry may utilize a considerable amount of herbage when accessible, and select plant species differently in relation to the palatability and the nutritional value of the plant species, and the type of feed offered (Adamović et al., 2005). The feeding strategy especially in organic poultry production is characterized with using a roughage, where fresh herbage from pasture is predominant, as a necessarily part and supplement to concentrate diet (Stojanović et al., 2006). The fresh herbage could provide 5% of the daily DM intake of meat chickens and 10% of DM intake of growing and laying poultry (Hristov et al., 2006).

The average forage consumption of birds with pasture available is about 30-40 g DM/hen/day, which severely affected the intake of commercially formulated feed, but did

not affect productivity. Antell and Ciszuk (2006) reported that the apparent ME of grass was 6.4 MJ/kg DM, in the nutrition of laying hens consumed pasture. The layers might consume 30–40 g DM/day in the form of herbage, worms, and insects in addition to 100 g of concentrates. Reducing the quantity of concentrates fed to layers by 15% had no detrimental effect on egg production when individual herbage consumption was up to 30 g DM/day. Jondreville et al. (2011) found that, on a DM basis, hens fed a complete diet ingested 5.6–8.1 g/day of herbage and 3.6–7.2 g/day of soil.

Almeida et al. (2012) compared slow and medium growth broiler genotypes foraging on two different types of vegetation (grass–clover or chicory), it was estimated that the slow-growing genotype had a daily intake of 5–8 g forage DM/day, whereas the medium-growing genotype had an intake of 9 g for females and 20 g DM for males.

Conclusion

Low pasture DMI is a major factor limiting milk production of high producing grazing cows. Supplementary feeding of grazing cows decreases pasture DMI while increases total DMI. Grazed forage should be the main feed of beef cattle and much of the requirements for energy and protein can be provided by pasture. Beef cattle grazing well managed grass/clover pastures without supplementing may gain approximately 1.0 kg/day. Decrease in protein availability results in decreasing in herbage intake by sheep. Growth rates of lambs are better on the grass/clover sward, with average growth rates on the grass swards ranging from 70 to 80% of those on the grass/clover swards. Intake of grazed forage is not able to satisfy energy and protein requirements of grazing goats all year around. The use of forages and concentrates with a medium to high level of CP is important at the late pregnancy and early lactation. A high level of concentrates usually reduces the grazing activity of goats. The extent to which pasture can contribute to the nutritional needs of the pigs depends on voluntary feed intake and ability to utilize the fibrous components. Gestating sows are able to obtain 30–45% of daily energy intake from high-quality pasture. Pasture provides a low amount of nutrients for growing-finishing pigs. The fresh herbage could provide 5% of the daily DM intake of meat chickens and 10% of DM intake of growing and laying poultry.

Acknowledgment

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Oral presentation

EFFECTS OF THE MIXTURE OF ESSENTIAL OILS AND ORGANIC ACIDS ON PERFORMANCE, CARCASS YIELD AND INTERNAL ORGANS IN BROILERS FED DIETS WITH SEPIOLITE

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Abstract

The purpose of this study was to determine the effects of dietary additive containing essential oils and organic acids on performance, carcass yield and internal organs in broilers fed diets with sepiolite. A total of 300 Ross 308 broiler male chicks aged one day were divided into one control group and two treatment groups each group containing 100 chicks. Each group was divided into 5 replicates, as subgroups, each comprising 20 chicks. The experimental period lasted 39 days. Sepiolite at the level of 1% was added to the basal diet as topdressed. Control diet was basal diet with sepiolite. The additive containing essential oil with organic acids (NafOil Anti Plus, thyme oil, orange oil, garlic oil and organic acids, Biotem Ltd Company). was added at 0.1 and 0.2% to the first and second treatment groups, respectively. Dietary supplementation of additive containing thyme oil, orange oil, garlic oil and organic acids didn't affect the final body weight, body weight gain, feed intake, feed efficiency and livability during the 39 days of experimental period. Carcass yield and relative weights of liver, heart, gizzard, spleen, Bursa fabricius and abdominal fat were not affected from the usage of additive in diets containin sepiolite. As a conclusion effectiveness of the mixture of essential oil and organic acids (NafOil Anti Plus) could be more pronounced when the additive is supplemented into diets with sepiolite in suboptimal conditions.

Keywords: Broiler, sepiolite, essential oils, organic acids, performance, carcass yield

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Introduction

Many feed additives known as natural growth promoters have been used increasingly in poultry diets as effective alternatives to antibiotics to maintain poultry health and enhance performance. Essential oils originate from leaves, roots, tubers or fruits of herbs, spices and other plants. The beneficial effects of essential oils in poultry may arise from the stimulation of feed intake, digestive enzymes and immunity and the properties of antibacterial, antiviral, antioxidant anti-inflammatory activities. Organic acids reduce pH, survival of pathogens, increase digestion of nutrients and therefore improve growth performance and provide people with health and nutritious poultry products (Brzoska et al., 2013; Fallah et al., 2013; Ogunwole et al., 2011; Petrolli et al., 2012). Sepiolite, a hydrated magnesium silicate is a natural feed additive (E-562) used as a binder and anti-caking agent up 2% in all feeds for all animal species (EFSA, 2013). Sepiolite reduces dust losses, increases digestion time and intestinal viscosity, improves fecal consistency and intestinal transit time (Pappas et al., 2010). Therefore this study was aimed to determine the effects of the additive containing essential oils with organic acids added to the diets with sepiolite on performance, carcass yield and internal organs in broilers.

Materials and Methods

A total of 300 Ross 308 broiler male chicks aged one day were divided into one control group and two treatment groups each group containing 100 chicks. Each group was divided into 5 replicates, as subgroups, each comprising 20 chicks. Feed and water were provided for ad libitum consumption and the diets were presented in mash form. The experimental period lasted 39 days. Sepiolite at the level of 1% was added to the basal diet as topdressed. Control diet was basal diet with sepiolite. Control diet was supplemented with the additive containing essential oil with organic acids (NafOil Anti Plus, thyme oil, orange oil, garlic oil and organic acids, Biotem Ltd Company) at 0.1 and 0.2% for the first and second treatment groups, respectively. Body weight (BW) and feed intake (FI) was recorded. Body weight gain (BWG) and feed conversion ratio (FCR) was calculated. The birds were observed Daily for evaluating mortality. European Production Efficiency (EPEF) was calculated. At the end of the experiment (d 39) 10 broilers from each group were weighed and slaughtered by severing the jugular vein. Hot carcass weights were determined and carcass yield was calculated. Absolute and proportional weights of abdominal fat, liver, heart, spleen, gizzard and Bursa Fabricius were also determined. Data were analyzed as a completely randomized block design, with 3 dietary treatments and 5 replicates using the ANOVA procedure of the SPSS. The effect of graded levels of dietary mixture of essential oils-organic acids on different variables using polynomial contrasts. Statistical differences were considered significant at P≤ 0.05 (Dawson and Trapp, 2001).

Results and Discussion

Broiler performance was improved using dietary topdressed sepiolite (Pappas et al., 2010). This effect was explained by the increased retention time of digesta which allows for a more effective endogenous digestive enzyme activity in the digestion and absorption of fats, proteins and carbohydrates. Therefore in this experiment the diets containing sepiolite were used to determine the effects of the additive containing essential oils with organic acids on performance, carcass yield and internal organs in broilers. Supplementation of the

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mixture of essential oils and organic acids to the diets with sepiolite on performance of broilers was shown in Table 1. Dietary supplementation of additive containing thyme oil, orange oil, garlic oil and organic acids didn't affect the final body weight, body weight gain, feed intake, feed efficiency, livability and European Production Efficiency values during the 39 days of experimental period. Similar to the present study, feed conversion ratio was not affected by dietary garlic essential oils (Rahimi et al., 2011; El Tazi et al., 2014). However some researchers (Dieumou et al., 2012; Ramadan, 2013) reported that garlic and thyme oil improved productive performance in broilers. Carcass yield and relative weights of liver, heart, gizzard, spleen, Bursa fabricius and abdominal fat were not affected from the usage of additive (Table 2). Carcass yield was not affected with the Similarly, some researchers reported that thyme oil (Ramadan, 2013), orange peel oil (Dalkılıç et al., 2015) didn't affect relative organ weights. Orange oil is used as antioxidant, antimicrobial and growth promoter agent (Aksu and Bozkurt, 2009). In another study (Ramadan, 2013) garlic oil increased relative weight percentage of bursa Fabricius and decreased liver weight percentage in broilers. However numerical increases were obtained in feed conversion ratio, EPEF and carcass yield. The differences among groups may be due to the dietary ingredients, type and dosage of additives.

Table 1. Supplementation of the mixture of essential oils and organic acids to the diets with sepiolite on performance of broilers

	Mixture of essential oils and organic acids, %			Pooled standard error of mean	P	
	0	0.1	0.2		Linear	Quadratic
BW at the beginning, g	42.45	42.80	43.50	0.235	0.075	0.714
BW at day 39	2931.90	2936.11	2933.06	10.879	0.989	0.886
BWG, g (day 0-39)	2889.45	2893.31	2889.56	10.791	0.997	0.880
FI, g (day 0-39)	4424.11	4417.78	4417.97	10.635	0.830	0.895
FCR, g/g (day 0-39)	1.531	1.527	1.529	0.005	0.876	0.764
Livability, %	97.00	98.00	99.00	0.655	0.244	1.000
EPEF	476.52	483.32	487.35	5.726	0.479	0.916

BW: body weight, BWG: body weight gain, FI: feed intake, FCR: feed conversion ratio, EPEF: European production efficiency factor
No differences were observed (P>0.05)

Table 2. Supplementation of the mixture of essential oils and organic acids to the diets with sepiolite on carcass yield and internal organs weight percentages of broilers

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	Mixture of essential oils and organic acids, %			Pooled standard error of mean	P	
	0	0.1	0.2		Linear	Quadratic
Carcass yield, %	74.73	75.46	75.31	0.200	0.246	0.303
Liver weight, %	1.923	1.891	1.928	0.021	0.931	0.463
Heart weight, %	0.526	0.559	0.524	0.011	0.959	0.151
Spleen weight, %	0.130	0.116	0.110	0.005	0.077	0.698
Bursa Fabricius weight, %	0.066	0.062	0.063	0.003	0.602	0.711
Gizzard weight, %	0.959	1.053	1.000	0.029	0.559	0.243
Abdominal fat weight, %	1.121	1.089	1.028	0.035	0.287	0.845

No differences were observed ($P>0.05$)

Conclusion

Dietary usage of the additive containing essential oils and organic acids didn't affect growth performance, carcass yield and relative weights of liver, heart, gizzard, spleen, Bursa fabricius and abdominal fat in broilers fed diets with sepiolite. However numerical improvement in growth performance was seen therefore it was concluded that the mixture of essential oil and organic acids (NafOil Anti Plus) added to the diets with sepiolite could be more effective in broiler nutrition when the additive is supplemented in suboptimal conditions.

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Oral presentation

EFFECTS OF PHYTOBIOTICS PREMIXES IN DRINKING WATER ON PERFORMANCE AND CARCASS YIELD OF BROILERS

Yalçın S.[1], Eser H,[2] Yalçın S.[3], Onbaşılar İ.[4]

Abstract

The aim of this study was to determine the effects of the usage of phytobiotics premixes in drinking water on performance, carcass yield and internal organs in broilers. A total of 960 Ross 308 broiler male chicks aged one day were divided into one control group and three treatment groups each group containing 240 chicks. Each group was divided into 6 replicates, as subgroups, each comprising 40 chicks. The experimental period lasted 37 days. Liquid phytobiotics (NafOil Pro) premixes was added to the drinking water at the level of 0, 2, 3 and 4 g/10 l. Phytobiotics premixes did not affect the live weight, live weight gain, feed consumption, feed conversion ratio, livability and European production efficiency factor. Carcass yield and relative weights of liver, heart, gizzard, spleen, Bursa fabricius and abdominal fat were not affected from the usage of phytobiotics premixes. As a conclusion effectiveness of phytobiotics premixes could be more pronounced when the additive is supplemented in suboptimal conditions.

Keywords: Broiler, phytobiotics, premixes, performance, carcass yield

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Introduction

Phytobiotics, effective alternatives to antibiotics have been used increasingly in poultry diets to maintain poultry health and enhance performance. Phytobiotics have characteristics of the stimulation of feed intake, digestive enzymes and immunity and the properties of antibacterial, antiviral, antioxidant anti-inflammatory activities. Therefore this study was aimed to determine the effects of the usage of phytobiotics premixes in drinking water on performance, carcass yield and internal organs in broilers.

Material and Methods

A total of 960 Ross 308 broiler male chicks aged one day were divided into one control group and three treatment groups each group containing 240 chicks in the poultry house of Mudurnu Süreyya Astarcı Vocational School of Higher Education of Abant İzzet Balsal University in Bolu in Turkey. Each group was divided into 6 replicates, as subgroups, each comprising 40 chicks. Chicks of each replicate groups were placed in separate floor pen measured as 2m x 2 m, width and length, respectively. Each pen had rice hulls litter. Feed and water were provided for ad libitum consumption and the diets were presented in mash form. The diets were formulated according to the commercial management guide (Ross 308 Broiler). The ingredients and chemical composition of the diets are presented in Table 1. The experimental period lasted 37 days. Continuous lighting was applied during the whole experiment. Average room temperature was 34°C on the first three days and then gradually reduced by 2-3°C per week to final temperature of 22°C. Liquid phytobiotics (NafOil Pro, BİOTEM Biyosid Ltd Co) premixes was added to the drinking water at the level of 0, 2, 3 and 4 g/10 l. NafOil Pro contain essential oils (anise, walnut, blackseed, thyme, eucalyptus, orange, garlic), organic acids (lactic acid, malic acid, citric acid, tartaric acid, salicylic acid, ascorbic acid), emulsifiers (monopropylene glycol, sodium stearol 2- lactylate) Crude protein contents of diets were determined according to the AOAC(2000). Metabolizable energy levels of samples were calculated using the Carpenter and Clegg's equation (Carpenter and Clegg, 1956). Body weight and feed intake was recorded. Body weight gain and feed conversion ratio was calculated. The birds were observed daily for evaluating mortality. European Production Efficiency Factor was calculated. At the end of the experiment (d 37) 12 broilers from each group were weighed and slaughtered by severing the jugular vein. Hot carcass weights were determined and carcass yield was calculated. Absolute and proportional weights of abdominal fat, liver, heart, spleen, gizzard and Bursa Fabricius were also determined. Data were analyzed as a completely randomized block design, with 4 dietary treatments and 6 replicates using the ANOVA procedure of the SPSS. The effect of graded levels of phytobiotics on different variables using polynomial contrasts. Statistical differences were considered significant at P≤ 0.05 (Dawson and Trapp, 2001).

Results and Discussion

Supplementation of the phytobiotics to the drinking water on performance of broilers was shown in Table 2. The phytobiotics supplementation to the drinking water didn't affect the final body weight, body weight gain, feed intake, feed efficiency, livability and European Production Efficiency values during the 37 days of experimental period. Carcass yield and relative weights of liver, heart, gizzard, spleen, Bursa fabricius and abdominal fat were not affected from the usage of liquid additive in drinking water. Similar results obtained with some studies (Ocak et al., 2008; Karimi et al., 2010; Al-Mufarrej, 2014) that there was no

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effect of oregano or black cumin powder in the diet on the performance of broilers. However some researchers (Windisch and Kroismayr, 2007; El-Ghany and İsmail, 2013) reported that phytobiotics improved the growth performance of broilers. Windisch et al. (2008) and Grashorn (2010) observed that the phytobiotics improved flavor and palatability of feed and may improve the feed intake and performance of broilers. The phytogenic bioactive compounds may stimulate the proliferation of absorptive cells in intestine and increase the production and activity of digestive enzymes and therefore improve the growth performance (Vidanarachchi et al., 2005; Jang et al., 2007). These differences may be due to the dietary ingredients, type and dosage of phytobiotics (Sugiharto, 2014).

Table 1. Ingredients and chemical composition of basal diets

Ingredients, (g/kg)	Broiler starter (1-14 day)	Broiler grower (15-28 day)	Broiler finisher (29-37)
Corn	368.050	347.051	375.307
Soyabean meal, 46% CP	170.000	70.000	63.374
Fullfat soya	150.000	160.000	140.000
Wheat	90.000	110.000	130.000
Corn glüten meal	56.635	45.259	0.000
Sunflowerseed meal, 36% CP	40.000	70.000	76.437
Bonkalite	40.000	60.000	60.000
Meat and bone meal	30.000	14.330	19.744
Poultry by products	0.000	35.000	45.000
DDGS	20.000	50.000	30.000
Guar meal	0.000	0.000	15.000
Fat	9.474	9.750	10.745
Poultry fat	0.000	0.000	8.000
Tallow	0.000	3.000	4.000
Acid oil	0.000	0.000	3.000
Limestone	8.345	9.403	6.979
Lysine sulfate	5.352	5.829	4.217
Liquid methionine	2.976	1.926	2.128
MCP	2.463	2.000	0.000
Salt	1.455	1.652	1.602
Threonine	1.300	0.850	0.517
Vitamin premix	1.000	1.000	1.000
Mineral premix	1.000	1.000	1.000
Choline	0.750	0.750	0.750
Sodium sulfate	0.700	0.700	0.700
Sodium bicarbonate	0.500	0.500	0.500
Chemical composition			
Crude protein, g/kg	241.70	229.74	202.50
ME, kcal/kg	3010	3132	3190

Table 2. Supplementation of phytobiotics to the drinking water on performance of broilers

	Phytobiotics added to the drinking water				Pooled standard error of mean
	0	2 g/10 l	3 g/10 l	4 g/10 l	
Live weight gain, g (day 0-37)	2690.38	2689.53	2690.89	2707.57	11.214
Feed intake, g (day 0-37)	4173.88	4159.37	4096.69	4173.37	17.260
Feed conversion ratio, g/g (day 0-37)	1.55	1.55	1.52	1.54	0.008
Livability, %	96.25	94.17	94.58	94.58	1.048
EPEF	458.25	450.30	458.45	455.84	5.190
Carcass yield, %	74.81	75.00	75.58	75.06	0.192

No differences were observed ($P > 0.05$)

Conclusion

In this study phytobiotics premixes did not affect growth performance, carcass yield and relative weights of internal organs. However the phytobiotics premixes (NafOil Pro) could be more effective in broiler nutrition when the additive is supplemented in suboptimal conditions.

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Poster presentation

THE EFFECT OF CELL WALL LIGNIFICATION ON *IN VITRO* DRY MATTER AND NEUTRAL DETERGENT FIBER DIGESTIBILITY

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Abstract

Fiber content and its digestibility have the greatest impact on overall digestibility because fiber is the slowest digesting component in feeds. Lignin is considered an anti-quality component in forages because of its negative impact on the nutritional availability of plant fiber. Lignin interferes with the digestion of cell wall polysaccharides by acting as a physical barrier to microbial enzymes. Lignification therefore has a direct and often important impact on the digestibility of the forage.

The objective of this study was to investigate the impact of lignin content on *in vitro* NDF digestibility and dry matter digestibility of alfalfa harvested at different development stage and in different cuts. The experiment was designed as two factorial trial: the first factor was stage of alfalfa, cultivar K 28 development (FB – full-bud stage; EBL – Early Bloom, 10-15% of flowering and MBL – Mid Bloom, 50-60% of flowering) and the second factor was cut (cut I, cut II, cut III and cut IV).

The NDF content was higher in the late harvested alfalfa in the first and the second cut, but in the third cut NDF determined in the third development stage was lower than in the first development stage. In all investigated seasons of growth, we observed increased lignin content as maturity advanced. In the present study we observed faster lignification of cell wall in the third and the fourth cut than in the first and the second cut. *In vitro* $DNDF_{30h}$ (Digestibility of Neutral Detergent Fiber, incubated 30 h) and $DNDF_{48h}$ (Digestibility of Neutral Detergent Fiber, incubated 48 h) declined with advancing maturity from 305.7 to 293.7 g kg^{-1} NDF and from 387.7 to 330.4 g kg^{-1} NDF, respectively. Also, late harvested

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alfalfa had lower *in vitro* DM (Dry Matter) digestibility than did early harvested alfalfa. The highest DNDF and DM digestibility was observed in the fourth cut.

Keywords: alfalfa, lignification, plant maturation, NDF digestibility

Introduction

Alfalfa (*Medicago sativa* L.) is the most important forage legume in the temperate climate (Jonker et al., 2010) because of high yield and high nutrient levels (Yu et al., 2003). It is grown on over 30 million hectares globally, and on about 200,000 ha in Serbia (Đukić, 2005). Dairy cow performance is determined by the amount of digestible nutrients that is consumed each day. The amounts of consumed digestible nutrients are related to both intake and digestibility. The intake and digestibility of dairy rations are related to the neutral detergent fiber (NDF) content and digestibility of forages. Mertens (2009) indicated that fiber content and its digestibility have greatest impact on overall digestibility because fiber is the slowest digesting component in feeds. This author concluded that NDF content and NDFD (Neutral Detergent Fiber Digestibility) are the key determinants of dry matter digestibility.

Oba and Allen (1999) compiled data from several experiments to evaluate the impact of NDFD on dairy cow performance. They concluded that increasing NDFD increased both intake and milk production. Mertens (2006) observed significant relationships between *in vitro* NDFD 48h and intake and milk production.

With advancing maturity, plants develop xylem tissue for water transport, accumulate cellulose and other complex carbohydrates, and these tissues become bound together by a process known as lignification. In particular, lignin in plant cell walls is more difficult for rumen bacteria to digest than cellulose or hemicellulose. The objective of this work was to determine *in vitro* dry matter digestibility and *in vitro* NDF digestibility of alfalfa (*Medicago sativa* L.) influenced by stage of maturity and different cuts.

Material and Methods

This experiment was carried out in the experimental field of Institute for forage crops in Kruševac (43°34`58``N, 21°19`35``E). The study area was situated at altitude of 166 m above sea level in Central Serbia. The experiment was designed in 2011. Soil type was with an organic matter content of approximately 3.5%; total nitrogen content of 0.16% and a pH of 6.5 in aqueous solution and 5.7 in 1N KCl. The soil was very poorly supplied with easily available phosphorus (4.9 mg P_2O_5 / 100 g of soil), with potassium content of 23.1 mg K_2O / 100 g of soil. Samples for chemical analysis were collected in 2012.

Alfalfa (*Medicago sativa* L.) – *cv* K 28 selected at Institute for forage crops, Kruševac, was sampled at three stages of maturity, corresponding to the cutting dates shown in Table 1. Plants from a pure stand were cut manually with scissors about 5 to 7 cm above the soil surface. Samples were dried to constant weight at 65° C for 48 h and dried samples   were

ground through a screen size of 1 mm. All analyses were done in duplicate and component concentrations were corrected to a 100º C dry matter basis.

To assess leaf and stem proportion, each plot was subsampled. These subsamples were dried at 65º C in forced-air oven, weighed, and then stems were separated from the leaves. The leaf and stem portions were reweighed and leaf and stem proportion were estimated for each sample.

aNDF was determined according to the method by Mertens (2002). Lignin was determined as the residue insoluble in 72% (w/w) sulfuric acid, applying the method of Van Soest and Robertson (1980). Two-stage pepsin-cellulase method was used for *in vitro* DM digestibility according to method by De Boevar et al. (1986). *In vitro* NDF digestibility for 30 and 48 h (NDFD) was determined according to method by Riveros and Argameneteria (1987).

Table 1. Cutting dates and estimated stages of maturity of forages

Maturity stage Cut	FB – Full Bud	EBL – Early Bloom 10-15% of flowering	MBL – Mid Bloom 50-60% of flowering
I	04 May (60)*	21 May (77)*	29 May (85)*
II	08 June (35)*	15 June (42)*	21 June (48)*
III	06 July (23)*	13 July (30)*	18 July (35)*
IV	08 August (26)*	16 August (34)*	21 August (39)*

*Number in parentheses indicate the number of days of the growing cycle

Table 2. Alfalfa leaf proportion, %

SM	FB	EBL	MBL
I cut	39.5	38.1	36.3
II cut	38.3	35.8	33.1
III cut	45.7	44.2	39.7
IV cut	48.9	46.4	43.3

Experiment was established as a randomized complete block design in three replications, with factorial arrangements of two main factors (3 stage of maturity × 4 cuts). The data were processed in an analysis of variance in a randomized block design (ANOVA, Stat. Soft. STATISTICA 6). The significance of differences between arithmetic means was tested by Fisher test (p< 0.01).

Results and Discussion

The data collected in this study show that content of aNDF increased with growth and development (Table 3). The greatest content of aNDF was in the cut II. In the cut II the content of aNDF increased from 489.0 to 585.8 g kg^{-1} DM.

As forage matures there is generally a decline in digestibility of the fiber fraction that is associated with an increase in lignin. People have tried to use lignin concentration as a means of predicting cell wall and whole plant digestibility. The lignin concentration of alfalfa harvested at three different stage of maturity from the cut I to the cut IV are presented

in Table 3. The both effects (stage of maturity and cut) were significant (p< 0.01) in these trials. The results of this investigation indicated that lignin content was increased with advancing maturity in all four cuts. The highest content of lignin was found in the cut III expressed as a percentage of dry matter and in the cut I expressed as a percentage of aNDF (Table 3).

Maturity affected in DNDF in both treatments. With advancing maturity *in vitro* DNDF declined from 305.7 to 239.7 g kg^{-1} NDF and from 387.7 to 330.4 g kg^{-1} NDF for DNDF$_{30h}$ and DNDF$_{48h}$, respectively. As maturity proceeds, leaf-to-stem ratio declines and as a result NDF digestibility declines because a greater portion of total NDF is NDF in stem tissue. *In vitro* DNDF differed significantly from cut I to cut IV (p< 0.01). Results obtained in these investigations indicated that the highest DNDF was in cut IV (308.8 g kg^{-1} NDF and 491.1 g kg^{-1} NDF for DNDF$_{30h}$ and DNDF$_{48h}$, respectively). These results are similar as for DMD, which was also the highest in cut IV.

DMD (Dry Matter Digestibility) decreased with advancing maturity of alfalfa, as a result of increasing cell wall content in the stem and due to the decline and reducing leaf to stem ratio with maturation, which is confirmed in this study (Table 3). The average value of DM digestibility of alfalfa ranged from 726.3 g kg^{-1} at the full bud stage of maturity to 656.2 g kg^{-1} at mid flowering stage. Results of this investigation indicated that the highest digestibility was in the cut IV, even though it was developed in the part of the year with low precipitation and high temperatures. The lowest digestibility of alfalfa was estimated in the cut II as a result of intensive biosynthesis of structural carbohydrates and lignin and possibly unfavorable leaf to stem ratio.

Table 3. Content of aNDF and lignin and *in vitro* digestibility of dry matter and NDF

		Cut I	Cut II	Cut III	Cut IV	*X*
aNDF, g kg^{-1} DM	FB	408.6[C, c]	489.0[C, b]	549.9[A, a]	420.6[NS, c]	**467.0[c]**
	EBL	477.8[B, b]	536.8[B, a]	490.6[B, b]	445.1[NS, c]	**487.6[b]**
	MBL	508.3[A, c]	585.8[A, a]	539.4[A, b]	440.2[NS, d]	**518.5[a]**
	X	**464.9[b]**	**537.2[a]**	**526.6[a]**	**435.3[c]**	
Lignin, g kg^{-1} DM	FB	70.4[C, c]	77.3[C, b]	86.2[C, a]	51.3[C, d]	**71.3[c]**
	EBL	86.0[B, b]	89.9[B, a]	88.9[B, a]	83.3[B, c]	**87.0[b]**
	MBL	91.6[A, c]	96.1[A, b]	100.3[A, a]	91.7[A, c]	**94.9[a]**
	X	**82.7[c]**	**87.8[b]**	**91.8[a]**	**75.4[d]**	
Lignin, g kg^{-1} NDF	FB	172.3[B, a]	158.0[C, b]	156.8[C, b]	121.9[C, c]	**152.2[c]**
	EBL	180.0[A, b]	167.5[A, c]	181.2[B, b]	187.1[B, a]	**178.9[b]**
	MBL	180.2[A, c]	164.0[B, d]	185.9[A, b]	208.3[A, a]	**184.6[a]**
	X	**177.9[a]**	**163.4[c]**	**174.3[b]**	**173.2[b]**	
DNDF$_{30h}$, g kg^{-1} NDF	FB	341.1[A, a]	271.8[A, b]	270.1[A, b]	340.0[A, a]	**305.7[a]**
	EBL	278.8[B, b]	255.7[B, c]	248.8[C, d]	306.6[B, a]	**272.5[b]**
	MBL	211.2[C, c]	210.4[C, c]	257.2[B, b]	279.8[C, a]	**239.7[c]**
	X	**277.0[b]**	**245.9[d]**	**258.7[c]**	**308.8[a]**	
DNDF$_{48h}$, g kg^{-1} NDF	FB	422.3[A, b]	297.7[AB, d]	332.3[A, c]	498.7[A,a]	**387.7[a]**
	EBL	353.4[B, b]	275.7[B, c]	274.2[C, c]	506.5[A, a]	**352.5[b]**
	MBL	248.8[C, c]	303.5[A, b]	301.1[B, b]	468.2[B, a]	**330.4[c]**
	X	**341.5[b]**	**292.3[c]**	**302.5[c]**	**491.1[a]**	
In vitro DMD, g kg^{-1}	FB	735.2[A, b]	676.4[A, d]	711.3[A, c]	782.4[A, a]	**726.3[a]**
	EBL	653.6[B, c]	620.9[B, d]	675.5[B, b]	758.7[B, a]	**677.2[b]**
	MBL	629.3[C, c]	605.1[C, d]	667.1[B, b]	723.3[C, a]	**656.2[c]**
	X	**672.7[c]**	**634.1[d]**	**684.6[b]**	**754.8[a]**	

FB - Full Bud; EBL - Early Bloom, 10-15% of flowering; MBL - Mid Bloom, 50-60% of flowering; Different letters (A, B, C) denote significantly different means (p< 0.01) in the column, between the stages of growth and different letters (a, b, c, d) denote significantly different means (p< 0.01) in the row between the cuts

For livestock feeding, harvest at bud to early flower is recommended to provide forage with high to medium nutrient concentration. Lamb et al. (2003) reported decreased crude protein and increased fiber content as well as changes in leaf to stem ratio in alfalfa forage harvested at advancing maturity stages. The results of these investigations indicated that alfalfa harvested at full bud stage had greater leaf proportion than alfalfa harvested at early bloom or mid bloom stage. Other researchers also have reported decreases in leaf proportion with advancing maturity (Lamb et al., 2003; Sheaffer et al., 2000).

The digestibility of alfalfa depends on its fiber content. Riday et al., 2002 argue that the genetic variation of fiber content is one of the main reasons of forage quality variation. Variation in alfalfa forage digestibility and forage intake is correlated with the variation in the cell wall content. Knowing the genetic and seasonal variation of the fiber content of alfalfa forage is an important part of the strategy for developing new, higher quality cultivars of this crop.

Stage of maturity affects the forage quality more than any other factor, but the conditions in which the plant is grown and agronomic factors modify the effect of development on the plant quality and can lead to the quality varies from year to year due to seasonal variations, even when the plants are harvested at the same stage of maturity. The quality of forages is generally defined on the basis of animal performance and it can be said that it is a function of the nutrients concentration, amount consumed and digestibility. Among them, the digestibility is one of the most important qualities of forage nutritive value.

Lignification of alfalfa stem and consequent reduced digestibility occurs with high temperatures (Van Soest, 1994). Temperatures in our study were higher compared with temperature data reported by (Yu et al., 2003). Latitude may influence nutrient supply of forages as well. Deinum et al. (1981) found that digestibility of timothy grass harvested at the same morphological stage was higher at higher latitudes (69ºN *vs* 51ºN), mainly as a result of lower weather temperatures and longer day length. The current study was conducted at lower latitude (43ºN) than the study of Yu et al. (2003). However, more research is required to understand the effect of temperature and latitude on nutrient supply of alfalfa hay.

A number of factors influence DNDF (Neutral Detergent Fiber Digestibility) levels, including forage species, maturity, genetics and environment (Jensen et al., 2005). A higher digestible fiber is less filling because it is retained in the rumen for a shorter period of time. Since it is less filling in the rumen, diets containing highly digestible fiber allow greater dry matter intake (Kendall and Combs, 2004).

Conclusion

In conclusion, the measurement of NDF digestibility accurately and precisely is important in generating a quantitative summative energy prediction. It is useful for ranking forage quality on the farm for allocation to various animal groups. It is important to understand that *in vitro* NDF digestibility is measuring how much NDF a ruminant can digest. $NDFD_{30}$ and $NDFD_{48}$ of alfalfa varied from 245.9 in the cut II to 308.8 g kg^{-1} NDF in cut IV and from 292.3 in cut II to 491.1 g kg^{-1} NDF in cut IV, respectively, and can greatly affect the energy content, intake potential and animal performance associated with forage. Since the higher proportion of leaf tissue was observed in the cut III and cut IV than in the cut II,   it

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could lead to the conclusion that alfalfa digestibility was related to the leaf to the stem ratio. Shorter harvest interval in cuts III and IV could be the reason for improving alfalfa quality, as well as dry matter and NDF digestibility.

Acknowledgement

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Poster presentation

DETERMINING MARGINAL PRICES FOR DDGS IN DIETS FOR BROILERS BY LINEAR PROGRAMMING MODELS

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Abstract

The need for alternative source of fuel has led to a significant production of ethanol using cereal grain crops. This fact has results in opening of a huge number of ethanol factories in the past years, especially in neighboring countries (Croatia, Hungary, Bulgaria). Using corn for fermentation base, the obtained byproduct is DGS, and after drying it is DDGS (Distillers dried grains with solubles). Mainly used so far in ruminant feeds, this by-product has become available for non ruminants according to the increased availabilities of nutrients. We should not omit the price as one of the key factors. Since the DDGS is a by-product (not a conventional feed for non ruminants), the price must be a stimulating factor for farmers to choose this ingredient. In this paper, we have used the models of linear optimization to define marginal price for DDGS. Based on marginal prices, it can be concluded that, with the prices and feedstuffs used in optimization models in this paper, DDGS had higher value than its price. In the described conditions the price of this by-product justifies its usage in diets for broilers.
Keywords: DDGS, by-product, broilers, diets, price

Introduction

Animal nutrition is one of the greatest challenges in modern animal husbandry, because on the one hand it is necessary to meet all physical and physiological needs of the animals, on the other hand it is necessary to do this with the least-cost. Thus, in modern animal husbandry, the number of other feeds, like by-products (beet pulp, oil-seed-cakes, DDGS etc.) is increasing. The oil crises of the twentieth century have caused the search for alternative fuels. One of the most important and the best replacement for gasoline is found in the use of ethanol. Ethanol for this purpose was produced from corn grain. The Energy Independence and Security Act of 2007 increased the U.S. renewable fuel standard to a

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targeted 36 billion gallons by 2022, of which 15 billion gallons can be derived from conventional sources such as corn (Fox 2008). In the European Union the favored use of renewable energy sources has encouraged increased production of biofuels. The increase of bio-ethanol production has resulted in large quantities of DDGS entering the feed raw material market (Świątkiewicz and Koreleski 2008). Serbia currently uses only 30% of the available capacity for the production of bioethanol, but in the near future, Serbia need to define a clear strategy on filling the existing bioethanol production capacity, but also expansion and construction of new factories (Semenčenko et al., 2014). The certainty of this is reflected in international obligations that Serbia accepted by ratifying the Stabilisation and Association Agreement(SAA) with EU.

Fox (2008) reports that Livestock accounted for the bulk of US consumption, with the distribution across species at approximately 42% for dairy cattle, 42% for beef cattle, 11% for swine, and 5% for poultry. Beside that the high nutrient quality, related to the typically gentle drying and processing it receives, DDGS obtained from the modern ethanol industry may be used as a feed for monogastric animals with good results. (Świątkiewicz, and Koreleski, 2008). Corn DDGS is an good by-product for use in poultry diets and contains approximately 85% of the energy value in corn, has high levels of protein. Also essential amino acids, and is high in available phosphorus are benefits of DDGS. Layer and broiler diets can easily contain up to 10% DDGS with little, if any formulation adjustments for energy and amino acids. Swiatkiewicz and Korelski (2008) have found that is an acceptable ingredient of poultry diets and can be safely fed at 5-8% in starter diets for broilers and turkey, and 12-15% in grower-finisher diets for broiler and turkey and diets for laying hens. However, these are conservative dietary inclusion rates assuming that diets are not formulated on a digestible amino acid basis. Recent research studies (Shim et al., 2011; Loar et al., 2010; Masa'deh et al., 2011) have shown that DDGS can be added to poultry diets at even higher dietary inclusion rates (e.g. 20%) as long as accurate nutrient profiles specific to the DDGS source are used, and diets are formulated on a digestible amino acid basis. Noll (2005) recommended a maximum dietary inclusion level of 10% corn DDGS for meat birds. Lumpkins et al., (2004) indicated that DDGS from modern ethanol plants was an acceptable feed ingredient for broiler diets and could be safely used at 6% in the starter period and 12 to 15% in the grower and finisher periods. Diets fed in the study by Lumpkins et al., (2004) were formulated on the basis of total amino acids, which might account for the birds' inability to use greater than 6% in the starter diet or more than 12 to 15% in grower and finisher diets. Campasino et al., (2015) found that inclusion of DDGS in broiler diets reduced energy and nitrogen digestibility, and lead to negatively influenced broiler performance. The inclusion of nonstarch polysaccharide-degrading enzymes (NSPase) improved growth performance in low-energy diets and nutrient digestibility in diets containing DDGS (Campasino et al., 2015).

Material and Methods

The marginal prices for DDGS sample were obtained using Panonmix®, a linear programming based software.
Four linear optimization models were formulated, all using the same feedstuffs and the same feedstuff prices: corn grain, 18 RSD/kg; wheat grain, 17,5 RSD/kg; solvent-extracted soybean meal, 45 RSD/kg; expellers soybean meal, 45 RSD/kg; heat-treated soybean, 50 RSD/kg; L-lysine, 200 RSD/kg; DL-methionine, 500 RSD/kg and mineral and vitamin premix, 250 RSD/kg.

Table 1. Broiler nutrient requirements used in linear optimization models

Model 1	≥ 19% crude protein; ≥ 5% crude fat; ≤ 5% crude fiber; ≤ 8% ash; ≥ 0,9% lys; ≥ 0,7 met + cys
Model 2	≥ 17% crude protein; ≤ 6% crude fiber; ≤ 8% ash
Model 3	≥ 12,97 MJ/kg ME; ≥ 1,29% lys; ≥ 0,99 met + cys; ≥ 0,88% thr; ≥ 1,37% arg
Model 4	≥ 13,39 MJ/kg ME; ≥ 1,16% lys; ≥ 0,91 met + cys; ≥ 0,78% thr; ≥ 1,22% arg

The first two models included requirements proposed by Serbian regulation on feed quality (Serbian regulation, 2014), and the last two included requirements for the Ross 308 broiler strain (Aviagen, 2014). Requirements used in models are shown in Table 1.

Table 2. Chemical Composition of DDGS

	As-fed Basis	**DM Basis**
DM	88,97%	100,00%
Moisture	11,03%	-
Crude Protein	28,88%	32,46%
Crude Fat	8,46%	9,51%
Crude Fiber	7,76%	8,72%
Ash	4,02%	4,52%
NFE	39,85%	44,79%
NDF	29,23%	32,85%
ADF	10,85%	12,20%
Lignin	0,54%	0,61%
NPN	1,19%	1,34%

DM - Dry matter; NFE - Nitrogen free extract; NDF - Neutral detergent fiber; ADF - Acid detergent fiber; NPN - Non protein nitrogen;

Crude protein, crude fat, crude fiber and ash values for DDGS sample were analyzed by Serbian regulation (1987), (Table 2.). Crude protein for example: determined according to Babcock et al., (2008), while metabolic energy value and amino acid composition were calculated. Metabolic energy value was calculated by method proposed by NRC (1994), and amino acid composition by AMINODat 4 (Evonik Degusssa, 2010).

Results and Discussion

Marginal prices for DDGS sample obtained by linear programming models were higher than DDGS actual price in all models. In first two models marginal prices for DDGS were substantially higher than the actual price (41% and 24%), and in two last models, the difference was moderate (7%). The marginal prices are shown in Table 3.

Table 3. Marginal prices of DDGS obtained by four linear optimization models

	Marginal price, RSD/kg	Marginal price, % of actual price
Model 1	38,2	141
Model 2	33,5	124
Model 3	28,9	107
Model 4	28,9	107

The results in (Table 3) show that DDGS had higher value than price in used models. Also it can be noted that this advantage is related to high level of protein. But depending on the various factors, (differences in the protein content of the corn grain, differences in residual starch and differences in fermentation efficiency) it is possible significant variation DDGS protein level. This range could be pretty wide 23 to 32% (Spiehs et al., 2002; Lumpkins et al., 2005; Batal and Dale, 2006; Fastinger, et al., 2006; Evonik Degussa, 2010;). Therefore, the protein level determined by chemical analyses were crucial for the usage of DDGS in optimization for broiler diets. The amino acid content in DDGS in general can vary substantially. For instance, the content of the first-limiting amino acid for poultry, methionine, has been reported to range from 0.42% to 0.65% (Spiehs et al., 2002; Lumpkins et al., 2005; Fastinger et al., 2006; Wang et al., 2007; Świątkiewicz, and Koreleski 2008; Evonik Degussa, 2010;). Campasino (2015) reported that inclusion of DDGS reduced energy and nitrogen digestibility when included in broiler diets, but inclusion of NSPase improved growth performance in low energy diets and nutrient digestibility in diets containing DDGS. In this case with inclusion of NSPase, in DDGS based diets for broilers, marginal price will be additionally burdened. In our models that inclusion would probably turned off the usage of DDGS and of NSPase.

In review (Tahir, 2012) points out that linear programming for minimizing feed cost with respect to a set of restrictions (requirements and ingredient minima and maxima) was developed in the 1950s (Baum et al., 1953; Heady et al., 1956; Dent and Casey 1967; Wang 1996;). Glamočić et., al (2003.) compared some models and noted that the lowest average price in the reporting period had the wheat middlings and the highest fish meal. The highest coefficient of variation 28.6% had a corn grain, while soybean meal had the lowest coefficient of variation 6.6%. It must be pointed out that DDGS in this paper was not compared with cheep alternative feedstuffs like sunflower meal and wheat middlings, and that just limited number of nutrients was used in models. Using least cost optimization software (Panonmix,) in this analyses, related to the conditions in the defined models, showed that any fluctuation of variable price will changing the marginal price and the share of DDGS in the mixture.

Conclusion

Based on marginal prices, it can be concluded that, with the prices and feedstuffs used in shown models, DDGS had higher value than its price, offering opportunity for decreasing feeding costs of broilers. It must be pointed out that DDGS was not compared with cheep alternative feedstuffs like sunflower meal and wheat middlings, and that just limited number of nutrients was used in models. Including alternative feedstuffs or using digestible amino acids requirements could substantially influence results.

Acknowledgement

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Poster presentation

ADVANTAGES AND DISADVANTAGES OF WHOLE MILK AND MILK REPLACER IN FEEDING OF CALVES

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Abstract

Growing calves is one of the most important and most sensitive breeding procedures in cattle production. When comparing results of feeding whole milk and milk replacer to calves until the 60th day of age, several advantages and disadvantages may be determined from the viewpoint of production, health and economic issues. Calves fed whole milk until the 30th day of age exhibited better productive, health and economic outcomes than calves fed whole milk until the 20th and until the 10th day of age. The best results referring to growth are obtained in a group of calves fed whole milk for the longest period, and the smallest growth rate was exhibited by a group of calves fed whole milk for the shortest period of time. Enhanced feeding of calves with whole milk, based on 20% of calf body weight during the first 30 days of life, resulted in better weight gain, and such calves in general achieve better growth rate than calves fed milk replacer. On the other hand, several authors reported that the highest daily gain was achieved in groups of calves fed pure milk replacer, and the worst results were obtained in calves fed whole milk, so they concluded that feeding of calves with milk replacer achieved better daily weight gain than feeding with whole milk. When referring to health condition of calves, the least cases of diarrhea were reported in calves fed whole milk, while the most cases of diarrhea occurred in calves fed milk replacer. If taking into account the results referring to weight gain and occurrence of diarrhea, it is concluded that calves fed whole milk had the best weight gain with a minimum occurrence of diarrhea. In terms of economic efficiency of feeding whole milk to calves, and when considering the current price of milk and good growth performances, as well as general health conditions and calves' welfare, it can be stated that feeding whole milk to calves is fully justified and competitive.

Keywords: calves, whole milk, milk replacer

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Introduction

Each organism has a need for nutrient and energy intake to synthesize for basic and production needs. The feeding in each animal category is accurately balanced so that each animal has a physical intake of quantity and quality of food needed. In calves, it is of the utmost importance colostrum supply within the first hours of life. Colostrum is rich in proteins, mass and antibodies needed for calves' immunity preservation. After 3 to 5 days feeding with colostrum the calves were fed by whole milk and within 10 days of age a transfer to the milk replacer is possible. Daily liquid food consumption is 8-10 l daily and after 6 weeks, the reduction of liquid food is required in order to encourage calves to a greater dry food consumption and, therefore, develop the rumen. Calves are separated between 6 and 9 weeks of age. Separation of calves based on a daily starter mixture consumption is a more widespread principle in practice, after the calf's consumption of up to 1 kg of starter mixture three days in a row.

The problems in first weeks of calf's life are diarrhea and pneumonia. The most common causes of diarrhea are bacteria (Escherichia coli, Salmonella sp.) and viruses (rota virus, corona virus). Diarrhea occurrences could emerge in bad milk replacer feeding, insufficiently heated milk replacer or superabundant meal. The combination of low temperatures and high air humidity, the temperature oscillations and poor hygienic conditions are the most common causes of pneumonia in calves.

Production Effects of Milk and Milk Replacer

Healthy offspring growth is one of the most important and the most demanding production lines in beef production. A proper cultivation is reflected in the subsequent production and reproductive performances and service utilization in adult animals. The production performance of the female calves are dependent on the proper liquid food nutrition (Soberon et al., 2012). Heinrichs et al., (1995) quote that in the USA at the end of 20th century more than half of dairy farm calves were fed by milk replacer. The most important reason for the use of milk substitutes (replacers) in feeding calves is a favorable economic impact (Radivojević et al., 2008).

In addition to the whole milk in feeding calves, surplus colostrum is used, milk of the after calved cows that still does not go into the milk cooling tank or waste milk (Drackley, 1999). Cow whole milk usually contains approximately 12,5 % dry matter, 3,2% proteins, 3,7% fat and 4,6% lactose. These contents may vary, depending on the meal, lactation season and other factors. Pasteurized milk can also be given to the calves (Quigley, 2010). The advantages of such feeding is a reduced number of pathogenic bacteria compared to fresh milk, which may result in a reduced occurrence of diarrhea and other diseases. Pasteurized milk feeding presents a certain technological procedure which reduces the number of microorganisms and improves the quality of milk if fed with waste milk (Quigley, 2001). Looper et al., (2001) and Jamaluddin et al., (1996) quote that feeding calves with pasteurized waste milk affects the achievement of higher daily gain weight in relation to the supply of calves with raw milk.

Besides that, feeding with milk replacer is more expensive than feeding with pasteurized waste milk (Godden et al., 2005). Niwinska et al., (2004) have compared the effect of raw whole milk with the effect of the milk replacer containing 19,5% crude proteins and 15,4%

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crude fat. Calves fed with milk achieved statistically significant weight gain and consumed a larger amount of concentrate compared to calves fed by a milk replacer. Lee et al., (2009) quote that in experiments they used milk replacer with the same formulations as the whole milk, and over a period of 70 days of the experiment, the calves fed by whole milk had a higher body weight, was higher, longer and wider than calves fed with milk replacer. Taking into consideration the cost of gain weight during the first month of life, the competitiveness of full milk in relation to milk replacer has been confirmed (Domaćinović et al., 2009).

Table 1. Overview of diarrhea occurrences and calculation of costs of calves weight gain in the 1st month (Domaćinović et al., 2009)

Experimental period	Group 1	Group 2	Group 3
Diarrhea occurrence			
1st month	6	8	7
Cost per kg of gain			
1st month x %	23,36 100	30,30 129,7	37,08 158,7

Although most of scientific researches have shown that calves fed by whole milk generally achieved better production results than calves fed by milk replacer, certain researches have shown that well-balanced milk replacer formulations can produce equally good or even better performances than calves fed with whole milk.

Researches (El – Jack and Ahmed, 2012; Wagenaar and Langhout, 2007; Langhout, 2003.; Compinis et al., 2002.; Davis and Drackley, 1998) show that milk replacers can bring benefits to calf breeders and milk producers through the high quality products, easy storage, good production results disease control and cost-effectiveness increase. If we compare the effects of milk replacers of the highest quality with the effects of whole milk in diet of calves, very similar performances will be determined (Green, 1996). El – Jack and Ahmed (2012) have monitored the effect of feeding calves with milk replacer, containing 22.9 % crude protein and 10 % raw fat to the body's growth and economy of production. Their results showed that the calves fed with milk replacer achieved significantly higher daily weight gain and final body weight at weaning compared to calves fed with whole milk. Also, the cost of milk replacer was significantly lower than the cost of whole milk.

Chemical Composition and Nutritive Value of Milk Replacers

Milk substitutes (replacers) must be characterized by a high biological value protein, good water solubility, good taste, as well as satisfactory microbiological quality. The composition and quality of milk replacer affect the growth, health and overall production indicators of calves that consumed it. Modern producers of milk substitutes, inferior amino acid composition of some components, compensate by adding synthetic amino acids, usually lysine and methionine in their formulations. Milk replacers given to the calves in their first weeks of life should be of the highest quality. Milk substitutes that contain content non milk proteins, should be given to feeding older calves. The proteins of plant origin contain a higher total percentage of crude protein and the protein of organic origin have a better digestibility and preferred amino acid composition. The content of crude fiber influences the quality of milk replacer, which can be from plant sources of protein. Milk substitutes containing less than 0.15 % crude fiber do not contain proteins of plant origin.

The percentage share of crude protein in the composition of milk replacers ranges from 18-30 %.

Table 2. Nutritive values of milk replacement and starter diet fed to calves (Domaćinović et al., 2009)

Nutrients	Milk replacement	Starter diet
Dry matter, %	95,0	89,0
Crude protein, %	23	15
Crude fat, %	17	-
Crude fibers, max. %	2,5	10
Crude ash, %	9,0	10
Lysine, %	1,7	-
Ca, %	0,9	0,8
P, %	0,7	0,6
Na, %	-	0,2

The plant protein sources commonly used are the soy protein concentrate, soy flour, hydrolyzed wheat gluten. Soy protein sources result in poor performances on the animal and these substitutes should be enriched with additional methionine in order to achieve the highest weight gain. Ghorbani et al., (2007) report that calves fed with 25 % soy milk in 49 days of age achieved approximately the same weight as the calves were fed with 100 % whole milk. Also, the same authors report that feeding with soy milk to 50 % does not adversely affect the health status of calves, which is crucial in the first 4 weeks of life, when the calves very sensitive to substances of vegetable origin.

The most common source of protein of milk origin are skimmed milk powder, whey protein concentrate, dehydrated whey powder. Digestibility of milk proteins is generally greater than the digestibility of proteins that are not of milk origin (Davis and Drackley, 1998). The amino acid profile of whey protein concentrate is more suitable for feeding calves than skimmed milk powder and casein (Lammers et al., 1998).

The most common sources of animal protein are egg protein, fish protein, blood plasma. Feeding calves with milk replacer containing the A certain proportion of protein derived from fish led to results with lower digestibility of organic matter and less weight gain compared to calves fed milk protein origin.

Fats and oils are an important source of energy in the milk replacers. Nowadays, vegetable oils, soybean, palm and coconut oils are used, while the fats of animal origin were commonly used once. Milk fat has a 95 % digestibility which makes it nutritionally most appropriate for feeding calves. Fat in milk replacers ranges from 10-20 % (Quigley 2001).

Table 3. Example formulas of milk replacers with different levels od fat (Quigley, 2001)

Ingredient	10 % fat	15% fat	20 % fat
Whey	36	24	13

Whey Protein Concentrate (WPC 34%)	45	48	50
Fat (7/60)	16	25	33
Additives	3	3	4
TOTAL	100 %	100 %	100 %

Lactose is the main carbohydrate in milk replacers and the only carbohydrate that new-born calves are able to digest. The content of lactose in milk replacers is 40-45 %. During the first three weeks of life, the calf does not have the enzymes amylase and maltase, and can not efficiently digest the starch and its degradation products, dextrin and maltose. Therefore, in this period, even only 2% of the starch in dry matter milk replacer may cause a decline in digestibility (Krishnamoorhy and Moran, 2011). Thermal treatment of the starch and glucose leads to a partial gelatinization and enzymatic hydrolysis, which leads to greater utilization of starch.

Milk Replacer Supplements
Newer formulations of milk substitutes contain numerous additives, and their selection and concentration depend on the specificity purposes. Apart from vitamins and minerals, amino acids, antioxidants, different flavors , probiotics , emulsifiers , and other additives such as acidifiers , medicines , herbal extract and others are added into the milk replacers. Adding probiotics into the formulation of the present milk replacers has almost become a rule, but their effectiveness is still questionable. The enrichment of milk replacers by probiotic bacterial culture of *Lactobacillus acidophilus* did not affect the body weight of calves at weaning, the occurrence of diarrhea, utilization of food as well as the general health status of the calves (Cruywagen et al., 1996). Gorgulu et al., (2003) have determined in their research that the calves fed milk enriched with probiotic culture *Lactobacillus sp.* had better health status, less frequent incidence of diarrhea and lower cost of veterinary treatment compared to calves whose milk is not enriched by probiotics. There were no significant differences in weight gain between the experimental groups. Adding the probiotic culture *Enterococcus faecium* into the milk meal of the calves, showed good results in an increase in weight gain and in reducing the incidence of diarrhea and the presence of certain pathogenic bacteria in the faeces (Jatkauskas et al., 2010).

In recent years, there were more and more researches on the impact of immunoglobulin from chicken egg yolk to the protection and prevention of diarrhea caused by rotavirus and other pathogens. Adding the egg yolk enriched with specific immunoglobulins in the feeding of calves in the first 2 weeks of life is the defense of diarrhea caused by rotavirus (Vega et al., 2011).

Toll – Vera and Vera (1996) have confirmed that the feeding of calves by an acidified milk replacer reduced the problems with diarrhea and improved the overall health of the calves. Organic acids, citric, formic and propionic acid, are most commonly used acidifiers in milk-replacers.

Conclusion

In the early stages of life, the calves should be provided with the best possible conditions for accommodation and nutrition and health monitoring. The benefits of feeding calves

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with whole milk are better weight gain, better health condition and lower price growth due to the current milk price. Disadvantages of feeding calves with milk replacers are poorer and questionable formulations in milk replacers. In order to produce equally good or even better production results in calves, the formulations in milk replacers have to contain the highest quality ingredients, primarily of milk origin and certain additives. But in that case, because of the high cost of those components, the cost of feeding calves with such milk replacers would be questionable.

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Poster presentation

PRESENCE OF AFLATOXIN IN MAIZE DURING 2014 AND 2015 HARVEST SEASON

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Abstract

The aim of this study was to compare the presence and the content of aflatoxin in maize samples from two harvest seasons (2014-2015) in Serbia. A total of 162 samples of maize were analyzed using ELISA test. Out of 112 samples from 2014 harvest, aflatoxin was present in 4 (3.57%) samples with one sample above EU and Serbian regulations. During 2015, the occurrence of aflatoxin was somewhat higher (12%) in 50 analyzed samples, with four samples above EU and Serbian regulations. Unlike aflatoxin presence, its content differed more considerably between two harvest periods. In 2014, aflatoxin content was ranged from 5 to 22 µg/kg, with the average value of 11 µg/kg. On the other hand, in samples from 2015 harvest, the average content was 59 µg/kg (ranged from 7 to even 210 µg/kg). Although results from 2015 show high contamination level with aflatoxin, its low presence may lead to a conclusion that the high results are just sporadic cases of contamination and does not reflect the situation in the field. Also, results from both periods indicate that the high aflatoxin contamination that occurred during 2012 harvest is just an exception and that our country's climate still is not favorable for aflatoxin production in the field.

Keywords: aflatoxin, maize, ELISA, Serbia

Introduction

Mycotoxins, a natural food and feed contaminants, became an important factor in terms of food safety. Mycotoxin production can occur during plant growth, maturity, harvesting, storage, processing of grains and is influenced by various factors (temperature, relative humidity, oxygen availability, damaged or broken grain kernels) (Bernardo, 2004; Lanyasunya et al., 2005). The Food and Agriculture Organization (FAO) estimated that

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25% of the world's crops are affected at least by one mycotoxin (WHO, 1999). Aflatoxins (AFB1, AFB2, AFG1, AFG2, AFM1, AFM2) are secondary metabolic products of some *Aspergillus spp.*, of which the most common and the most famous is *Aspergillus flavus*. These mycotoxins and belong to a group of potent carcinogenic and teratogenic compounds (Kurtzman et al., 1987; Martins, 1989). The most potent known hepatocarcinogenic substance is aflatoxin B1 (AFB1) which has been proven to be also genotoxic (Van Egmond and Jonker, 2004; Zain, 2011). Aflatoxins are commonly found in peanuts, cottonseed, maize and their products. In wheat, rice, soybeans oats, and sorghum aflatoxins only rarely are noted (Sweets and Wrather, 2009).

Aflatoxin losses to livestock producers from aflatoxin-contaminated feeds include death and subtler effects of immune system suppression, reduced growth rates, and losses in feed efficiency. Other adverse economic effects of aflatoxins include lower yields for food and fiber crops (Anon, 1989).

Probably the most important way of exposure of children to aflatoxins is through cow's milk. The children are the most sensitive group since they consume large amounts of milk, especially babies. Namely, aflatoxin B1 from animal feed is converted to a hydroxylated compound (aflatoxin M1) which appears in the milk of lactating cows. It was found that aflatoxin M1 appear in milk within a few hours after aflatoxin B1 containing feed is consumed. The level of aflatoxin M1 in milk disappears within a few milkings after the source of aflatoxin B1 is removed from the diet (Harris and Staples, 2008).

Aflatoxin M1 is highly stable during normal milk processing. It was found that aflatoxin M1 concentration in milk cannot be reduced by vat pasteurization or high temperature short time pasteurization (Harris and Staples, 2008).

Serbia is one of the world's top producers and exporters of maize. It is ranked 15 based on maize production of 7 million metric tons (MT) (IndexMundi, 2016c) while it is ranked 10 regarding maize production growth rate of 16.76% (IndexMundi, 2016a). On the other hand, as maize exporter, Serbia is even better ranked. Based on maize export of 2.2 million MT, it is ranked 8 (IndexMundi, 2016b), and the rank of 7 was awarded according to maize export growth of 29.41% (IndexMundi, 2016).

Infection of maize by *Aspergillus spp.* usually occurs through the silk in hot weather conditions. The fungi then grow down the silk channel and around the developing ear. Yellow-brown silks that are still humid are the most susceptible to colonization and invasion down the silk channel. Fresh, not pollinated silks are relatively resistant, while brown, dry silks can be colonized, but growth of the fungus down the silk channel is limited (Vincelli, 1995). The fungus appears to grow from the ear tip toward the base by colonizing the silk first, then the glumes and finally the kernel surface. After the silk dies, the growth of the fungi is more powerful in hot weather. If conditions are favorable for the production of aflatoxin, it is more difficult to prevent infection from *Aspergillus spp.* and subsequent aflatoxin production (Anderson, 1983; Gardisser, 1989).

Contributing factor to *Aspegillus* infection is kernel damage by insects or birds which provides their easier colonization by the fungi. Even if kernels stay uninfected at moment of harvest, the presence of *Aspergillus flavus* spores on kernel surfaces may lead to post harvest infection, and consequently, aflatoxin contamination. Herman and Trigo-Stockli

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(2002) reported that aflatoxin production by *Aspergillus spp.* is highest at 25-32 °C, although the development of fungi usually stops when the temperature is below 13 °C and kernel moisture content is below 15%.

To reduce the risk of exposure, many countries have regulated the maximum level (ML) of AFB1 in maize. Currently, the legal limits of AFB1 in maize are highly variable from the European Union (EU) countries to other countries (the EU has a limit of 20 µg/kg) (European Commission, 2003). In Serbia, proposed ML of AFB1 in maize is somewhat higher (30 µg/kg) (Serbian Regulation, 2014), since the April 2014. Before that, Serbian legislative permitted the ML of total AFs in maize of 50 µg/kg (Serbian Regulation, 2010). Aflatoxin has long been monitored by the United States Food and Drug Administration, and a level of 20 µg/kg has been set as the limit of aflatoxin content for maize, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals (FDA, 2000).

Although aflatoxin contamination in maize is generally uncommon in Serbia, occasional incidents did occur and lead to significant economic losses for individual producers. Our limited monitoring data and experience in dealing with aflatoxin contamination indicate that the incidence of aflatoxin contamination in Serbia is similar to other European countries. One particular incident happened in 2013 which lead to a very high aflatoxin contamination of maize which was reported by many authors (Jajić et al., 2013; Kos et al., 2013; Lević et al., 2013). This lead to a maize export decreasing and major financial losses of Serbian industry (Jajić et al, 2013).

The aim of this work was to provide the information on the occurrence of aflatoxin in maize in 2014 and 2015 harvest season, relating to the high aflatoxin contamination during 2012/2013 period.

Material and Methods

Samples
Samples of maize (162) were collected randomly from different locations in Serbia during 2014, 2015 and 2016. Immediately after sampling, about 100 g of each sample were prepared by grinding in a laboratory mill in such a way that >93% passed through a sieve with pore diameter of 1.0 mm. Then, sample was homogenized by mixing and packed in plastic bags. Samples were stored in a freezer at -20 ºC until analysis. Prior to each analysis, the samples were allowed to reach room temperature.

Extraction
Exactly 20 g of samples were weighed in a 150 ml beaker. Aflatoxin was extracted with 100 ml of 70% methanol on an Ultra Turrax T18 homogenizer for 3 min at 11,000 rpm. Crude extract was then filtered through 6 Advantec filter paper.

Analysis
The immunochemical analysis was performed using the Veratox, Aflatoxin (Total), Quantitative Test Kit (Neogen, Lansing, MI, USA) with four calibration standard solutions (0, 5, 15 and 50 µg/kg). Analytical procedure was carried out according to manufacturer's procedure. Optical densities were obtained using the reader of microtiter plates with a 630 nm filter (BioTec Instruments, USA).

Quality control
In order to ensure quality of obtained results, method for determination of aflatoxin was validated. Validation parameters of the method were estimated according to European Commission (2006). Limit of detection (LOD) and limit of quantification (LOQ) were established by the manufacturer where LOD was 2 µg/kg, while LOQ was 5 µg/kg. LOQ was experimentally verified by analyzing blank sample of maize, spiked with aflatoxin B1 standard at level of 5 µg/kg. Samples containing less than 5 µg/kg of aflatoxin were considered as negative. Average recovery value, based on analysis of certified reference material TR-A100 (Trilogy, USA) was 108.7% which is within acceptable limits according to Commission Regulation 401/2006 (European Commission, 2006). Precision was estimated in terms of repeatability and reproductivity. Both parameters can be described as "acceptable" according to Commission Regulation 401/2006 (European Commission, 2006). Therefore, the method was suitable for the determination of aflatoxin in maize samples.

Results and Discussion

The monitoring of aflatoxin was done by analyzing a total of 162 samples of maize, using immunochemical ELISA test (results are presented in table 1). Out of 112 samples from 2014 harvest, aflatoxin was present in 4 (3.57%) samples with one sample above EU and Serbian regulations. During 2015, the occurrence of aflatoxin was somewhat higher (12%) in 50 analyzed samples, with four samples above EU and Serbian regulations. Unlike aflatoxin presence, its content differed more considerably between two harvest periods. In 2014, aflatoxin content was ranged from 5 to 22 µg/kg, with the average value of 11 µg/kg. On the other hand, in samples from 2015 harvest, the average content was 59 µg/kg (ranged from 7 to even 210 µg/kg).

Table 1. Occurrence of aflatoxin in maize samples during 2014 and 2015 harvest seasons in Serbia.

Harvest season	2014	2015	2014-2015
No. of samples	112	50	162
Average, µg/kg	11	59	39
Minimum, µg/kg	5	7	5
Maximum, µg/kg	22	210	210
No. of positive samples (%)	4 (3.6)	6 (12.0)	10 (6.2)
No. of samples > 0,02 µg/kg (%)	1 (0.9)	4 (8.0)	5 (3.1)
No. of samples > 0,03 µg/kg (%)	0 (0.0)	4 (8.0)	4 (2.5)

Overall presence of aflatoxins in two monitored harvest periods was 6.2% with the average aflatoxin content of 39 µg/kg. Although the average aflatoxin was very high, only 5 (3.1%) samples exceeded EU legislated maximum level. This number was slightly lower (2.5%) regarding Serbian legislation.

In recent years, aflatoxin contamination in maize was monitored in Serbia and its neighboring countries. Analysis of historical monitoring data may indicate potential patterns of aflatoxin incidence in our region.

Kos et al. (2013) were monitoring aflatoxin contamination from 2009 to 2012 harvest season using ELISA analytical technique. They did not detect aflatoxins in 180 samples collected during 2009 to 2011 harvest seasons. On the other hand, in 200 samples from 2012 harvest season they found aflatoxin presence in 68.5%, with content ranged from 1.01 to 86.1 µg/kg. Kos et al. (2014) also analyzed 40 maize samples from 2012 harvest. Aflatoxin was found in 60% of samples. The authors found that 25% of samples contained AFs at level of 1 to 10 µg/kg, 58% of samples at level between 10 and 50 µg/kg and 17% of samples with AFs content above 50 µg/kg.

In Romania, 56 cereal samples (corn, wheat, barley and oats) were investigated for *Aspergillus spp.* presence and aflatoxin content. Maize samples were the most infected cereal (80%). Aflatoxin was also detected mostly in maize, but at low levels (<10 µg/kg) (Tabuc et al., 2010). Also, Curtui et al. (1998) did not detect aflatoxin in 30 analyzed samples of maize On the other hand, Pleadin et al. (2014) analyzed 633 maize samples originating from 2013 in Croatia. They reported the mean value for AFB1 of 81 µg/kg. Also, they found very worrying level of 2072 µg/kg, as maximum level they detected in samples.

These literature data indicate that in recent years in Serbia and surrounding countries, aflatoxin contamination was at similar levels. Until 2012 harvest season, aflatoxin was not detected at concerning levels, although there were some indications of *Aspergillus spp.* presence in Romania. As reports from 2013 showed, there was a high aflatoxin contamination in both Serbia and Romania during 2012 harvest season. These results were explained by the uncommon high frequency and incidence of *Aspergillus flavus* infection of maize which was caused by extremely high temperature and drought (Lević et al., 2013). During the observed period (2014 – 2015) the weather conditions were not favorable for *Aspegillus spp.* growth and aflatoxin production, particularly in 2014. According to the report of the Republic Hydrometeorological Service of Serbia (2014), during the vegetation period (April-September) on the territory of Serbia, a very heavy and intensive rainfall was recorded. Summer of 2014 (June – August) was characterized as moderately warm and extremely humid and rainy weather. The highest daily temperatures were below long-term average. Moderately warm and rainy weather also prevailed during September. The vegetation period of 2015 (April – September) on the territory of Serbia was warmer with less precipitation compared to the long-term average. Normal raining conditions prevailed at the largest part of the territory of Vojvodina and in southeast Serbia. In general, for this production year could be said that weather conditions were mostly just about average (Republic Hydrometeorological Service of Serbia, 2015).

The results from this study indicate that the aflatoxin presence and its content returned to the levels before 2012 harvest season. Although results from 2015 show high contamination level with aflatoxin, its low presence may lead to a conclusion that the high results are just sporadic cases of contamination and does not reflect the situation in the field. Also, results from both periods indicate that the high aflatoxin contamination that occurred during 2012 harvest is just an exception and that our country's climate still is not favorable for aflatoxin production in the field.

Acknowledgment

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Poster presentation

MYCOTOXINS IN COMPLETE FEEDING MIXTURES FOR MILKING COWS

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Abstract

The aim of this research was to investigate the presence of aflatoxin (AF) and deoxynivalenol (DON) in samples of complete feeding mixtures for milking cows. Samples originating from northern Serbian province Vojvodina, were collected during two different periods (2013/2014 and 2015/2016). A total of 136 samples were analyzed using ELISA test kits for the presence of aflatoxins (107) and deoxynivalenol (29). The results show higher presence of AF in 2013/2014 period (87.7%) than in 2015/2016 period (2.9%) which can be explained by favorable weather conditions for aflatoxin production during the first period. On the other hand, DON presence was quite similar and very high, during both periods (100% and 81.8%). Regarding content of mycotoxins, aflatoxins showed considerably high content in 2013/2014 period with the average value of 19 µg/kg, (ranged from 6 to 119 µg/kg) and 28 samples above EU and Serbian regulations. In the second period, only one sample was positive, with AF content of 6 µg/kg. Although, DON presence was quite high in both periods, its content was well below EU and Serbian regulations (ranged from 270 to 621 µg/kg in the first period, and from 280 to 1453 µg/kg in the second period). High presence of aflatoxin in mixtures for dairy cows reflects on the production and quality of milk and it is necessary to continuously monitor its occurrences. On the other hand, due to the nature of cow metabolism, the presence of DON is not significant when it comes to state of health of the cow and milk production and quality.

Keywords: ELISA, milking cows, mycotoxins, Serbia

Introduction

Mycotoxins are well-known natural food and feed contaminants. Among mycotoxins, aflatoxins (AFs) are the most important and most studied ones. These compounds are secondary metabolic products of some *Aspergillus spp.* molds, usually *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius*. Aflatoxins belong to a group of potent carcinogenic and teratogenic compounds (Kurtzman et al., 1987; Martins, 1989). The most potent known hepatocarcinogenic substance is aflatoxin B1 (AFB1) which has been proven to be also genotoxic (Van Egmond and Jonker, 2004; Zain, 2011). Aflatoxins are frequently found in peanuts, cottonseed and maize. In wheat, rice, soybeans oats, and sorghum aflatoxins are only rarely noted (Sweets and Wrather, 2009).

Deoxynivalenol (DON) is the most widely spread mycotoxin from the trichothecene group, most frequently produced by *Fusarium graminearum* and *Fusarium culmorum* molds.

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Maize is one of the most sensitive cereals to the presence of these molds. A disease of maize caused by this molds is known as *Gibberella* ear rot (JECFA, 2001). Animals differ in terms of susceptibility to DON. Pigs and poultry are highly sensitive while ruminants are relatively resistant (Pestka, 2007).

In Serbia, maize is the main carbohydrate feedstuff used in dairy cow diets, so this feedstuff probably poses the highest risk of aflatoxin and DON intake in these animals.

Probably the most important way of exposure of children to aflatoxins is through cow's milk. The children are the most sensitive group since they consume large amounts of milk, especially babies. Namely, aflatoxin B1 from animal feed is converted to a hydroxylated compound (aflatoxin M1) which appears in the milk of lactating cows. It was found that aflatoxin M1 appear in milk within a few hours after aflatoxin B1 containing feed is consumed. The level of aflatoxin M1 in milk disappears within a few milkings after the source of aflatoxin B1 is removed from the diet (Harris and Staples, 2008). In addition, aflatoxin M1 is highly stable during normal milk processing and was found that aflatoxin M1 concentration in milk cannot be reduced by vat pasteurization or high temperature short time pasteurization (Harris and Staples, 2008).

To reduce the risk of exposure, many countries have regulated the maximum level (ML) of AFB1 and DON in maize. Currently, the EU legal limit for AFB1 in maize is 20 µg/kg (European Commission, 2003). In Serbia, proposed ML of AFB1 in maize is somewhat higher (30 µg/kg) (Serbian Regulation, 2014). Regarding dairy feed, the EU has set the limit of 5 µg/kg (European Commission, 2003), which identical to Serbian legislation (Serbian Regulation, 2014).

European Union regulated DON content in cereals and cereal products with the exception of maize by-products at 8 mg/kg. In complementary and complete feeding stuffs is 5 mg/kg with the exception of complementary and complete feeding stuffs for pigs (0.9 mg/kg) and complementary and complete feeding stuffs for calves (older than 4 months), lambs and kids (2 mg/kg) (European Commission, 2006a). In April 2014, the Serbian legislation was entirely harmonized with the European legislation, regarding DON content in feed (Serbian Regulation, 2014).

The aim of this research was to investigate the presence of aflatoxin (AF) and deoxynivalenol (DON) in samples of complete feeding mixtures for milking cows collected during two different periods (2013/2014 and 2015/2016) in northern Serbian province of Vojvodina.

Material and Methods

Samples
Samples of complete feeding mixtures for milking cows (136) were collected randomly from different locations in northern Serbian province of Vojvodina during two different periods (2013/2014 and 2015/2016). Immediately after sampling, about 100 g of each sample were prepared by grinding in a laboratory mill in such a way that >93% passed through a sieve with pore diameter of 1.0 mm. Then, sample was homogenized by mixing and packed in plastic bags. Samples were stored in a freezer at -20 ºC until analysis. Prior

to each analysis, the samples were allowed to reach room temperature.

Extraction
Exactly 20 g of samples were weighed in a 150 ml beaker.   Aflatoxin was extracted with 100 ml of 70% methanol on an Ultra Turrax T18 homogenizer for 3 min at 11,000 rpm. Crude extract was then filtered through 6 Advantec filter paper. DON was extracted the same way, but using distillated water as opposed to 70% methanol.

Analysis
The immunochemical analysis was performed using the Veratox for Aflatoxin (Total), Quantitative Test Kit (Neogen, Lansing, MI, USA) with four calibration standard solutions (0, 5, 15 and 50 µg/kg), and Veratox for DON 5/5 NE, Quantitative Test Kit (Neogen, Lansing, MI, USA) with five calibration standard solutions (0, 0.25, 0.5, 1.0 and 2.0 µg/kg). Analytical procedure was carried out according to manufacturer's procedure. Optical densities were obtained using the microtiter plates reader with a 630 nm filter (BioTec Instruments, USA).

Quality control
In order to ensure quality of obtained results, method for determination of aflatoxin was validated. Validation parameters of the method were estimated according to European Commission (2006). Regarding aflatoxin, limit of detection (LOD) and limit of quantification (LOQ) were established by the manufacturer where LOD was 2 µg/kg, while LOQ was 5 µg/kg. LOD for DON was 100 µg/kg and LOQ was 250 µg/kg. LOQ was then experimentally verified by analyzing blank sample of feeding mixture, spiked with aflatoxin B1 standard at level of 5 µg/kg and DON standard solution at level of 250 µg/kg. Samples containing less than 5 µg/kg of aflatoxin and 250 µg/kg of DON were considered as negative. Average recovery value for aflatoxin, based on analysis of certified reference material TR-A100 (Trilogy, USA) was 108.7% which is within acceptable limits according to Commission Regulation 401/2006 (European Commission, 2006). Average recovery values for aflatoxin and DON, based on analysis of certified reference materials TR-A100 (Trilogy, USA) and BRM 3024 (Romer Labs, USA), were 108.7% (aflatoxin) and 107.9% (DON). Obtained recovery values were within acceptable limits according to Commission Regulation 401/2006 (European Commission, 2006b).

Precision was estimated in terms of repeatability and reproductivity. Both parameters can be described as "acceptable" according to Commission Regulation 401/2006 (European Commission, 2006). Therefore, the method was suitable for the determination of aflatoxin and DON in feed samples.

Results and Discussion

A total of 136 samples were analyzed using ELISA test kits for the presence of aflatoxins (107) and deoxynivalenol (29). The results (Table 1) show considerably high presence of AF in 2013/2014 period (87.7%) and then very low presence in samples collected during 2015/2016 period (2.9%). On the other hand, DON presence was very high during both periods (100% and 81.8%). Regarding content of mycotoxins, aflatoxin displayed noticeably high content in 2013/2014 period with the average value of 19 µg/kg, (ranged from 6 to 119 µg/kg) where 28 samples were above EU and Serbian regulations. In the second period, only one sample was positive, with AF content of 6 µg/kg. Although, DON presence was quite high in both periods, its content was well below EU and Serbian regulations (ranged from 270 to 621 µg/kg in the first period, and from 280 to 1453 µg/kg

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in the second period).

Table 1. Occurrence of aflatoxins and deoxynivalenol in samples of complete feeding mixtures for milking cows during two different periods in Serbia.

Mycotoxin	Aflatoxin		Deoxynivalenol	
Collecting period	2013/2014	2015/2016	2013/2014	2015/2016
No. of samples	73	34	18	11
Average, µg/kg	19.3	6	429	946
Minimum, µg/kg	6	6	270	280
Maximum, µg/kg	119	6	621	1453
No. of positive samples (%)	64 (87.7)	1 (2.9)	18 (100.0)	81.8%
No. of samples above SRB and EU regulations (%)	28 (38.4)	0 (0.0)	0 (0.0)	0 (0.0)

As previously mentioned, maize is the main source of mycotoxins in complete feeding mixtures, therefore the climate factors that influence mold growth and mycotoxin production in this feedstuff were considered. Since the sample collecting periods were 2013/2014 and 2015/2016, the maize used in feeding mixtures predominantly originated from 2013 and 2015 harvest.

Infection of maize by *Fusarium spp.* most frequently takes place in the phase of maize flowering (Sutton, 1982). Humid weather conditions in the period from silking to ripening enables maize contamination (Vigier et al., 1997). The ear is the most sensitive to contamination at the beginning of silking (Reid and Hamilton 1996). The silking period in Serbia takes place during the month of July and the first half of August. On the other hand, infection of maize by *Aspergillus spp.* usually occurs through the silk in hot weather conditions (Vincelli, 1995). After the silk dies, the growth of the fungi is more prevailing in hot weather (Anderson, 1983; Gardisser, 1989). Herman and Trigo-Stockli (2002) reported that aflatoxin production by *Aspergillus spp.* is highest at 25-32 °C, although the development of fungi usually stops when the temperature is below 13 °C and kernel moisture content is below 15%.

In 2013, the critical period for *Fusarium* infection of maize (July-August) was warmer than usual with much less rainfall in relation to long-term average. The amount of rainfall was the smallest in the northern parts of Vojvodina region. The most unfavorable period for most crops lasted from mid-July to the end of the second week of August, and the most affected were the spring crops. Precipitation that overcame during the last week of August only partially mitigated the effects caused during the dry season (Republic Hydrometeorological Service of Serbia, 2013). These weather conditions probably were not favorable for *Fusarium* infection. However, the dry weather conditions supported *Aspergillus spp.* growth and aflatoxin production. Regarding 2015, the vegetation period (April – September) on the territory of Serbia was warmer with less precipitation compared to the long-term average but normal raining conditions prevailed in Vojvodina region. Overall, in this production year the weather conditions were around average (Republic Hydrometeorological Service of Serbia, 2015). Since the samples originated from region of Vojvodina, normal raining conditions that prevailed during critical period for *Fusarium* infection of maize, could lead to DON production. On the other hand, these weather conditions were much less favorable for *Aspergillus* mold growth and later production of aflatoxins.

In our samples, the presence of AF in 2013/2014 collecting period was higher (87.7%) compared to 2015/2016 period (2.9%) which can be explained by favorable weather conditions for aflatoxin production during the first period. On the other hand, DON presence was quite similar and very high, during both periods (100% and 81.8%). Although, the weather conditions were unfavorable for *Fusarium* growth during critical period in 2013, the humid weather in the second decade of September probably resulted in DON production. Regarding content of mycotoxins, aflatoxins showed considerably high content in 2013/2014 period, ranged from 6 to 119 µg/kg and 38.4% of samples above EU and Serbian regulations. This was possibly an outcome of hot and dry weather during summer months of 2013. In the second period, when the weather conditions were humid, as expected, only one sample was positive with low AF content. As previously stated, DON presence was quite high in both periods but its content was well below EU and Serbian regulations. Somewhat higher DON concentrations found in the second period are probably a result of more humid weather conditions.

High presence of aflatoxin in mixtures for dairy cows reflects on the production and quality of milk and it is necessary to continuously monitor its occurrences. On the other hand, due to the nature of cow metabolism, presence of DON is not significant when it comes to state of health of the cow and milk production and quality. This study indicates the existence of a serious risk related to the occurrence of aflatoxin and DON in the food chain of Serbia and importance of frequent monitoring of these mycotoxins. Since the Serbian regulation for control of mycotoxins in animal feed was adopted and harmonized with EU regulations, it can be predicted that in the future there will be more frequent control of mycotoxins and more data about their occurrence in Serbia.

Acknowledgment

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Poster presentation

EFFECT OF *PEGANUM HARMALA* SEEDS ON BLOOD FACTORS, IMMUNE RESPONSE AND INTESTINAL SELECTED BACTERIAL POPULATION IN BROILER CHICKENS

Goudarzi M.[1], Nanekarani Sh.[1]

Abstract: This experiment was designed to study the effects of feeding different levels of *Peganum harmala* seeds (PHS) and antibiotic on serum biochemical parameters, immune response and intestinal microflora composition in Ross broiler chickens. A total of 240 one-d-old unsexed broiler chickens were randomly allocated to each of the 4 treatment groups, each with 4 replicate pens of 15 chicks. The dietary treatments included of control (C) - without PHS and antibiotic - the diet contains 300 mg/Kg Lincomycin 0.88% (A) and the diets contain 2g/Kg (H1) and 4g/Kg (H2) PHS. The chicks were raised on floor pens and received diets and water ad libitum for 6 weeks. Blood samplings were performed for determine of antibody titer against Newcastle disease on 14 and 21 days and for biochemical parameters on 42 days of age. The populations of Lactobacilli spp. and Escherichia coli were enumerated in ileum by conventional microbiological techniques using selective agar media. Inclusion of PHS in diet resulted in a significant decrease in total cholesterol and significant increase in HDL relative to the control and antibiotic groups. Antibody titer against NDV was not affected by experimental treatments. The *E. coli* population in birds supplemented with antibiotic and PHS was significantly lower than control but the *Lactobacilli spp.* population increased only by antibiotic and not by PHS. In conclusion, the results of this study showed that addition of *Peganum harmala* seeds powder seem to have had a positive influence on some biochemical parameters and gastrointestinal microflora.

Keywords: antibiotic, biochemical parameters, broiler, immune system, *peganum harmala.*

Introduction

Growth promoters such as antibiotics play an active role in commercial production of poultry. Although, good results are obtained with these substances, their use might have unfavorable effects, in other side it may also result in production of residual problems in the tissues of birds and animals (Burgat, 1991). For this reason, most antibacterial performance promoters recently have been banned in many countries. It is indispensable to minimize these components, and deals with replacers without any adverse effect on production. The medicinal plants have been used traditionally in the therapy of some diseases in human for a long time. Also in recent years the effects of medicinal herbs have been studied on various indices such as performance, immunity and serum biochemical profiles in broilers (Nanekarani et al., 2012; Goodarzi et al., 2013 and 2014). In practice, herbs, their extracts and the components extracted from them have been used as an alternative for antibiotic growth promoters (Ocak et al., 2008; Ayasan et al., 2013).

Peganum harmala - known as "Espand" in Iran- belongs to family Zygophylaceae is a traditional medicine. It has been employed for the treatment of a range of human diseases (Aslam et al., 2014). This herb contains several the beta carboline alkaloids such as

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harmaline, harmine, harmalol and harmol. They have some of pharmacological and biological activities such as antibacterial and antifungal (Darabpour et al., 2011), anticoccidial (Tanweer et al., 2014), disinfectant (Shahverdi et al., 2005), growth promoting (Tanweer et al., 2012), cholesterol lowering and hepatoprotective effects (Eini et al., 2014), Glucose lowering (Singh et al. 2008), monoamineoxidase inhibition (Salari et al., 2012), hypothermic (Abdel-Fattah et al., 1995), platelet aggregation inhibitory Saeed et al., 1993), immunomodulatory effects (Ghareghani Poor et al., 2014) and anti-inflammatory (Monsef et al., 2004).

Arshad et al., (2008) announced that the extract of *Peganum harmala* has limited antimicrobial activity against E. coli in vivo. Tanweer et al., (2014) concluded that P. harmala has the anticoccidial effect in broiler chicks. Tanweer et al., (2013) reported that the blood HDL and LDL concentration increased and decreased, respectively, by using of methanolic extract of Harmal in diet. In spite of these findings, there has been a dearth of information on the effect of harmala on, serum biochemical parameters, and intestinal microflora in comparison with an antibiotic growth promoter in broiler chickens. Therefore, the present study was design to compare and survey the effect of two levels of P. harmala and Lincomycin antibiotic onance, serum biochemical parameters, and intestinal microflora in broiler chicks.

Material and Methods

A total of 240 one-d-old unsexed broiler chickens (mean initial weight: 37.5 ± 1 g) were randomly allocated to each of the 4 treatment groups, each with 4 replicate pens of 15 chicks. The dietary treatments included of control (C) - without *Peganum harmala* seeds (PHS) and antibiotic - the diet contains 300 mg/Kg Lincomycin 0.88% (A) and the diets contain 2g/Kg (H1) and 4g/Kg (H2) PHS. The basal diet (Table I) was formulated according to the nutrient requirements (NRC 1994) based on corn and soybean meal: starter (1 to 21 d) and grower (22 to 42 d). *Peganum harmala* seed powder and antibiotic were added on top of the basal diets. The *P. harmala* were supplied from a local market and the dry seeds were cleaned of foreign materials and seeds and milled to a soft powder. The chicks were raised on floor pens (0.096m2/bird) for 6 weeks and during this period, they received diets and water ad libitum. The bird had access to feed and water through a tube feeder and a manual waterer in each pen. The chicks were reared under a lighting program which included of 23 hours of light and 1 hour of darkness. The ambient temperature was 32oC $\pm$ 1 during the first week, and it was reduced three degrees per week in following weeks and finally it was maintained at 22oC.

At the end of period after 8 hours of starvation, the birds were killed by serving the jugular vein and carotid artery on one side of the neck and allowed to bleed. The blood samples were collected from 8 birds in each treatment to assess of biochemical parameters. The blood was centrifuged at 2000 ×g for 15 min to obtain serum (SIGMA 4 - 15 Lab Centrifuge, Germany). Individual serum samples were analyzed for total protein, total cholesterol, high-density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride (Pars-Azmoon Co., Tehran).

The birds were vaccinated against Newcastle disease virus (NDV) subcutaneously with 0.2 mL per bird at 7 days of age. The blood samples were collected from the brachial vein of two randomly selected birds of each replicate at 14 and 21 days of age. The samples were centrifuged at 2000 ×g for 15 min to obtain serum (SIGMA 4 - 15 Lab Centrifuge)

Table 1. The ingredient and calculated composition of basal diets

Item	Starter (0-21)	Grower (22-42)
Ingredient, g/kg		
Corn	543.5	656.3
Soybean meal (43.8%)	378.1	292.8
Soybean oil	40.0	17.5
Di calcium phosphate	12.9	10.9
Oyster sell	14.1	13.2
NaCl	4.4	3.1
Mineral premix1	2.5	2.5
Vitamin premix2	2.5	2.5
DL-Methionine	1.4	0.6
Lysine	0.6	0.6
Analysis results		
Metabolizable energy (kcal/kg)	3000	3000
Crude protein (g/kg)	215.7	187.6
Calcium (g/kg)	9.4	8.4
Available phosphorus (g/kg)	3.8	3.3
Methionine(g/kg)	4.9	3.7
Lysine(g/kg)	12.0	1.0
Methionine + Cysteine (g/kg)	8.4	6.8

1-Ingredients per kg: Mg, 60 g; Fe, 80 g; Cu, 10 g; Zn, 50 g; Co, 2 g; I, 1 g,
2- Ingredients per kg : vitamin A, 1000,000 IU; D3, 1500000 IU; E, 15000 IU; K, 3g; B1 2g; B2, 4 g; B6, 3g; B12, 0.015 g; pantothenic acid, 10 g; nicotinic acid, 2 g; folic acid, 1 g; choline, 250g ; Se, 100 g

Germany). Antibody titers against NDV were measured by hemagglutination Inhibition Test according to procedure described by (Thayer et al., 1998).

For a determination of some selected micro-organisms in intestinal digesta from 32 birds (2 birds per replication) the digesta samples were collected from ileum and used for microbial assays within 1 h from collection. Digesta samples were serially diluted in 0.85% sterile saline solution for enumeration of *Lactobacilli spp.* and *Escherichia coli* (E. coli) by conventional microbiological techniques using selective agar media. All microbiological analyses were performed in duplicate and the average values were used for statistical analysis. *Lactobacilli spp.* was anaerobically assayed using MRS agar (Fluka 80961). Lactobacilli spp. was confirmed by using API 50 CH kit (Biomerieux_ SA, Marcy-l'Etoile/France). *E. coli* were enumerated through the use of Plate Count MUG Agar (Fluka 80961) and TBX Agar (Fluka 92435). Results were expressed as base-10 logarithm colony-forming units per gram of digesta.

All data were analyzed by one-way ANOVA using the GLM procedure of SAS for Windows version 9.1 (SAS, 1998). The data were analyzed base on following model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij,}$$

where Y_{ij} is the dependent variable, μ is the general mean, T_i is the treatment effect of the ith treatment, and ε_{ij} is the random error. The significance of differences between means was compared by using of the Duncan's multiple range tests of SAS. Significance was declared at P≤ 0.05 for all variables measured.

Results and Discussion

Inclusion of 4 and 2 g/Kg PHS in diet resulted in a significant decrease in total cholesterol relative to the control and antibiotic groups (Table II). The HDL was increased by using 2 g/Kg PHS in diet. The other parameters include triglyceride, LDL and total protein were not affected by dietary treatments.

Tanweer et al., (2013) found that 250 mgL-1 methanolic extract of *Peganum harmala L.* in drinking water reduced total cholesterol, triglycerides and LDL cholesterol and increased HDL cholesterol in broiler chicks. Eini et al., (2014) reported similar results in male Wistar Rat. Qazan (2009) found that the concentration serum cholesterol decreased in the chicks fed a diet containing 10% *Peganum harmala L.* leaves. Abaza et al., (2003) fed 2.5 kg P. harmala seeds and 2.5 kg chamomile flower heads per ton of broiler diet and observed reduced serum cholesterol as compared to the control group. In contrast to these findings, Hasanzadeh et al., (2013) did not observe significant difference in total cholesterol and HDL by using aqueous and ethanolic extract of Harmala seeds. The variation in findings of reducing cholesterol is probably due to the difference in experimental designs, ages and species of animal and types and parts of the plant used. The key enzyme involved in regulating cholesterol metabolism is 3-hydroxy-3methyl-glutaryl-CoA (HMG-CoA) reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway. By competitively blocking this enzyme, the HMG-CoA reductase inhibitors interfere with cholesterol formation. As a result, they decrease total cholesterol, LDL, apolipoprotein B (a membrane transport complex for LDL-C), very low-density lipoprotein (VLDL), and plasma triglycerides. They also increase serum concentrations of HDL (Ciftci et al., 2010). It is possible that the HMGCoA reductase has been inhibited by different alkaloids harmine, harmaline, harmol, present in *P. harmala*. This plant has antioxidant property (Jinous and Fereshteh, 2012) which reduce LDL oxidation and thereby reducing total cholesterol content. Berrougui et al., (2006) reported that the harmaline had a markedly higher antioxidant capacity than harmine in scavenging or preventive capacity against free radicals as well as inhibiting the aggregation of the LDL protein moiety (apolipoprotein B) induced by oxidation.

Table 2. Effect of experimental diets on biochemical parameters

Variable	Treatments(mg/dl)				
	C	A	H1	H2	SEM
Triglyceride	69.50	74.00	80.50	85.00	3.31
Total Cholesterol	119.30ab	125.50a	108.00b	113.25b	2.32
LDL - Cholesterol	15.00	13.75	14.00	13.75	0.69
HDL - Cholesterol	84.75ab	82.00b	91.00a	86.75ab	1.46
Total protein	3.20	3.25	3.18	3.20	0.05

*Values in the same row not sharing a common superscript differ significantly (P≤0.05). SEM = Standard error of mean

As shown in Table III the using of antibiotic and PHS in diet failed to induce any significant effects on antibody titers against NDV at 14 and 21 days of age (P > 0.05).

Result of this trial showed that, humoral immune responses were not affected by dietary treatments. As harmala has been reported to have antimicrobial (Darabpour et al., 2011) and antioxidant activities (Tanweer et al., 2014), an increase in immune responses of chicks was

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anticipated. Similar results have been reported in some previous researches (Rahimi et al., 2011; Goodarzi et al., 2013) with other herbs. Unfortunately, there are a few reports on the effects of harmala on bird immune responses. The result of this study is in contrast to the results of Tanweer et al., (2014) who reported that the methanolic extract of *P. harmala* significantly improved antibody titer against ND at day 21 and 28 when used at the rate of 250 mg/L of drinking water. Ghareghani Poor et al., (2014) reported that *P. harmala* extract with optimal dose of 100 mg/kg can act as immunostimulants and enhance the immune response of cultured fish.

Table 3. Effect of experimental diets on antibody titers against NDV at 14th and 21st days

Antibody titers (log10)	Dietary treatments				
	C	A	H1	H2	SEM
14 days	0.696	0.750	0.706	0.737	0.014
21 days	0.812	0.817	0.807	0.828	0.013

SEM = Standard error of mean

Data on ileum bacteria populations of broiler chicks at Day 42 of age (table IV) showed that the using antibiotic and PHS in diet had significant (P<0.05) effect on *E. coli* and *Lactobacilli spp.* populations. The *E. coli* population in birds supplemented with antibiotic and PHS was significantly lower than control. The effect of antibiotic on it was bigger than PHS. The difference between H1 and H2 groups were not significant. The *Lactobacilli spp.* population increased only by antibiotic and not by PHS. The differences between control and PHS groups were not significant (P>0.05).

Table 4. Effects of dietary treatments on ileum bacteria populations of broiler chicks at d 42 of age.

Variable	Dietary treatments				
	C	A	H1	H2	SEM
E. coli (CFU g-1)	6.875[a]*	5.850[c]	6.425[b]	6.325[b]	0.102
Lactobacilli spp. (CFU g-1)	4.675[b]	5.225[a]	4.700[b]	4.850[b]	0.082

*Values in the same row not sharing a common superscript differ significantly (P≤0.05).
 SEM = Standard error of mean

It has been reported that the *Peganum harmala* has antibacterial, antifungal and antiviral effects (Shonouda et al., 2008). It traditionally has been used as an antiseptic and disinfectant agent by burning its seeds in Iran (Arshad et al., 2008). An ethanolic *P. harmala* extract has been shown to have high antibacterial activity against *MRSA* (methicillin resistant *Staphylococcus aureus*) (Moghadam et al., 2010) and *CRSA* (cefixime resistant *S. aureus*) (Prashanth and John, 1999). Goodarzi et al., (2013) and Darabpour et al., (2011) showed inhibitory effect of alcoholic extract of *P. harmala* seeds on the growth of *E. coli*. Arshad et al., 2008) announced that the extract of *Peganum harmala* has limited antimicrobial activity against *E. coli* in vivo, but long-term continuous feeding may induce undesirable effects. Abdel Aziz et al., (2010) reported that the β–carboline alkaloid isolated from the aerial parts of *Peganum harmala* showed significant antibacterial activity against *Streptococcus pyogenus*. It has been reported that harmane as a highly aromatic planar alkaloid exerts its antibacterial activity through interchalate with DNA (Cowan, 1999). Thus, this antibacterial mechanism must be considered for active extract of *P. harmala*.

Conclusion

The results of this study showed that *Peganum harmala* seeds have positive effect on some serum biochemical parameters and ileum bacteria populations in broiler chicks. The adding of *Peganum harmala* seeds in diet resulted in a significant decrease in the total cholesterol and a significant increase in the HDL. Also, the using PHS in diet led to the lower *E. coli* population in ileum. There is not any significant different between 2 and 4 g/Kg levels of PHS.

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Poster presentation

EFFECTS OF BORON (ORTHOBORIC ACID) SUPPLEMENTATION ON MEAT PROTEIN AND LIPID PROFILES OF LAYING HENS

Kaynar O., Aslan H., Macit M., Yenice G.

Abstract

Boron is a metalloid element from Group IIIA of the periodic table. The principal uses for boron compounds include industrial purposes. It is believed to be boron an essential nutrient and influences the concentrations or activity of many macromolecules and plays a role in a large number of metabolic events and growth by affecting energy substrate utilization.

This study was carried out to determine the effects of boron (orthoboric acid) supplementation into the diets of hens in the late laying period on protein and lipid profiles of meats. Two hundred and eighty eight Lohman commercial laying hens 62 weeks old were fed with 0, 50, 75 and 150 mg/kg of boron for 12 weeks. In the present study boron supplementation up to 150 mg/kg in to the diets did not affect protein concentrations and profiles of meats negatively. However, increased TG, PL percentages, while decreased FFA, DAG and COL percentages in the total lipids.

Keywords: SDS-PAGE, HPTLC, cholesterol, triglyceride, phospholipid

Introduction

Boron is a semi-conductive element from Group IIIA of the periodic table. It has an elemental property intermediate between metals and non-metals (Bolaños et al., 2004). The chemistry of boron is unique and after carbon, possibly the most capable element can able to forms nearly 230 compounds with other elements (WHO, 1998). Boron is always found in nature bound to oxygen or as borates. It is colorless and odorless with either a white granular powder or transparent crystalline form, which are readily soluble in water. Boric acid is the prevalent form of boron in animals and humans. It is a weak Lewis acid (Coughlin, 1996: Bolaños et al., 2004) that forms complexes with biological substances containing cis-hydroxyl groups (Bolaños et al., 2004). During transport in the body, or in vitro-vivo systems, boric acid is weakly attached to organic molecules containing cis-hydroxyl groups in concentration dependent and reversible manner (IEHR, 1997; WHO, 1998) and such affinity may account for the mechanism by which boric acid performs some of its biological effects (WHO, 1998; Bolaños et al., 2004). Boron's unique chemistry allows it to react with many other biomolecules (Devirian and Volpe, 2003) and by this way, affects the metabolism or utilization of a number of substances involved in life processes, including nitrogen, nucleic acids, glucose and triglyceride (Nielsen, 1996).

The importance of the boron for plant growth was first reported in the early 1920s and since then boron has been accepted as an essential element for the growth of all vascular plants. However animal studies examining the effects of boron on metabolism have been started in recent years (Hunt and Nielsen, 1981) and The National Academy of Science Institute of Medicine is categorized boron as a possible trace mineral nutrient, the primary role of boron in animals remains unknown. Recent studies on the biological significance of boron

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to various metabolic, nutritional, hormonal and physiological processes such as steroid biosynthesis, immune response, growth and mineralization indicated that boron is significant element for humans and animals (Nielsen, 1997). Although the evidence is similar, boron is not consistently accepted as an essential nutrient for higher animals as it is for plants. Actually, major complication to wide reception of boron essentiality by animal nutritionists is the absence of an evidently defined specific biochemical function. While many researches about boron metabolism in animals has been made in the last few years, the primary role of boron remains undefined and the detailed mechanism by which boron action on animals has not yet been fully described. Aim of this research was to determine the effects of boron (orthoboric acid) supplemented into the diets of hens in the late laying period on protein and lipid profiles of meats.

Material and Methods

Animals
Two hundred and eighty eight Lohman commercial laying hens that are 62 weeks old were fed with 0, 50, 75 and 150 mg/kg of boron (B) for 12 weeks (Table 1a, b). The research was carried out in 18 replicates and four laying hens were used for each replicate. During the experiment, 16-hour lighting was applied and feed and water were given ad-libitum.

Table 1a. Ingredient and nutrient composition of the diet

Item	%	Nutrient*	%
Corn	29.9	Dry matter	88
Wheat bran	40	Crude Protein	16
Sunflower meal	4	Crude fiber	7
Soybean meal	15	Ash	13
Sunflower oil	1	Ether extract	1
Salt	0.3	NaCl	0.35
DCP 18	1	L-Lysine	0.65
Calcium Carbonate	8	DL-Mehionine	0.32
Vitamin-mineral	0.8	Cysteine	0.30
Premix**		Ca	3.50
		P	0.60
		Na	0.16

*Calculated from tabular values.

**Per kg contains: Vitamin A; 6.000.000 IU, Vitamin D3; 1.200.000 IU, Vitamin E; 15.000 mg, Vitamin K3; 2.000 mg, Vitamin B1; 1.500 mg, Vitamin B2; 3.500 mg, Niacin; 12.500 mg, Cal-D-Paln; 5.000 mg, Vitamin B6; 2.500 mg, Vitamin B12; 7.5 mg, D-Biotin; 22.5 mg, Folic Acid; 500 mg, Cholin Chloride; 62.500 mg, Canthaxanthin; 1.000 mg, Apo Ester; 2.50 mg, Vitamin C; 25.000 mg, Manganese; 40.000 mg, Fe; 30.000 mg, Zn; 30.000 mg, Cu; 2.500 mg, Co; 1.00 mg, I; 500 mg, Se; 75 mg.

Table 1b. Laboratory results of the diets**

Groups	0 mg/kg B	50 mg/kg B	75 mg/kg B	150 mg/kg B
Dry Matter	88.9	89.2	89.0	88.8
Crude Protein	16.2	16.4	16.0	17.1
Oil	3.0	3.1	3.0	2.9
Ash	11.4	12.0	11.7	12.4
ADF	7.6	7.5	7.6	7.9
NDF	24.4	21.4	20.2	26.9
ME[1]	2650	2641	2636	2640

**Calculated

The meat samples of *pectoralis major* (breast) and *illiotibialis* (leg) muscles were taken aseptically from each experimental group in sterile plastic bags to determine lipid and protein profiles at the end of the study. All samples were homogenized and processed within the same day and stored at -86 ^{0}C until analyzed.

Methods

1. Homogenization of meat samples
One gram of meat samples were mixed with 4 mL of SDS 10 % and then homogenized at 5000 rpm for 2 min using a tissue homogenizer. During homogenization all samples were kept in ice bath.
2. High Performance Thin Layer Chromatography (HPTLC) of meat lipids
2.1. Extraction of Meat Lipids
Five hundred microliters of n-hexane:iso-propanol 3:2 (v/v) mixture was added to 1000 μL of meat homogenate (Hara and Radin, 1978) in an eppendorf tube. After vortexing vigorously, the tubes were centrifuged at 5000 x g for 5 min at +4 ^{0}C and the upper phase was used for chromatographic analysis of meat lipids.
2.2. HPTLC
For separation and identification, 20 x 10 cm HPTLC plates were used. Standard lipid mixture comprised, cholesterol, palmitic acid, 1-2, dipalmitoyl phosphatidylcholine, 1-3, dipalmitin and triolein. 3-6 μg per component from standards and 2.5 μl meat lipid extracts were spotted with a micropipette 2 cm away from the bottom of the HPTLC plates. The lipids were developed by using n-hexane: diethyl ether: formic acid; 80:20:2 (v/v). After completion of the developing stage, the entire plate was sprayed with a 10 % $CuSO_4$ (w/v) in 8 % H_3PO_4 (v/v) and lipid classes were visualized by charring at 180 ^{0}C for about 10 min (Kaynar et al., 2013). Meat lipids were separated into the following classes: Triacylglycerol's (TG), free fatty acids (FFA), cholesterol (COL), diacylglycerol's (DAG) and phospholipid's (PL).
2.3. Evaluation of HPTL chromatograms
HPTL chromatograms were scanned with Epson Perfection V700 Photo scanner at 600 dpi resolutions as 16-bit grayscale TIFF image format and analyzed with TL 120 software. Results were obtained as percentage of individual lipid class in total lipid composition of meat samples (Kaynar et al., 2013).
3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of meat proteins:
3.1. SDS-PAGE

Proteins of meat samples were separated according to their molecular weight by SDS-PAGE by using 4 % stacking and 10 % resolving gel (Laemmli, 1970). The meat homogenates were diluted 1:5 with electrophoresis denaturing sample buffer (4 ml dH$_2$O, 1 ml 0.5 M tris-HCl (pH 6.8), 0.8 ml glycerol, 1.6 ml 10 % SDS, 0.4 ml 2-β-mercaptoethanol, 0.2 ml 0.05 % bromphenol blue) and 15 microliters of each mixture was applied in each well. The electrophoresis was carried out in tris-glycine running buffer (Trizma base 1.515 g, glycine 7.2 g, SDS 0.25 g/500 ml, pH 8.3) at 20 mA/gel constant current for 90 min. After electrophoresis proteins were visualized by silver staining.

3.2. Evaluation of SDS-PAG electrophoretograms

SDS-PAG electrophoretograms were visualized by BioRad GelDoc XR gel analysis system at 600 DPI resolutions as TIFF image format and analyzed with TL 120 software. Results were obtained as g/100 g concentration and percentage of individual protein.

4. Statistical analyses

The effects of boron supplementation on the protein and lipid profiles have been tested with One-way ANOVA. Differences between groups are determined with the Multiple Comparison Range Test of Duncan. All statistical analyses have been made with SPSS. 20.0 package software.

Results

Lipid profile

After chromatographic development lipids of the both meats were separated into the following classes: TG, FFA, COL, DAG and PL. However, percentages of lipid classes were different in both meats (Table 2).

Although ratios of lipid classes in both meat samples were different, effects of B supplementation on lipid classes were nearly the same: Percentages of TG and PL were increased in parallel with supplemented boron concentration while percentages FFA, DAG and COL percentages in total lipid decreased (Table 2).

Protein profile

Brisket

Thirty-five protein bands between 15-215 kDa were detected in brisket (Figure I). Five of the 35 proteins called as "major proteins (> 1 g/100 g tissue)" were 167, 49, 37, 16 and 15 (Table 3). Other 30 proteins called as "minor proteins (< 1 g/100 g tissue)" (Table 3). Total protein concentrations of 0 (Ctrl), 50, 75 and 150 mg/kg boron supplemented groups were 23.600, 23.493, 23.382 and 23.436 g/ 100 g tissue respectively (Table 3).

Table 2. Effects of boron supplementation (mg/kg) on lipid profiles of meats (%)

Lipid	0	50	75	150
		Brisket		
TG	74.428 ± 1.585^b	80.365 ± 1.275^a	81.102 ± 0.939^a	81.713 ± 0.595^a
FFA	6.258 ± 0.591^a	5.430 ± 0.710^{ab}	5.248 ± 0.888^{ab}	4.988 ± 0.361^b
COL	13.855 ± 1.185^a	8.887 ± 0.943^b	8.485 ± 0.748^b	8.105 ± 0.517^b
DAG	2.190 ± 0.407^a	1.828 ± 0.331^{ab}	1.655 ± 0.351^{ab}	1.580 ± 0.374^b
PL	3.272 ± 0.491	3.495 ± 0.388	3.507 ± 0.132	3.617 ± 0.310
		Leg		
TG	72.896 ± 2.927	74.583 ± 1.102	75.225 ± 0.597	75.500 ± 1.080
FFA	4.405 ± 0.528	4.335 ± 0.278	4.100 ± 0.141	3.825 ± 0.386
COL	11.233 ± 0.479^a	10.453 ± 0.461^b	9.675 ± 0.479^c	9.575 ± 0.299^c
DAG	7.453 ± 2.555^a	6.453 ± 0.349^b	6.450 ± 0.300^b	6.200 ± 0.327^b
PL	4.010 ± 0.472^b	4.178 ± 0.595^{ab}	4.575 ± 0.386^{ab}	4.925 ± 0.544^a

Different superscripts within the same columns differ (p<0.05)

Leg
Thirty-for protein bands between 17-210 kDa were detected in leg (Figure II). Four of the 34 proteins called as "major proteins (> 1 g/100 g tissue)" were 153, 31, 18 and 17.5 kDa. Other 30 proteins called as "minor proteins (< 1 g/100 g tissue)". Total protein concentrations of Ctrl, 50, 75 and 150 mg/kg boron supplemented groups were 20.187, 19.916, 19.970 and 20.027 g/100 g tissue respectively (Table 3).
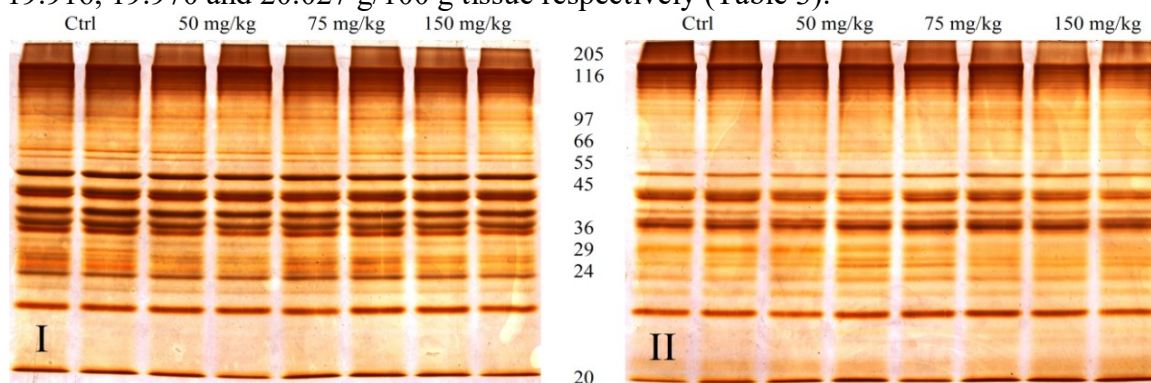
Figure. Electrophoretograms of brisket (I) and leg (II) protein profiles of laying hens

Discussion

The major source of boron entry into the human and animal body is via consumed food such as fruits, vegetables, pulses, legumes and nuts. Boron is readily absorbed following oral exposure in animals (Ku et al., 1991; Usuda et al., 1998) and as an unmetabolized form (Emsley, 1989) distributes throughout the soft tissues of the body (Ku et al., 1991).

Table 3. Effects of boron supplementation (mg/kg) on protein profiles of meats (g/100 g tissue)

	Brisket				Leg			
	0	50	75	150	0	50	75	150
				Major Proteins				
Amount	6.097	6.113	6.190	5.984	4.575	4.458	4.446	4.469
%	25.835	26.021	26.473	25.533	22.663	22.384	22.263	22.315
				Minor proteins				
Amount	17.503	17.380	17.192	17.452	15.612	15.458	15.524	15.558
%	74.165	73.979	73.527	74.467	77.337	77.616	77.737	77.685
TOTAL	23.600	23.493	23.382	23.436	20.187	19.916	19.970	20.027

Boron accumulates in various internal organs at varies levels; concentrations of B for the heart, liver, lung, kidney and brain were 28, 2.31, 0.6, 0.6, 0.06 ppm, respectively (Hamilton et al., 1972; Indraprasit et al., 1974; Nielsen, 1997; Massie et al., 1990). This suggests that any function carried out by boron differs from organ to organ (Newnham, 1991). Boron shows the main effect on lipid metabolism between macromolecules. When the studies related to this topic investigated, the administration of two boron-derived hypolipidemic agents (Tetrakis-μ-bisdicopper (II) and N,N-dimethyl-n-octadecylamine borane) significantly lowered serum low density lipoprotein (LDL) cholesterol and triglyceride levels in rats (Hall et al., 1989). Besides, they were inhibited LDL binding and entrance into liver cells, fibroblasts and aorta cells and promoted high-density lipoprotein (HDL) binding in liver cells. In conclusion, boron-containing drugs promoted cholesterol removal from tissues and decreased lipid accumulation (Hall et al., 1989).

Boric acid supplementation to the drinking water of adult male rats at levels of 2 mg boron/day for 2 weeks resulted in a trend for lower plasma total cholesterol and HDL3-cholesterol levels, as well as significant decreases in plasma triglyceride and total HDL-cholesterol concentrations (Naghii and Samman, 1997). The administration of 4 g/day of borax to daily diet in dogs, in the first week, apolipoprotein B-100 (Apo-B100) level and after the second week VLDL and TG levels were decreased (Basoglu et al., 2002). Hall *et al.* (1989) reported that when B was orally administrated (8 mg/kg/day) to rats, daily for 14 days, LDL cholesterol and TG levels decreased. LDL bonding and LDL entrance into liver cells were also decreased, whereas aorta cells showed increased HDL bonding and accumulation in liver cells. By this way remove cholesterol from tissues and decrease lipid accumulation (Devirian and Volpe, 2003). Moreover, boron supplementation in to the diets lowers total serum cholesterol level in Japanese quails (Eren et al., 2006), increases plasma

total cholesterol and triglycerides concentrations in pigs (Armstrong et al., 2000), increases blood total cholesterol in broilers (Kurtoğlu et al., 2005), decreases total cholesterol levels in laying hens (Eren and Uyanık, 2007) and decreases serum triacylglycerol concentration in rats (Naghii and Samman, 1997). In one study on cows, significant decreases were observed in serum triglyceride and very low density lipoprotein (VLDL) levels of animals treated orally with sodium borate (Basoglu et al., 2002). The other effect of boron is on protein metabolism. The boron supplementation into diet decreases serum albumin concentration in laying hens (Eren and Uyanık, 2007). However, total plasma protein concentrations of heifers were unaffected by increased borate (0, 150, 300 ppm) supplementation in drinking water (Green and Weeth, 1977).

In the present study supplementation of boron to the diets of laying hens, did not affected protein concentrations and profiles of individual proteins of meats negatively or positively up to 150 mg/kg concentrations. However, boron influenced lipid metabolism by bringing the state of lipolysis to lipogenesis on meats (Increased TG, decreased FFA and DAG ratios). Moreover boron supplementation into diet increased biological and nutritionally important PL ratio and the decreased atherogenic COL ratio in total lipids of both meats. In particular, the reduction of tissue cholesterol was in line with the result of "boron prevents the accumulation of cholesterol in the tissue" as reported by Hall et al. (1989). However, how boron affects that the rate of other lipid classes in tissue could not be reached any conclusions. Besides, supplementation of boron in to diet does not affect the concentration of total protein in meat with parallel as stated by Green and Weeth (1977).

Conclusion

Several studies as our work have been performed to investigate the effects of boron intake on animals and humans over the last decade. According to our results, boron supplementation in to diet may be advised to improve the quality of meat for hypercholesterolemic patients by reducing "atherogenic cholesterol content" and/or to increase biological and nutritionally important "phospholipids" ratio in meats of hens without affecting protein metabolism negatively. However, the importance of the boron as a nutritionally essential trace element is not clear yet and more studies to clarify both its metabolic effects considering the hormonal mechanisms and its activity are needed.

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Poster presentation

USAGE OF DIETARY MODIFIED DRIED VINASSE IN BROILERS

Yalçın S.[1], Onbaşılar İ.[2], Yalçın S.[3]

Abstract

The aim of this study was to determine the effects of the usage of modified dried vinasse on performance, carcass yield and some blood parameters in broilers. A total of 192 daily Ross 308 male broiler chicks were allocated into one control group and three treatment groups each containing 48 chicks. The modified dried vinasse (BroMass, Integro Food and Feed Manufacturing Company, İstanbul, Turkey) was used at the level of 2, 4 and 6% in the diets of the first, second and third treatment groups, respectively. The experimental period lasted 6 weeks. The inclusion of modified dried vinasse at the level of 6% in the diets increased body weight gain. Modified dried vinasse at the levels of 4 and 6% decreased feed consumption and improved feed conversion. Dietary treatments did not significantly affect carcass yield, the relative weights of gizzard, liver and spleen and total protein in blood serum. Total cholesterol level in blood serum was reduced with dietary modified dried vinasse. As a result of this study it was concluded that the use of modified dried vinasse can be considered as a safe and an alternative protein source for protein supplements in broilers.

Keywords: Broiler, modified dried vinasse, performance, carcass yield, blood parameters

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Introduction

Vinasse is a by-product from industrial production of yeasts, alcohol, citric acid or other substances by fermentation of molasses. It is used in feed industry as a binder and dust reducer. The modified dried vinasse contains 45-60% modified vinasse produced from bakers yeast industry. Modified dried vinasse contains less than 2% potassium and it is rich in protein and energy. The main sources of nitrogen are betaine (9-41%) and glutamic acid (15-26%) (Weigand and Kirchgessner, 1980; Yalçın et al., 2010). It is very common to add up to 3% vinasse to the diets of monogastric animals and up to 5% to the compound feed of ruminants in France (Stemme et al., 2005). Therefore the aim of this study was to determine the effects of modified dried vinasse on performance, carcass yield and some blood parameters in broilers.

Material and Methods

A total of 192 daily Ross 308 male broiler chicks were allocated into one control group and three treatment groups each containing 48 chicks. Each group was divided into four replicates as subgroups, each comprising 12 chicks. Feed in mash form and water were provided ad libitum during the 6 week experimental period. The modified dried vinasse (BroMass, Integro Food and Feed Manufacturing Company, İstanbul-Turkey) was used at the level of 0, 2, 4 and 6% in the diets. The ingredients and chemical composition of the diets were shown in Table 1. Nutrient composition of diets and BroMass were determined according to the AOAC (2000). Metabolizable energy levels of samples were estimated using the Carpenter and Clegg's equation (1956). Amino acids of BroMass were determined with modified OPA derivatization using the HPLC system (Heems et al., 1998) of Agillent 1100 series (Agillent Technologies, Waldbronn, Germany). Chicks were weighed individually at the beginning of the experimental period and weekly. Live weight gain, feed consumption and feed conversion ratio (kg feed per kg live weight gain) were calculated. At the end of the experiment 16 broilers from each group were weighed and slaughtered. Hot carcasses were weighed to determine the carcass yield. Absolute and proportional weights of liver, gizzard and spleen were also determined. Total protein and total cholesterol in blood serum were determined. Statistical analysis were done using SPSS program (SPSS INC., Chicago, IL, USA). The effect of graded levels of modified dried vinasse on different variables using polynomial contrasts. Statistical differences were considered significant at P≤ 0.05 (Dawson and Trapp, 2001).

Results and Discussion

Modified dried vinasse (BroMass) was high in crude protein (31.90%) and contained high amounts of glutamic acid. The composition of BroMass was given in Table 2. Mean values for live weight gain of groups fed diets containing 0, 2, 4 and 6% modified dried vinasse were 2393, 2393, 2450 and 2501 g, respectively (Table 3). Group fed 6% BroMass had higher weight gain during the 6 week experimental period. BroMass at the levels of 4 and 6% decreased feed consumption and improved feed conversion. The inclusion of modified dried vinasse in diets did not significantly affect carcass yield, relative weights of gizzard, liver and spleen and total protein in blood serum. Total cholesterol in blood serum was decreased with dietary modified dried vinasse significantly (P<0.05). Waliszewski et al (1997) reported that 4% of cane condensed molasses solubles can prove profitable for the

broiler feed industry, chiefly in sugar cane producing countries where its prıce is low and it is considered a waste by-product that my pollute.

Conclusion

As a result of this study it was concluded that the use of modified dried vinasse can be considered as a safe and an alternative protein source for protein supplements in broiler diets. Modified dried vinasse (BroMass) can be used up to 6% in the diets of broilers without causing any adverse effects on performance and carcass yield.

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Apendix:

Table 1. Ingredients and chemical composition of the diets

Ingredients g/kg	Broiler starter (0-21 day) BroMass, %				Broiler grower (22-42 day) BroMass, %			
	0	2	4	6	0	2	4	6
Corn	482.5	474.5	465.5	458	471.0	462.0	453	444.5
Soybean meal	257.0	244.5	229.5	214.5	218.5	211.0	195.5	185.0
Fullfat soya	194.0	193.0	195.5	197.5	237.0	230.0	233.0	229.5
BroMass	0	20.0	40.0	60.0	0	20.0	40.0	60.0
Sunflower seed oil	32.0	34.0	36.0	37.0	40.0	44.0	46.0	49.0
Limestone	15.0	14.5	14.0	13.5	14.0	13.5	13.0	12.5
DCP	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Salt	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL-Methionine	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Lysine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chemical composition (Analyzed, per kg)								
Crude protein, g	22.30	22.37	22.30	22.33	22	22,12	21.94	22.17
ME, kcal	3140	3120	3125	3134	3200	3200	3216	3208

Table 2. Composition of BroMass

Dry matter, %	90.40		Amino acids, g/100 g	
Crude protein, %	31.90		Aspartic acid	0.956
Crude fiber, %	5.90		Glutamic acid	6.566
Ether extract, %	1.8		Glycine	0.708
Crude ash, %	5.50		Threonine	0.401
			Methionine	0.236
			Lysine	0.573

Table 3. Effect of dietary BroMass on the performance of broilers during 0-42 days

	BroMass, %				SEM	P		
	0	2	4	6		Linear	Quad	Cubic
BWG, g	2393 b	2393 b	2450 ab	2501 a	16.2	0.06	0.337	0.595
FI, g	4111 a	4000 b	3869 c	3894 c	27.6	<0.001	0.028	0.175
F:G, g/g	1.72 a	1.67 b	1.58 c	1.56 c	0.02	<0.001	0.090	0.295

BWG: Body weight gain, FI: Feed intake, F:G: Feed to gain ratio
Means within a row followed by the different superscripts differ significantly ($P < 0.05$).

Table 4. Effect of dietary BroMass on serum protein and cholesterol levels of broilers

	BroMass, %				SEM	P		
	0	2	4	6		Linear	Quad	Cubic
Protein, g/100ml	3.23	3.37	3.15	3.28	0.039	0.907	0.952	0.047
Cholesterol, mg/100 ml	126a	120b	119b	119b	0.941	0.005	0.052	0.520

Means within a row followed by the different superscripts differ significantly (P < 0.05).

Poster presentation

DETERMINATION OF OPTIMAL CONCENTRATION OF ORGANIC SELENIUM ON EGG PRODUCTION IN LAYING HENS

Januzi V.[1], Sena L.[1]

Abstract

This study proved the effect of supplementation of food with organic Selenium at different concentrations. The experiment was conducted for a period of 11 weeks at a poultry farm near Podujeve. A total of 300 Lohman Brown layers were divided into three groups as follows: Control Group (C), Experiment 1 (E1), Experiment 2 (E2). For the three groups the same feed formula was applied, but if the control group was fed with the basic feed, the feed formula of the other two consequent ones was supplemented with Organic Selenium (Se) Sel-Plex®, Alltech, inc., respectively 0.3 and 0.4 ppm. During the experimental period the following parameters were consistently recorded, monitored and evaluated: live weight, egg production, egg mass, egg quality and feed conversion ratio (FCR). At the end of the study it was shown that the performance indicators of poultry including egg production, egg weight, egg mass, and weight of hens were not affected by the level of supplemented Selenium in the diet of poultry. In the group supplemented with 0.4ppm Selenium the food was saved in the mass of 1,63% compared to control and 1.20% compared to E1 (a significant difference of P ≤0.05). A tendency for improving egg quality is seen by increasing the Se dose. Although, the egg weight of E2 was respectively 3.7 and 2% larger than control and E1, so can say that there is only a trend for higher weight, because the differences are statistically unconfirmed. The hens of the best group reached the lower weight compared to standard hybrid.

Keywords: e*gg production, egg quality, layer, Selenium.*

Introduction

Selenium is an essential element in poultry nutrition. Selenium exists in nature in organic and inorganic form. Selenium is traditionally introduced in poultry diets through inorganic form. Lately organic forms of Se are used as an alternative of inorganic supplementation (Payne, et al., 2005). Using organic has led to increased utilization rate and its deposit in the body of poultry and eggs (Cantor et al., 2000; Payne et al., 2005; Utterback et al., 2005 and Maysa, M. Hanafy. et al., 2009). Supplementation of the laying hen diets with organic Se has helped improve the health state and productivity as well as in the production rich in Se eggs (Yaroshenko et al., 2003 and Sara et al., 2008).

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Due to antioxidant attributes, selenium affects the quality of eggs. In commercial layers (Rutz et al., 2003) Sel-Plex increases the concentration of Se in the egg shell which results in the improvement of its quality. It increases the concentration of Se in the yolk and egg white, resulting in improvement of internal egg quality during storage (Mohiti-Asli M., 2008). Se plays an important role in bird reproduction indicators.

The objective of the study is to investigate the effects of organic selenium (Sel- Plex) addition in layers diet on their performance indicators.

Material and Methods

The study was conducted on a commercial egg production farm near Podujeve for a period of 11 weeks. Based on the principle of comparative analogy, age, productivity and health status, there were formed 3 groups of 100 hens each. Chickens were of the egg hybrid of Lohman Brown in the 19th week of production. Throughout the experiment chickens were kept in the same environment (farm), 4-floors chicken battery, with 5 chickens each cage, as well as environmental conditions, and equal treatment and service.

The composition of the diet was the same for all groups (difference was in levels of Se): control (C) the basal diet contained a trace mineral premix that provided no supplemental Se, while the other two experiment groups were supplemented with of organic Se portion respectively 0.3ppm (experiment 1 - E1) and 0.4ppm (experiment 2 - E2). Layers food was prepared in the food factory near poultry. It uses organic selenium Sel-Plex®, Alltech, inc.

Table 1. Calculated Composition and analysis of basal diet

Ingredients	(%)
Yellow corn	60
Soybean meal 48%	20
Sunflower meal	10
Limestone	7.7
Vitamin and mineral premix	1.0
Sodium chloride	0.3
Di-calcium phosphate	1.0
Total	100
Calculated analysis	
Metabolizable energy (kcal/kg)	2732.50
Crude protein (%)	17.70
Crude fiber (%)	3.20
Crude fat (%)	2.67
Calcium (%)	3.33
Available phosphorus (%)	0.38
Lysine (%)	0.75
Methionine (%)	0.34
Cysteine (%)	0.29

At the end of the experimental period the weight of the eggs was monitored by selecting 10 eggs / group on the random basis principle. Measurements were made on the quality of the interior and exterior of the egg. It was determined the weight of the egg, the weight of the egg white, the yolk, the shell and the shell thickness, the index format, the yolk index

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and Haugh Unit, and intensity of yolk with Roshe meter.

While the egg mass (EM) for each group was calculated with the following formula:
EM = EW (Egg Weight) x EN (Egg Number)/NC (Number of hens)

Recorded Indicators were as follows:

1. The daily production of eggs for each group

2. The egg weight/group;

3. Internal and external egg quality;

4. Feed consumption/group in kg;

5. Feed Conversion Rate/egg unit;

6. Body weight; To do this, 5% of the flock was weighed every week.

The obtained results were statistically processed via ANOVA and descriptive analysis, for the comparison tTest was applied.

Results and Discussion

In the table below we present the impact of the level of selenium in the performance indicators at the end of the experiment.

Table 2: Performance indicators of hens through various groups

Parameters	Control	0.3 ppm Se (E1)	0.4 ppm Se (E2)
Egg/hen /week	5.57±0.60	5.58±0.53	5.65±0.54
Egg/hen total	61.26	61.38	62.13
FCR	145.98±14.80	145.36±12.80	143.64±13.01
Egg production (%)	80.18±9.55	79.73±7.53	80.69±7.66
Mortality (%)	5	2	2
Final body weight	1.79±0.15	1.74±0.11	1.86±0.15

The table above shows a slight lead of E2 with regards to the other two groups in the performance indicators.

If we analyze the results of production yield per hen per week, or for the entire period as well as the egg production (%), will see that the group, whose portion was supplemented with 0.4ppm Se proved more successful. E2 group produced averaging 1.42% and 1.22% more eggs per hen compared with the Control and E1, respectively. However, these differences are statistically non-significant for P≤0.05.

Our results for egg production agree with those of Cantor et al. (2000) and Patton (2000) who reported no difference in egg production when hens fed a basal diet supplemented with 0 or 0.30 ppm of Se. In accordance with our results Jiakui and Xialong (2004), Chinsrari et al. (2009) have not found any significant effects of additions of Se yeast.

Regarding Feed Conversion Rate (FCR), it is apparent of E2 supremacy over the other two groups, but the differences are significant only between C (Control) and E2 (Experiment 2) for P≤0.05. Therefore, supplementation ration level of 0.4ppm resulted more effective in the use of feed/egg, saving respectively 1.63% and 1.20% of food compared with C and E1. According to studies of Ganpule and Manjunatha (2003), it showed that

supplementation of poultry rations with organic selenium significantly contributed to improving FCR.

It is noticeable that there was a tendency for higher body weight in the group that received 0.4ppm Se (E2), however, the differences were not significant (P <0.05 level). It should be noted that poultry the best group (E2) reached lower weight compared to standard hybrid.

Egg quality indicators

Table 4. The weight of the egg components and egg qualities by groups

Parameters of egg quality	Control (C)	Experiment 1 (E1)	Experiment 2 (E2)
Egg weight (g)	61.02±5.04	62.00±5.15	63.28±5.34
Albumen weight(g)	37.89±4.88	39.10±4.29	38.98±3.14
Yolk weight(g)	14.95±1.05	15.15±0.90	15.91±1.32
Egg shell weight (g)	8.18±0.93	8.05±0.61	8.45±0.68
Shell thickness (mm)	0.58±0.04	0.59±0.03	0.59±0.01
Egg shape index	1.36±0.00	1.40±0.00	1.40±0.00
Yolk index	47.85±1.30	49.10±0.82	49.55±1.18
Haugh Unit (HU)	48.20±1.30	49.58±0.81	50.65±1.22
Yolk intensity (Roche)	11	11	11
Egg mass (kg)	3.74	3.81	3.93

With an increase in Se dose, there is a tendency for improvement of the egg quality. E2 eggs have weight of 3.7 and 2.1% greater than Control and E1, respectively. However, we may say that there is only a tendency towards higher weight of the egg and its components in the group that received 0.4 ppm Se given that the differences were not significant (p <0.05 level). This finding is also confirmed by other studies (Arpasova, et al., 2009; Skrivanet. Al., 2006; Utterbacket. Al., 2005). Egg shape index has shown better form in E1 and E2. Regarding the intensity of yolk, it is the same for all groups and it is within normal confidence limits. Egg mass in the best group (E2) is higher than C for 190g (4.8%) and 120g (3.1%) higher than E1. In groups that were treated with Sel-Plex® (E1 and E2) resulted in an increase of the average value of the yolk index and Haugh Unit as compared to Control group. The results of our study are compatible with other studies (Rutz et al, 2003, 2005, Maysa M. Hanafy et al. 2009), in which the addition of organic selenium in the diet of poultry has led to the improvement of the eggs quality indicators (egg yolk index and HU, etc.).

Conclusion

Dietary chicken supplementation with organic Se in two different concentrations of 0.3 and 0.4ppm showed no effect on the poultry performance indicators. The group, in which the diet was supplemented Se in a 0.4ppm showed tendency for improvement of egg production, egg weight and its components, the egg mass and for higher body weight. In this group FCR, yolk index, and Haugh Unit were improved.

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Poster presentation

TEST RESULTS OF EXPLOITATIONAL EXAMINATIONS OF TRACTOR MOWERS DURING ALFALFA MOWING IN THE CONDITIONS OF CENTRAL SERBIA

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Abstract

Alfalfa is one of the most important forage crops used for the production of high quality forage. Mowing is the first step in the process, hence it is important to perform at an optimum time in terms of agrotechnical measures required because it reduces the negative impact of external factors. Quality of mower operation depends on the state of crops, defined operating parameters, technical safety and operator training. The consequences of poor compliance of relevant parameters are reflected in a significant distortion of the quality of work and increased losses. The paper presents the results of testing of tractor mower for mowing alfalfa in exploitation conditions in central Serbia in the 2014/15 period. The aim of this study was to determine the quality of the examined mower according to the defined parameters. Based on these results, it was concluded that the total losses measured in alfalfa mowing varied in the range of 1.20 to 4.06% of biological yield. Minimum cutting height was measured in oscillatory mowers (Type C) 4.94 cm, and the highest – in the rotary mower disc (Type B) 9.17 cm.

Keywords: mower, quality of the work, losses, cut height

Introduction

Alfalfa is one of the most important forage crops, which are grown in Serbia on the surface area of 108.834 ha, with yield of 5.2 t ha^{-1} (Stat. Yearb. Serb., 2015). Most often it is used for storing hay (in 90% of cases), silage, for industrial processing and manufacturing various dehydrated products, and less for grazing (Đukić, 2005). In order to successfully obtain alfalfa hay is very important to do the mowing at the optimum agrotechnical point in time, with the correct choice of type and the brake units which feature height adjustment, because this reduces the negative impact of external factors. Too low cutting height reduces the capacity for regeneration and growth of alfalfa, while too high mowing alfalfa has resulted in higher losses of nutrients and getting lower quality feed (Crasi et al., 2001). Period of exploitation, yield and quality of alfalfa hay depends on the amount and manner

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of mowing during the growing season (Kallenbach et al., 2002; Nagy, 2003; Veronesi et al., 2006). Zoranović et al., (1996) suggest that total losses in alfalfa mowing varies in the range of 0.90-2.29%, average 1.59%. Stems cutting height was 5.35 to 7.54 cm, 6.42 cm on average. The same authors state that with increasing operating speed increases cutting height. Wiersma et al., (2001) point out that alfalfa should be cut to a height of 5.08 cm in order to reduce losses and to obtain high yields of quality mass. Mitrovic et al., (2011) state that the oscillation mower for mowing achieved average speed of the aggregate of 4.4 km h^{-1}, rotary mower with drums 7.9 km h^{-1}, and rotary mower with disc 6.5 km h^{-1}. Potkonjak et al., (2009), state that the lowest cutting height achieved classical mower average of 6:42 cm, and the highest by rotary mowers -7.50 cm for mowers with 6 discs and 7.47 cm in mowers with 14 discs. Jugović et al., (2013) examined the rotary mower with drums and come to the conclusion that the minimum cutting height of 5.15 cm recorded at a speed of movement of the aggregate 5.89 km h^{-1}, and maximum 6.50 cm, recorded at 9.29 km h^{-1}. The same authors state that when mowing with the mower realized losses incurred due to the height adjustment of 2.65%, while the losses incurred due to fragmentation mass decrease with increasing the speed of movement of 1.06 per 0.82%. Vuković et al., (2015) point out that the greatest total losses in alfalfa mowing measured when mowing with a rotary mower with drums 3.04%, with losses due to the grinding weight of 1.86% and a height of cut from 1.18%. According to the same authors, the lowest total losses were measured were at orbital mowers 25.1%, with losses due to fragmentation of 0.40% and a height of cut of 0.85%.

Material and Methods

In conditions of central Serbia (44^0 06 '00 "N, 20^0 51' 00" E) in 2014/15., the two-year test three types of mower for mowing alfalfa were made. The study included a rotary mower drums with RK 135 (Type A), rotary mower with disc FPM 627.716 (Type B) and classical oscillatory mower with a cutting pinion IMT 627.667 (Type C). In the first phase conditions were determined, the yield was determined with 1 m^2 in five repetitions on the diagonal plots and converted to an area of 1 ha, so that the average yield of alfalfa was 3.78, respectively 4.12 t ha^{-1} (dry land farming). In the second stage operation effects were determined depending on predefined parameters. Strengthened losses when mowing, operating speed and cutting height. The losses are determined by measuring the area of 1m^2 using a rectangular frame working width tested mowers. Total losses are presented as the sum of the losses incurred due to the height adjustment and losses caused by the grinding plant mass, and were evaluated according to the change of regime operating speeds. The cutting height is determined on rectangular surfaces 1 m^2 whose width is equal intervention lawn, measuring the height of the remains of the stems after cutting collapse. All values were taken in five repetitions. Speed of work was determined by chronometry, measuring the paths of length 30 m, and the base time to plot a total length of 80 m and time of turning on headlands. Mowers are operated by tractors power 30 or 43 kW.

Results and Discussion

Table 1 shows the basic information about the technical characteristics of the tested mower types and test conditions. The data presented in Table 1 indicate that all the mowers worked

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in similar production conditions with the average yield which was in the range of 3.78-4.12 t ha^{-1}.

Table 1. The characteristics of mowing machines and conditions of research

Parameters		Type of mowing machine		
		Drums rotary mower Type A	Discs rotary mower Type B	Classic with fingers Type C
Cutting width	[m]	1.35	1.65	1.60
RPM shaft rotations	[min^{-1}]	540	540	540
Number of oscillation	[min]	/	/	800-1.000
Drum rotation rpm	[min^{-1}]	2.250	3.000	/
Working efficiency	[ha h^{-1}]	1.2	2-3	1.25
Working speed	[km h^{-1}]	8-12	up 16	4-8
Cutting height	[cm]	4-7	3-9	3-9
Power requirement	kW	18	21	18
Transport speed	[km h^{-1}]	20	20	20
Weight	[kg]	325	366	170
Number of drums/discs		2	4	/
Number of knives	/	6	8	/
Height of stem	[cm]	51.28; 57.91	52.3; 58.86	53.5; 60.43
Yield of alfalfa	[t ha^{-1}]	3.78; 3.96	3.90; 4.12	3.81; 3.97

Table 2 shows the values of losses in alfalfa while mowing with tested mowers.

Table 2. Losses during mowers operation

Type of mowing machine	Years	Parameters	Samples losses (%)					Average
			Repetition					
			1	2	3	4	5	
Drums rotary mower Type A	2014	Due to cutting height	0.96	1.39	1.46	1.58	1.83	1.44
		Due to chopping	2.47	2.35	1.98	1.90	1.87	2.12
		Total losses	3.43	3.74	3.44	3.48	3.70	3.56
	2015	Due to cutting height	1.12	1.48	1.51	1.64	1.95	1.54
		Due to chopping	2.82	2.53	2.23	1.97	1.90	2.29
		Total losses	3.94	4.01	3.74	3.61	3.85	3.85
Discs rotary mower Type B	2014	Due to cutting height	1.48	1.73	2.14	2.59	2.98	2.18
		Due to chopping	1.46	1.37	1.25	1.10	0.88	1.21
		Total losses	2.94	3.10	3.39	3.69	3.86	3.39
	2015	Due to cutting height	1.65	1.88	2.31	2.57	3.10	2.30
		Due to chopping	1.51	1.40	1.32	1.15	0.96	1.27
		Total losses	3.16	3.28	3.63	3.72	4.06	3.57
Classic with fingers Type C	2014	Due to cutting height	0.59	0.72	0.83	1.10	1.34	0.92
		Due to chopping	0.61	0.50	0.45	0.38	0.30	0.45
		Total losses	1.20	1.22	1.28	1.48	1.64	1.37
	2015	Due to cutting height	0.61	0.76	0.88	1.12	1.39	0.95
		Due to chopping	0.63	0.58	0.49	0.40	0.32	0.48
		Total losses	1.24	1.34	1.37	1.52	1.71	1.43

Based on the results set forth in Table 2 after repetitions, it can be seen that least values of losses incurred due to the cutting height measured with the oscillating mowers (Type C) and were in the range 0.59 to 1.39%, while the losses incurred due to fragmentation of alfalfa ranged from 0.30-0.63% of the yield of alfalfa. Total losses are the sum of the losses incurred due to the height of cut and due to fragmentation varied in the range of 1.20-1.71% of the yield.

In rotary mower with disc (Type B), measured was that the highest value of losses incurred due to cutting height in relation to all tested mowers, viewed by repetitions for both years. Losses due to cutting height ranged in the range of 1.48 to 3.10% of the yield of alfalfa, while losses of grinding mass were in the range of 0.88-1.51%. Total losses measured when mowing with a new mower varies whether they are in the range of 2.94% to 4.06%, which is a specific maximum value of the total losses for all examined types of mower during both years.

Height realized losses in alfalfa mowing was also analyzed with the operation of rotary mowers with drums (Type A). Losses incurred due to the amount of cuts ranged in the range of 0.96-1.95% of the yield of alfalfa. Losses incurred as a result of the grinding weight in alfalfa mowing with this type mowers were in the range of 1.87-2.82%, which are the highest values for all types tested mowers. Total losses varied in the range of 3.43-4.01% of the yield. Similar results in their research are cited by different authors (Zoranović et al., 1996.; Wiersma et al., 2001.; Kallenbach et al., 2002; Nagy, 2003; Veronesi et al., 2006; Barać et al., 2012; Jugović et al., 2013, Vuković et al., 2015).

Based on the results in Table 3 it can be seen that the increase in speed of movement grew and cutting height for all types tested mowers. Minimum cutting height was measured in oscillatory mowers (Type C) during 2014 and amounted to 4.94 cm and speed of 3.95 km h^{-1}, and highest 9.17 cm in alfalfa mowing rotary mower with four discs (Type B) in 2015 at an operating speed of 11.38 km h^{-1}.

Table 3. Height of stem alfalfa cutting

Type of mowing machine	Years	Parameters		Repetitions					Average
				1	2	3	4	5	
Drums rotary mower	2014.	Cutting height	[cm]	4.98	5.28	5.92	6.83	8.21	6.24
		Working speed	[km h^{-1}]	8.10	8.95	9.41	9.98	10.13	9.31
Type A	2015.	Cutting height	[cm]	5.29	5.87	6.19	7.23	8.79	6.67
		Working speed	[km h^{-1}]	8.53	9.38	9.87	10.48	10.69	9.79
Discs rotary mower	2014.	Cutting height	[cm]	6.19	6.52	6.85	8.29	9.10	7.39
		Working speed	(km h^{-1})	8.54	8.97	9.94	10.96	11.25	9.93
Type B	2015.	Cutting height	[cm]	6.47	6.97	7.41	8.52	9.17	7.71
		Working speed	[km h^{-1}]	8.61	9.38	10.26	10.79	11.38	10.08
Classic with fingers	2014.	Cutting height	[cm]	4.94	5.97	6.25	6.84	7.46	6.29
		Working speed	[km h^{-1}]	3.95	5.42	6.38	7.23	7.90	6.18
Type C	2015.	Cutting height	[cm]	5.43	6.35	6.47	7.51	7.95	6.74
		Working speed	[km h^{-1}]	4.17	5.63	6.72	7.56	8.17	6.45

The results coincide with the results of other authors (Zoranović et al., 1996.; Wiersma et al., 2001.; Crasi et al., 2001; Potkonjak et al., 2009; Mitrović et al., 2011; Jugović et al., 2013,).
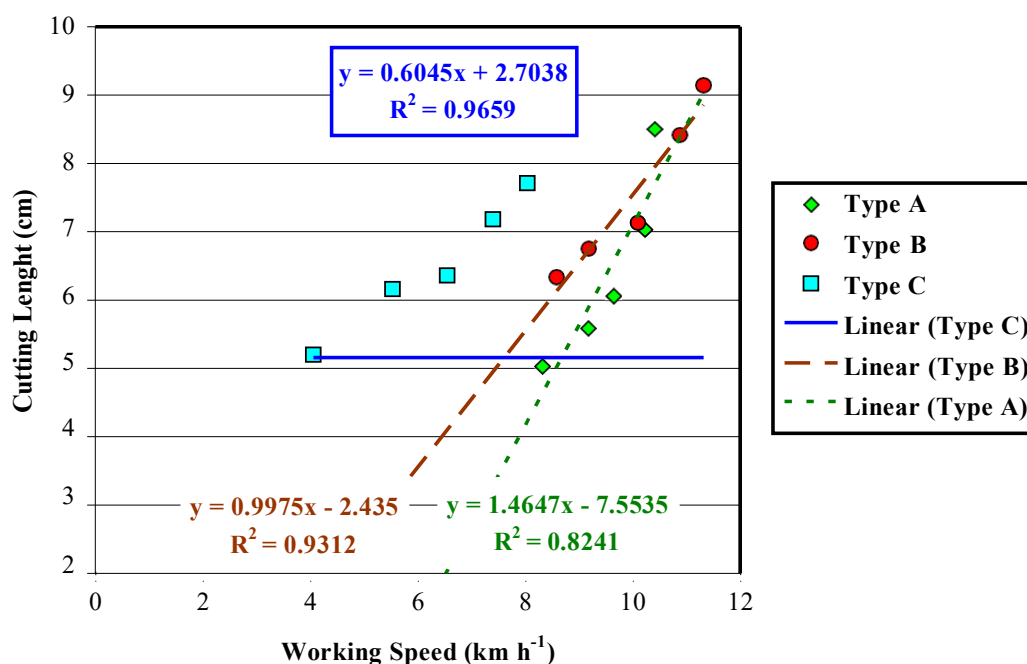
Figure 1. Relationships between the working speed and cutting length

Fig. 1 presents dependency of alfalfa cutting length achieved by three tested machines on the variations of their working speeds of. Aggregate Type C was the most sensitive toward changes of the working speed – its results (experimental data points) were characterized by the steepest trend line y = 1.46 x – 7.55. Simultaneously, the machine Type A was the least sensitive against the speed changes, having trend line y = 0.60 x + 2.70. R-square factors reached very high values in all cases: 0.96, 0.998 and 0.82 for machines of the type A, B and C respectively, thus verifying applicability of presented trend lines.

Conclusion

Based on these results it can be concluded that there are significant differences between the mowers in terms of quality of work. A minimum value of losses incurred due to the cutting height were measured with the oscillating mowers (Type C) and amounted to 0.59%, while the highest values were measured with the rotary mower disc (Type B) 3.10%. A minimum value of losses incurred due to fragmentation of meat were measured in the orbital mowers (Type C) 0.30%, and highest in rotary mowers with drums (Type A) 2.82%. The smallest total losses were measured at orbital mower with one cutting pinion (Type C) and amounted to 1.20%, the highest were in a rotary mower with four discs (Type B) 4.06%. Minimum cutting height was measured in oscillatory mowers (Type C) and amounted to 4.94 cm and speed of 3.95 km h⁻¹, while the maximum 9.17 cm was measured in alfalfa mowing with a rotary mower discs (Type B) and an operating speed of 11.38 km h⁻¹. R-square factors reached very high values in all cases: 0.96, 0.998 and 0.82 for machines of the type A, B and C respectively, thus verifying applicability of presented trend lines. With the increasing speed of the tested mowers the height of cuts and losses due to cutting height were increasing, and losses were decreasing due to fragmentation of the mass.. Since the upper limit of 5% of the total losses, there is a general conclusion that the test can be successfully used for mowing alfalfa in conditions of central Serbia.

Acknowledgement

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Poster presentation

COMPARISON OF DIFFERENT TYPES OF MOWERS IN ALFALFA CUTTING

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Abstract

Alfalfa is one of the most valuable feeds for nutrition of all kinds and categories of domestic animals. Production of good quality, highly nutritive alfalfa hay cannot forego the utilization of appropriate cutting machines. This study features results of the exploitation researches into the use of classic cutting oscillatory mower OLT-"Standard"; two types of rotary mowers with drums BRK 165 and PÖTTINGER CAT 185; and rotary mower with discs JF-STOLL SB 200. The aim of research is to obtain optimal values of exploitation parameters for each type of tested mower by conducting comparative analysis of different technical characteristics of the tested mowers from the aspect of quality of work, losses and production effectiveness. Average incision height by oscillatory mower was 6.57 cm at average moving speed of 5.97 km h^{-1}. Average incision height by rotary mower with drums was 6.17 cm at moving speed of 9.95 km h^{-1} for cutting apparatus BRK 165, whereas the rotary mower Pöttinger CAT 185 had average incision height of 6.33 cm at average moving speed of 10.12 km h^{-1}. Maximum incision height of 7.15 cm was recorded for the rotary mower with discs JF-Stoll SB 200 at average moving speed of 11.5 km h^{-1} . The lowest value of production effectives of 0.7 ha h^{-1} was recorded for the oscillatory mower, whereas the highest value of production effectiveness of 1.82 ha h^{-1} was recorded for rotary mower with discs JF-Stoll SB 200. Average values of production effectiveness for rotary mowers with drums were 1.27 ha h^{-1} (BRK 165) and 1.5 ha h^{-1} (Pöttinger CAT 185).

Keywords: mowers, cutting, losses, productivity

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Introduction

The operation of mowing is the most important procedure in alfalfa hay preparation process, being one of the best quality bulky forage used for nutrition of domestic animals. Adequate types of oscillatory and rotary mowers are used for this purpose and they need to meet demanding standards for high production effectiveness, economical and universal usage, and good quality of work with as little losses as possible; all in order to make a product containing high nutritious values. Hosseini and Shamsi (2012) deem that mowers belong to a group of agricultural machines consuming lots of energy; therefore adequate attention has to be paid to adjustment of given parameters in order to guarantee optimal energy consumption and quality of work. The importance of the alfalfa lies in the fact that it is used for production of high quality bulky forage, as well as for production of components for concentrated nutrients for monogastric animals after several phases of biomass processing (Đukić et al., 2008). The greatest dilemma in alfalfa production is how to achieve optimal ration between yields and quality of green fodder feed and hay (Orlaf, Putnam, 2004). Period of alfalfa exploitation, yield and quality fodder feed mainly depend upon number of mowing during vegetation period (Kallenbach et al., 2002; Nagy, 2003; Veronesi et al., 2006). Timing of mowing in certain stages of growth affects the yield and hay quality. Mowing in later stages (full blooming) gives greater yield, but poorer quality (Lloveras, 2001). Alfalfa hay is very sensitive to the way of drying. Total losses of nutritious material during hay drying on the field with suitable weather are 40 - 50%, states Radojević (2005). Height and way of incision are also important. Alfalfa stem is basically of weaker quality, portion of leaves is smaller, thus better quality of forage is made by higher incision mowing. (Crasi et al., 2001).

Radivojević (2000) claimed that in order to have an even cut, it is necessary to harmonize the relative aggregate moving speed with speed of mower's cutting device oscillation speed. Mower's moving speed ranges from 2/3 of cutting device oscillation speed. In case of slower moving speeds ($V = 5$ km h^{-1}), double cutting of the already cut off plants happens due to uncoordinated relation with oscillation speed. Barać et al. (2007) state that the moving speed of the oscillatory mowers is limited to 5 do 7 km h^{-1}, whereas the rotary mowers work without jamming and at greater speeds of 12 km h^{-1}, so that they have greater working effectiveness. As recommended by Wiersma and Wiederholt (2001), alfalfa need to be mowed on incision height of 2 inch (5.08 cm), in order to get maximum yield and good quality of mass. Thomas et al., (2006) state that incision height while mowing with rotary mower with discs impacts on nutritious values of alfalfa hay. Mowing at height lower than 2 inch ensures greater yield, but the content of raw proteins is reduced. While testing rotary mower with drums BRK 135, at the alfalfa yield of 3.22 t ha^{-1}, Mitrović and Irić (2003) claimed that mowing speed was in range of 6.55 – 9.4 km h^{-1} with productivity of 0.6 – 0.78 ha h^{-1}. While testing rotary mower with drums BRK 135, Jugović et al., (2013) learnt that the lowest incision height of 5.15 cm was made at mower moving speed of 5.89 km h^{-1}; whereas the highest incision height of 6.5 cm was made at mower moving speed of 9.29 km h^{-1}. The same authors state that during mowing with this mower, the losses caused by incision height were 2.65%, whereas losses caused by crushing while increasing moving speed were reduced from 1.06 to 0.82%. Testing a oscillatory mower with classic cutting apparatus and rotary mower with two drums, Barać et al., (2012) learnt that the average stem incision height at oscillatory mower with classic cutting apparatus was 6.32 cm, whereas it was 9.53 cm for rotary mower. Total loss for the oscillatory mower ranged from 1.12 to 1.64% of yield, whereas it was 3.09 to 3.5% for rotary mower.

Material and Methods

Tests of oscillatory mower OLT and rotary mower with drums BRK 165 were performed on a land parcel with average alfalfa yield of 3.3 t ha^{-1}; whereas tests of rotary mower with drums Pöttinger CAT 185 and rotary mower with discs JF-Stoll SB 200 were performed on a land parcel with average yield of 4.3 t ha^{-1}. Both parcels are located in Rasina Region, area of village Mačkovac (43^0 33' 33''N; 21^0 12' 53''E) in vicinity of Kruševac, and is private property of agricultural producer Aleksandar Mršić. The alfalfa green mass yield was determined by measurements taken from a length meter of swath width and calculated per hectare. The mowing machine speed was determined chronometrically. The cutting height was determined on the spot of loss determination, by measuring all sides of appropriate samples surface. The average of each sample is based on the determined parameters. The alfalfa mowing losses were measured on the surface of one length meter multiplied by real mower swath width on the same place of swath height determination. The total losses are represented as sum of losses caused by the cutting height and crushing process. Determination of the loss was conducted in three samples. The production effectiveness was defined applying the chronometric method, i.e. by measuring working time of the tested mower at certain moving speeds.

Results and Discussion

Table 1 presents basic data related to the state of tested mowers and alfalfa crops at the experimental plots, showing that all mowers worked in similar production conditions.

Table 1. Characteristics of tested mowers and experimental conditions.

Parameters		Type of mowers			
		A	B	C	D
Cutting width	(mm)	1530	1650	1850	2000
Power requirement	(kW)	20	20	37	30
Working speed	(km h^{-1})	4-8	8-10	8-10	10-16
Cutting height	(cm)	3-10	4-10	4-10	4-10
Number of drums/discs		-	2	2	5
Number of blade on the drums/discs		-	3	3	2
Drum/disc rotation	(min^{-1})	-	1.920	2.250	3.000
Height of stem	(cm)	73	73	78	78
Yield of alfalfa	(t ha^{-1})	3.3	3.3	4.3	4.3

A - Oscilatory mower;   B - Rotary with drums BRK 165;
C - Rotary with drums Pöttinger CAT 185;   D - Rotary with discs JF-Stoll SB 200

Average values of incision for mowers are given in Table 2 and illustrated in Figure 1. If the optimal alfalfa incision height was 6 cm, it is obvious from the given results that tested mowers had no significant discrepancies. The lowest average incision height of 6.17 cm at average moving speed of 9.95 km h^{-1} was made by rotary mower with drums BRK 165 (type B), whereas maximal value of 7.15 cm at average moving speed of 11.5 km h^{-1} was made by rotary cutting apparatus with discs JF Stoll SB 200 (type D). Oscillatory mower OLT (type A) achieved average incision height of 6.57 cm at average moving speed of 5.97

km h^{-1}, whereas the average incision height of rotary mower with drums Pöttinger CAT 185 (type C) was 6.33 cm are moving speed of 10.12 km h^{-1}. Presented results are in conformity with the results given by other authors (Barać et al., 2007; Wiersma and Wiederholt, 2001; Thomas et al., 2006; Crasi et al., 2001).

Table 2. Height of stem cutting

Type of mowers	Parameters		Test			Average
			(1)	(2)	(3)	
A	Cutting height	(cm)	5.43	6.75	7.52	6.57
	Working speed - (v)	(km h^{-1})	3.85	5.62	8.45	5.97
B	Cutting height	(cm)	5.52	6.16	6.84	6.17
	Working speed - (v)	(km h^{-1})	8.44	10.22	11.18	9.95
C	Cutting height	(cm)	5.61	6.25	7.15	6.33
	Working speed - (v)	(km h^{-1})	8.57	10.32	11.47	10.12
D	Cutting height	(cm)	6.75	7.19	7.51	7.15
	Working speed - (v)	(km h^{-1})	10.38	11.26	12.85	11.5

A - Oscilatory mower;   B - Rotary with drums BRK 165;
C - Rotary with drums Pöttinger CAT 185;   D - Rotary with disc JF-Stoll SB 200
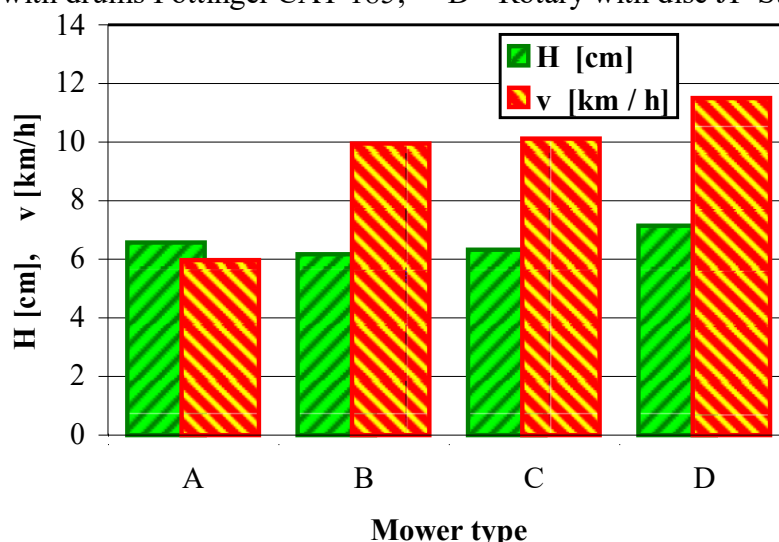
Fig. 1 Average height of stem cutting and working velocity.

When assessing work quality of mowers, losses made during mowing operation need to be also taken into consideration. Total losses presented for this study represent sum of losses caused by incision height and crushing losses, Table 3 (Figure 2). According to the results given in the Table 3, it is obvious that increase of moving speed of all tested mowers result in increase of losses caused by incision height with simultaneous reduction of crushing losses. The lowest average value of total losses, 1.25% of yield, was made by oscillatory mower; whereas total value of losses ranged between 1.02 to 1.59%. Highest average value of total losses of 3.34% of yields was noted at rotary mower with drums Pöttinger CAT 185. Value of losses for given cutting apparatus range from 3.17 to 3.63%. Another type of tested mower with drums BRK 165 made average value of total losses of 2.98% (2.84-3.13%). Average value of losses caused by rotary mower with discs was 3.2% of yields, and it ranged from 3.06 to 3.42 % of yields. The results fit the results of other studies (Hosseini and Shamsi, 2012; Radojević, 2005; Jugović et al., 2013).

Table 3. Losses during mowing process (% of alfalfa yield)

Type of mowers	Type of losses (%)	Test			Average
		(1)	(2)	(3)	
Oscilatory	G_{vr}	0.58	0.74	1.27	0.86
	G_{us}	0.44	0.41	0.32	0.39
	Total ($G_{vr} + G_{us}$)	1.02	1.15	1.59	1.25
BRK 165	G_{vr}	0.93	1.24	1.47	1.21
	G_{us}	1.91	1.73	1.66	1.77
	Total ($G_{vr} + G_{us}$)	2.84	2.97	3.13	2.98
Pöttinger CAT 185	G_{vr}	1.02	1.39	1.65	1.35
	G_{us}	2.15	1.84	1.98	1.99
	Total ($G_{vr} + G_{us}$)	3.17	3.23	3.63	3.34
Jf-Stoll SB 200	G_{vr}	1.21	1.42	1.98	1.54
	G_{us}	1.85	1.69	1.44	1.66
	Total ($G_{vr} + G_{us}$)	3.06	3.11	3.42	3.2

G_{vr} – incision height losses; G_{us} – crushing losses;
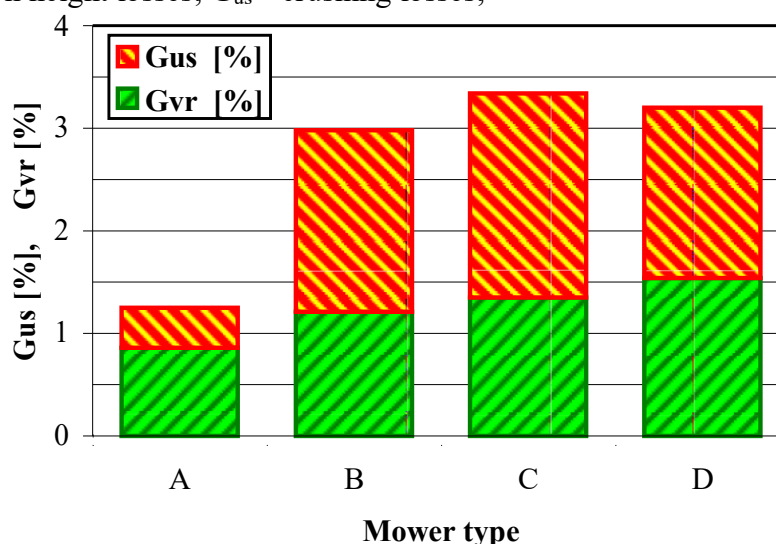
Figure 2. Average losses during mowing process (% of alfalfa yield)

Production effectiveness results for tested mowers are given in the Table 4 and graphically presented in Figure 3. Swath width, mowing speed, green mass yield, technical condition and exploiting reliability of mowers, as well as mower driver's experience, affect the production effectiveness. The shorter period of green mass mowing, the higher production effectiveness of the tested mowers. During our tests, the maximal production effectiveness was noted for work of rotary mower with discs JF Stoll SB 200. The average values ranged from 1.68 to 2.02 ha h^{-1}, with average of 1.82 ha h^{-1}. With regards to rotary mowers with drums, it was noted that cutting apparatus Pöttinger CAT 185 has achieved better results. Average production effectiveness of this mower was 1.5 ha h^{-1}, ranging from 1.3 to 1.67 ha h^{-1}. With regards to rotary mower BRK 165, the production effectiveness ranged from 1.13 to 1.39 ha h^{-1} with average production effectiveness of 1.27 ha h^{-1}. Minimal values of production effectiveness were given by oscillatory mower OLT. Production effectiveness of given model of mowers ranged from 0.47 to 0.97 ha h^{-1}, with average production effectiveness of 0.7 ha h^{-1}. Presented results are in conformity with the results given by other authors (Mitrović and Irić, 2003; Barać et al., 2012).

Table 4. Productive effectiveness of tested mowers

Type of mowers	Parameters		Test			Average
			(1)	(2)	(3)	
Oscilatory	Working speed - (v)	(km h^{-1})	3.85	5.62	8.45	5.97
	Working width - (B$_r$)	(m)	1.44	1.41	1.35	1.4
	η_{pr}	(-)	0.85			
	Productivity - (W$_{pr}$)	(ha h^{-1})	0.47	0.67	0.97	0.7
BRK 165	Working speed - (v)	(km h^{-1})	8.44	10.22	11.18	9.95
	Working width - (B$_r$)	(m)	1.57	1.48	1.46	1.5
	η_{pr}	(-)	0.85			
	Productivity - (W$_{pr}$)	(ha h^{-1})	1.13	1.28	1.39	1.27
Pöttinger CAT 185	Working speed - (v)	(km h^{-1})	8.57	10.32	11.47	10.12
	Working width - (B$_r$)	(m)	1.79	1.74	1.71	1.71
	η_{pr}	(-)	0.85			
	Productivity - (W$_{pr}$)	(ha h^{-1})	1.3	1.53	1.67	1.5
Jf-Stoll SB 200	Working speed - (v)	(km h^{-1})	10.38	11.26	12.85	11.5
	Working width - (B$_r$)	(m)	1.88	1.84	1.82	1.85
	η_{pr}	(-)	0.86			
	Productivity - (W$_{pr}$)	(ha h^{-1})	1.68	1.78	2.01	1.82

η_{pr} – Working time efficiency coefficient;
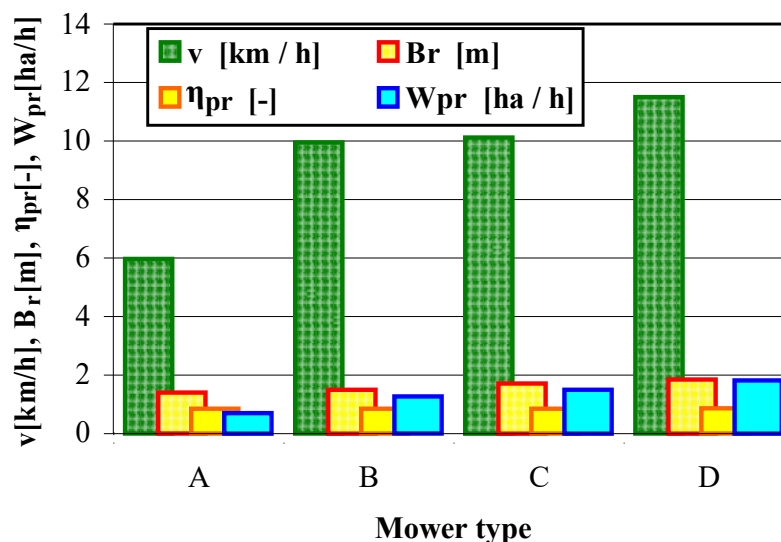
Fig. 3. Productive effectiveness of tested mowers

Conclusion

According to results of tested exploiting parameters of different types of mowers used for alfalfa mowing, the following conclusions may be made:

- The lowest average incision height of 6.17 cm at average moving speed of 9.95 km h^{-1} was made by rotary mower with drums BRK 165; whereas maximal value of 7.15 cm at average moving speed of 11.5 km h^{-1} was made by rotary mower with discs. Both values are in range of optimal ones.
- Total losses made by rotary mowers are significantly higher than the ones caused by oscillatory mower OLT, which had average total losses value of 1.25% of yields. With regards to rotary mowers, the maximal losses of 3.34% of yield was caused by rotary mower with drums Pöttinger CAT 185. Value of other losses made by other kinds of mowers was 3.2% for rotary mower with discs JF Stoll 200; whereas the value of total losses of rotary mower BRK 165 was 2.98% of yield. Since the maximal allowed total losses during alfalfa mowing are 5% of yield, it may be concluded that total loss values of all tested mowers are within optimal limits.
- The final assessment of work quality of the mowers are based upon achieved values of production effectiveness. Thus, it may be concluded that the lowest value of production effectiveness 0.7 ha h^{-1} was achieved by oscillatory mower OLT. Maximal production effectiveness of 1.82 ha h^{-1} was achieved by rotary mower with discs JF Stoll SB 200. Value of production effectiveness of rotary mower with drums is also significant in comparison to oscillatory mower.

According to presented results attained by testing different types of mowers, it may be concluded that use of rotary mowers, primarily rotary mowers with discs, is recommended for alfalfa mowing in the researched area (Rasina Region).

Acknowledgement

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Oral presentation

AUTOMATED WARNING SYSTEM FOR
LAMENESS DETECTION IN DAIRY COWS

Piette D.[1], Norton T.[2], Berckmans D.[3]

Abstract

Lameness in dairy cows is a major cause of production loss and reduced welfare. Today farmers have to invest a lot of time and money in manually detecting the lame cows in the herd. Consequently, a tool that detects lameness in real-time and automated way can create added value for the farmer.

In previous work, an algorithm was developed that analyses 3D top view images from the back of the cow to calculate a back posture score that gives information on the lameness of the cow. The scope of this work is to generate an automated warning system for lameness in dairy cows based on the back posture scores of the cows. The back posture scores of 209 Holstein Friesen cows were gathered over a period of one year in order to establish a historical back posture dataset for every cow. The gold standard for lameness (manual scoring) was collected for all cows by the same experienced observer on a weekly basis over the last six weeks of data collection.

This work presents the warning system prototype developed for real-time lameness monitoring on a dairy farm with 2500 cows. The algorithm developed performs with a specificity of 83.9% and a sensitivity of 76.1%.

Keywords: lameness, dairy cow, automated, warning system

Introduction

Lameness in dairy cows is amongst the leading causes of compromised animal welfare and reduced productivity (Van Hertem et al. 2014, Van Nuffel et al. 2015). With farm sizes increasing and livestock production intensifying, dairy farmers do not have the time to observe each individual cow on a daily basis. This has led to an underestimated prevalence of lameness in many dairy farms. In addition, lame cows in the herd are often not detected or are either detected when they are already in a progressed state of lameness (Archer et al. 2010; Leach et al. 2013). Considerable research has already been performed to automatically detect lame cows in the herd and to detect lameness in an early stage of the disease (Chapinal et al. 2011; Maertens et al. 2011, Ghotoorlar et al. 2012). Nevertheless, limited literature is available that focuses on translating this research into meaningful lameness monitoring solutions that create valuable information for the farmer.

The objective of this study is to build upon the previous work by Viazzi et al. (2013; 2014) in which an algorithm was developed to calculate back posture values that reflect the degree

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of lameness of dairy cows, using images of a cow's back from a 3D top view camera. In particular, this paper presents the design of a prototype automated lameness warning system based on the back posture values generated by Viazzi et al.'s algorithm (2013; 2014). For the design of the system, the authors of the paper collaborated with a commercial farm with 2500 cows. The presented warning system is the result of close collaboration and communication with the farmer to design a system that creates valuable information that can be implemented by the farmer into his daily farm management.

Material and Methods

Back posture values were collected during milking time on a dairy farm with 2500 Holstein Friesen cows. When the cows leave the rotary milking parlor after milking, they walk through an alley before returning to the barn. A 3D Kinect camera with top view of the cows' back is mounted in this alley and captures images of each cow's back as it walks under the camera. These images are used to calculate an average back posture value for each recording session of every cow based on the algorithm described by Viazzi et al. (2014). Back posture data was gathered for 1908 cows from August 2014 until October 2015. Since cows on this farm are milked twice a day, up to two back posture values are available per cow and per day in case image recording and processing were successful for both milking sessions (Viazzi et al. 2014). In the case where two back posture values were available for a cow on a certain day, the average back posture value for that day was calculated.

The gold standard for lameness used in this paper is manual scoring using the Sprecher Scale (Sprecher et al. 1997). This scale uses a 5 point scoring system where a score of 1 is given to a cow that is healthy and a score of 5 is given to a cow that is severely lame. Manual scoring of the 1908 cows in the farm was carried out by one experienced observer every Friday between September 18th 2015 and October 23rd 2015. Due to time constraints it was not possible to score all 1908 cows in one day, which is why some cows lack manual scores on one or more days. In total 5592 manual scores were collected of which 6% (359) were higher than 3, indicating a low prevalence of lameness on this farm.

In order to ensure the quality of the gold standard, only cows with a reliable manual score dataset were withheld for further analysis. First, cows with fewer than 4 manual scores during the scoring period were rejected, leaving 886 (out of 1908) cows for further analysis. Second, the evolution of manual scores was observed for each cow. Cows showing manual scores that were varying continuously between 1 (healthy) and 2 or higher (lame), were rejected from further analysis due to unreliable manual scoring. This resulted in a final dataset of 209 cows.

Figure 1 shows the back posture values and manual scores of newly lame cow 7126. During the scoring period, the manual scores of this cow evolve from 1 (healthy) to 2 and 3 (lame), which is why it is called 'newly lame'. This emerging lameness can also be seen in the back posture values of the cow that increase simultaneously with the manual scores. The objective of the automated lameness warning system is to detect this evolution from healthy back posture values to lame back posture values. Therefore a healthy baseline is calculated using historical data from the cows. A threshold is then defined to decide how much a back posture value can deviate from the healthy baseline before an alarm is generated.
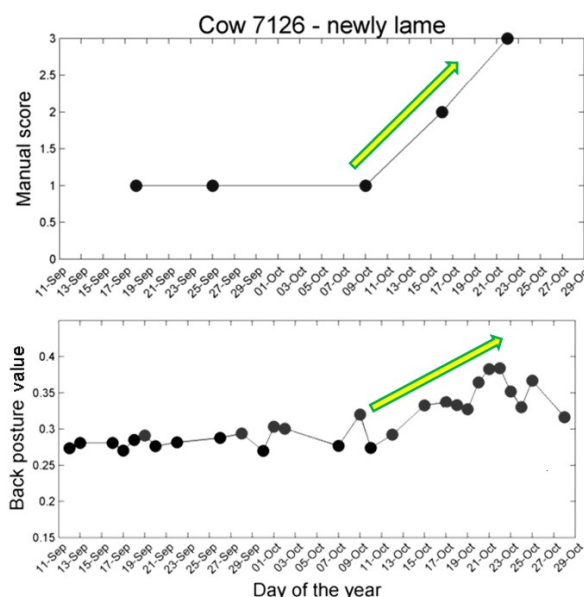
Figure 1: Illustration of manual score and back posture value in a newly lame cow

When an alarm is generated, the variable 'alarm' is set to 1, otherwise it is set to 0. Validation is then performed on a data point basis. When both a back posture value and a manual score are available for a certain data point, this point is labeled as true positive, true negative, false positive or false negative as follows:

- True positive: alarm = 1 & manual score > 2
- True negative: alarm = 0 & manual score 2
- False positive: alarm = 1 & manual score 2
- False negative: alarm = 0 & manual score > 2

Next, sensitivity and specificity of the system are determined.

Alarms generated on a daily basis for each cow are combined in a daily warning list of all cows. The cows on this list are ranked according to the number of alarms they had in the last week and according to their average back posture value of the last 7 days. This means that cows with a more urgent and severe lameness problem are on top of the list and vice versa. A selection of the top 50 cows in the list is shown to the farmer in a Graphical User Interface (GUI) that is specially designed to meet the needs of the farmer.

Results and Discussion

The warning system performs with a sensitivity of 76.1% and a specificity of 83.9%. The GUI that was developed for the farmer is presented in figure 2. The GUI contains three main sections. The most important section is the middle table of the GUI, which contains the warning list of the top 50 cows. From left to right the different columns in the table contain the following information: cow number, average back posture value of the past week, alarm rate of the past week, relative activity, deviation in milk yield and data completeness. The alarm rate tells the farmer the amount of days on which back posture gave an alarm, divided by the number of days for which back posture values were available. The relative activity is a variable generated by the farmer's herd manager software and shows how the activity of each cow changes in time. The deviation in milk yield is calculated by dividing the current milk yield by the average milk yield of the past

3 days and gives the farmer the information whether a cow is recently increasing or decreasing in milk yield. Data completeness gives the number of days for which back posture value was available in the past 7 days, divided by 7. When selecting a cow in the warning list, additional information about that cow is presented on the left and right side of the GUI. The left side visualized the historical data of the cow. The right side lists the treatment history of that cow.

All the information that is shown in the GUI was added and presented this way at the request of the farmer. The reason why milk yield deviation and relative activity are shown in the GUI is because before the implementation of the warning system the farmer used these variables to evaluate lameness in his farm. When introducing the warning system to the farmer, it was important to that he still had this information as a reference. The reason for this is that in the beginning of implementation the farmer needs time to get to know the warning system and trust its.
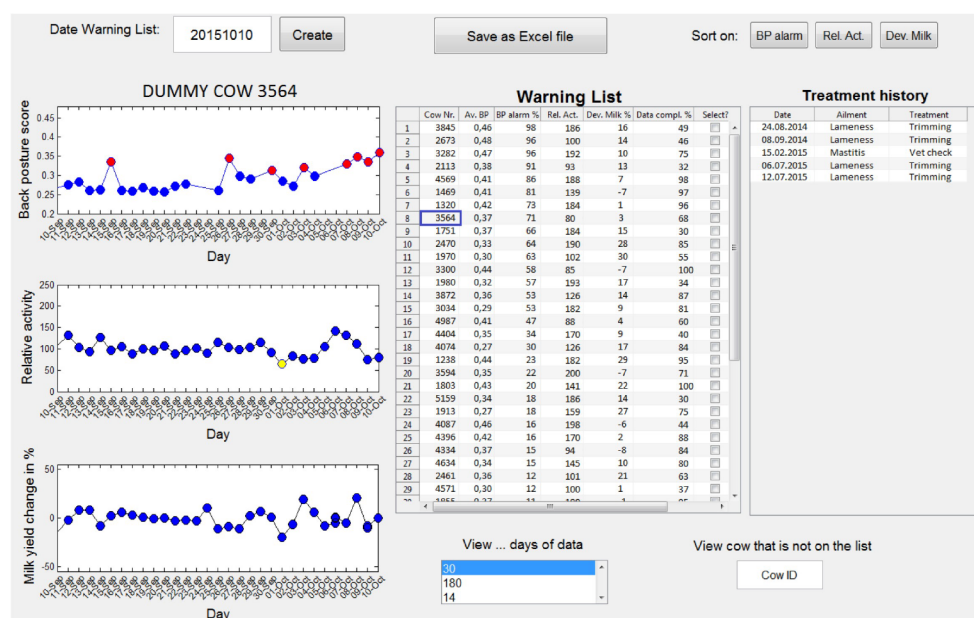
Figure 2: Graphical User Interface (GUI)

The farmer used the GUI from November 2015 until April 2016 without implementing the information from the GUI in the daily management of the farm. During this habituation period the GUI was frequently adapted to the needs of the farmer before coming up with the final version that is presented here. While the farmer was getting accustomed to the GUI and the warning system he based his lameness management decisions on relative activity and milk yield and compared the list of cows he detected using his method to the list of cows that was presented by the warning system. He concluded that 80% of the cows he detected as lame using his method equally appeared on the warning list in the GUI. The farmer had built up trust in the warning system and started implementing information from the GUI in his daily lameness management on the farm in May 2016, of which the results cannot yet be presented in this paper.

Conclusion

This study presents a farm prototype for automated detection of lameness in dairy cows. Using historical back posture values healthy baseline can be defined. Deviations from this baseline based on a threshold result in alarms that can be raised with 76.1% sensitivity and 83.9% specificity. These alarms are then translated into a warning list that can be presented to the farmer using a GUI. In this study a farmer with 2500 dairy cows used the warning system for a 6 months period in order to build up confidence in the system. At the same time he continued using his own "traditional" method to detect lameness in the farm. During this period he noticed that 80% of the cows he detected were also picked up by the warning system. Having gained confidence in the warning system the farmer started implementing the information from the warning system in his daily lameness management on the farm. Future work will determine the results of implementing the lameness management decisions from the warning system in the farm.

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Oral presentation

THE MOST PREVALENT LAMENESS DISORDERS IN DAIRY CATTLE

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Abstract

Lameness in dairy cattle is reported to be the third most common cause of culling or premature removal from the herd, behind reproduction disorders and mastitis. Occurrence of lameness may have a significant impact on cows welfare and dairy production. The incidence of lameness in dairy cows in intensive housing conditions varies throughout the world, ranging from 1 to 55% with the average of about 25%, as the result of the impact of a number of different predisposing factors and causes. Many researchers point out that the most important claws health disorders causing lameness are: digital dermatitis, sole ulcer, white line disease, toe necrosis/apical syndrome and sole hemorrhage. If lameness continues for a long time, it leads to high economic losses due to premature culling, decreased milk production, weight loss, reduced fertility, decreased slaughter value and high treatment costs. In this paper, the most significant aspects of the occurrence, causes and control of given disorders are presented. The importance of early identification of disease, which enables early treatment, significant shortening of convalescence and economic losses reduction, in this paper is also highlighted.

Key words: lameness disorders, causes, consequences, control, dairy cattle

Introduction

Lameness in cattle is a clinical manifestation of a vast spectrum of diseases specified in a total 43 causes (Blowey et al., 2004) and more than 80 potential hazards (Bell et al., 2008). Most commonly, it is a consequence of some claw disorders. It is one of the most serious health, production and welfare problems in intensive dairy cattle farming (Hristov et al., 2012). Despite improvements in husbandry conditions, it is still among three most common cause of premature removal of dairy cows from the herd, behind reproduction disorders and mastitis (Stanković et al., 2014; Zlatanović, 2015). Frequently, lame cows have simultaneously one or both of the mentioned problems, as it found in study by Relić et al. (2015). The incidence of lameness in dairy cows varies in the world, ranging from 1 to 55% with the average about 25% (Alban, 1995; Clarkson et al., 1996; Manske et al., 2002; Mohamadnia et al., 2005; Barker et al., 2010; Ito et al., 2010).

Impact of lameness and claw lesions in cows on health and production is well described by Huxley (2013), with plenty scientific data about impact on milk yield, body weight and carcase quality, nutrition, reproduction and culling with reference to overall economic impact of lameness. The extent of milk yield reductions is difficult to summarize, but they

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are mostly between 270 and 574 kg during the whole lactation (Zlatanović, 2015). Lame cows can experience discomfort and pain of long duration (Green et al., 2002), so lameness has been classified as the most representative animal-based indicator of compromised welfare in dairy cattle (Whay et al., 2003; Hristov et al., 2012).

In this paper, the most significant aspects of the occurrence, causes and control of claw disorders are presented. The importance of early identification of disease, which enables early treatment, significant shortening of convalescence and economic losses reduction, is also highlighted.

Characteristics and treatment of the most common claw disorders in dairy cattle

The most common claws disorders causing lameness are: digital dermatitis, sole ulcer, white line disease, toe necrosis/apical syndrome and sole hemorrhage (Anon., 2012; Zlatanović et al., 2012).

Digital dermatitis (*Mortellaro disease*) is the most important infectious cause of lameness in dairy cattle worldwide due to its prevalence, the cost of management and control, and the adverse effect on cow welfare and production performance (Zlatanović, 2015). It was first identified in Northern Italy in 1974 by Cheli and Mortellaro, and has now been reported across the world (Laven, 2001). It tends to mask or overshadow other more painful lesions present concurrently.

Review of recent perspectives regarding the etiology of digital dermatitis in ruminants is described by Wilson-Welder et al. (2015). Bovine digital dermatitis is a multifactorial polymicrobial infectious disease of the foot. Usually, it is caused by bacteria from the family *Spirochaetaceae* - genus *Treponema*, which containing 45 unique species of which 12 were predominant (Krull et al., 2014). Treponemes which have been successfully cultured to date include representatives of several clusters such as *T. medium/T. vincentii*-like, *T. phagedenis* - like, and *T. putidum/T. denticola* -like (Wilson-Welder et al., 2015). *Dichelobacter nodusus* assumed to be associated with tissue damage and can be readily co-detected with treponemes in cows with interdigital dermatitis and heel horn erosion, and thus is hypothesized to act in synergy with treponemes to initiate bovine and ovine digital dermatitis (Wilson-Welder et al., 2015). Other types of bacteria have also been identified in lesions including *Borrelia burgdorferi* (Blowey et al., 1994; Collighan and Woodward, 1997), *Bacteroides* and *Mycoplasma* species (Collighan and Woodward, 1997), *Campylobacter* species (Evans et al. 2009) and *Candidatus amoebophilus asiaticus* (Berry et al., 2012).

In the past, there were tendencies to treat digital dermatitis lesions with a mixture of antibiotics (generally with variable results), topical astringents (such as formalin which has welfare implications), and footbaths (variety of agents including antibiotics). However, up to now the best approach has not been determined. Studies of bovine digital dermatitis - associated Treponemes in the laboratory have found that penicillin based antibiotics are by far the most effective. Treatments for digital dermatitis include systemic and topical antibiotics (Laven and Proven, 2000) and are summarized by Refaai et al. (2013). In herds where a high proportion of animals are infected individual treatment is very time-consuming, so many farmers instead use footbaths to treat the entire herd (Laven and Proven, 2000). Elimination of digital dermatitis is rare, so repeated application of treatments is required to prevent recurrence of infection (Laven and Logue, 2006; Zlatanović, 2015).

Sole ulcer (*pododermatitis circumscripta, Rusterholz Ulcer*) is considered as the most important, prevalent, and costly of the non-infectious lesions in dairy cattle. In the initial stage the disease has an aseptic nature but later because of complications with bacterial infection, takes the form of suppurative-necrotic pododermatitis (Kos, 2009). The incidence of sole ulcer is ranged from 13.9% in United Kingdom (Greenough, 1987) to 21.5% in North America (van Amstel and Shearer, 2006). In some herds up to 40% (Anon., 2012) and in some Friesian herds up to 80% (Enelvodsen et al., 1991) of mature cows can be affected. Sole ulcers and white line disease are often studied together and reported as claw horn disruption lesions (CHDL) that often represent over 65% of all lesions diagnosed in lame cows (Foditsch et al., 2016).

Sole ulcer occurs worldwide where dairy cows are kept on hard or concrete flooring in tie or free stalls, particularly if conditions are unhygienic as is often during winter months. The most important predisposing factor is the presence of subclinical mastitis, but the primary cause is compression i.e. incarceration of dermis between the radius bone and sole horn (Kos, 2009). This condition compromises the horn production and its hardness (a hole is forming in the sole). As the damaged corium undergoes repair, granulation tissues erupt through the hole. Softening of the sole horn also occurs, which increases the rate of horn wear. The sole horn also softens when horn is exposed to the fluid component in slurry (Anon., 2012).

Iatrogenic forms of the lesion are produced when inexperienced claw trimmers remove too much horn from beneath the heel (and/or the posterior region of the abaxial wall), resulting in abnormal pressure on the dermis. Excessive wear of the softened sole horn flattens and thins the sole. Also, heel erosion is another potential contributing cause of a sole ulcer. Recently, *Treponema*-like organisms have been identified in the lesions (Anon., 2012).

Treatment of the claw with sole ulcer is aimed at removing pressure from the affected area by therapeutic claw trimming and applying a "lift". The simplest form of lift is a wooden or rubber block glued or nailed to the unaffected medial claw, thereby removing all weight-bearing from the ulcer region. Various models of easier-to-apply plastic slippers have been developed. Protruding granulation tissue need not be excised and must not be treated with any caustic agent, because this can retard healing. Bandages should not be applied, because this results in continued weight-bearing at the ulcer site. Covering the lesion causes it to remain moist and promotes maceration and bacterial infection. Many ulcers never fully resolve, and affected cows may have chronic, low-grade lameness and need corrective foot trimming 3–4 times/yr for their productive lives (Anon., 2012; Zlatanović, 2015).

White line disease refers to a group of lesions affecting the junction between the sole and the wall of the claw. White line disease is a non-infectious condition that occurs when the sole separates from the side wall of the hoof, allowing foreign material to penetrate and infect the white line region. Lesions of the white line include hemorrhage, fissure, and abscessation, which represent different stages of the breakdown of the integrity of the white line. Pathomorphological findings of white line disease with digital and inner organ infections in culling dairy cows were studied by Nouri et al. (2013).

Incidences of white line disease in older cows reach up to 35%. It is commonly observed lesion and has frequently been reported as a major cause of lameness, particularly where cattle are housed, fed concentrates and higher yielding (Collick et al., 1997; Amory et al., 2008; Kujala et al., 2010).

Treatment consists in cutting out any black mark in the white line (until healthy horn is exposed), and providing a free drainage of local abscess. Abscessation at the coronary band is usually indicative that white line disease is present. The same applies to a retroarticular abscess. Healing is uncertain, sometimes impossible (Anon., 2012).

Toe necrosis/apical syndrome: this term covers three different etiopathologies for a condition having a similar appearance. However, *Treponema*-like organisms have been isolated from all of these lesions (Anon., 2012). According to Paetsch (2014) toe necrosis syndrome is separation of the apical white line with tissue necrosis and clinical lameness. This definition includes complications such as pedal osteitis, middle and proximal phalangeal osteomyelitis, tendonitis, tenosynovitis, cellulitis, and embolic pneumonia. Toe tip necrosis is a disorder most commonly affecting the lateral claw of the hind feet of 10 to 12 month old beef cattle that develops within several days to a few weeks after processing, weaning, and transportation to a feedlot (Gyan et al., 2015). It is thought that long periods of standing without exercise allow the blood to pool in the feet and damage the tissues (Anon., 2012).

Treatment is cost effective only in animals with no obvious complication. The cavity should be cleansed, dried, and packed with an antibiotic powder. Further actions depend on the presence of necrotic tissue and/or bone necrosis. There are several reports of the toe (not the claw) having been amputated with satisfactory recovery. Systemic antibiotics and application of a lift to the sound claw is advised (Anon., 2012).

Sole hemorrhage: this is very common non-infectious lesion in lame cows. It is regarded as a typical sign of laminitis but is frequently overshadowed by bruising of the sole. Blood stains in the sole of the hoof are the most commonly seen abnormality of the sole. When subclinical laminitis was first described, sole hemorrhage was considered to be an invariable clinical sign. It was thought that the arteriovenous shunts in the solear papillae were compromised, allowing blood to perfuse down the tubules, giving a brush-like appearance. Since then, solear hemorrhages have increased in prevalence. This may be due to cows being forced to stand on concrete for longer periods than previously. Bruising/trauma more likely occurs if the horn is softened by moisture, or if the quality of the horn is reduced because of some nutritional error or because the thickness of the sole is reduced too much during hoof trimming. Interpreting the true cause of solear hemorrhages can be important (Anon, 2012; Zlatanović, 2015).

Control of lameness and claw disorders in dairy cattle

The treatment of laminitis and claw disorders mentioned above is not fully effective. Early identification of lesions permits early treatment, which significantly reduces the recovery period, but diagnosis in an individual animal is often not possible at the time (Zlatanović, 2015).

Prevention is most important in the control of lameness in cows. Preventative management in dairy herds is described by Ishler (2016) and the most important elements are presented here. At the first place, it requires knowing the prevalence of lameness and the animal group affected, which can be determined using a lameness scoring system by Sprecher et al. (1997). Then, factors that potentially affect claw condition must be considered.

The incidence of lameness and laminitis can be controlled through good nutrition and use of proper feeding and other management practices. Management practices such as

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vaccination, transportation, and reduced exercise can impose stress. Stress can reduce the animal's resistance to disease and can be a factor in lameness. Nutrition problems such as sudden changes in the ration, low or poor quality fiber, high energy feeding, and mineral and vitamin imbalances can cause stress, especially in early lactation. Disease, pain, and animal aggression can also be factors (Hristov et al., 2011; Ishler, 2016).

Important roles in claw disorders prevention have providing a comfortable area for resting, reclining and ruminating, and enough clean, dry and non-slippery walking space. First calf heifers may need to be managed differently from older animals to minimize development of a pecking order, and overcrowding. Mechanical development of hemorrhaging and/or ulceration can also occur in heifers as a result of trauma incurred from being transposed from earthen lots to concrete floors. Preventing laminitis in heifers may consist of a separate heifer group where animals are acclimated to their new environment allowing for increased resting time and minimized aggression.

Regular claw trimming may increase the functional life of a dairy cow. A professional hoof trimmer who uses correct equipment and procedures should be employed. Good record keeping is a key to monitoring a cow's condition (Zlatanović, 2015; Ishler, 2016).

Conclusion

The most common claws disorders in intensive dairy farms are digital dermatitis, sole ulcer, white line disease, toe necrosis and sole hemorrhage. Considering their prevalence, treatment costs and low healing rate, the best control method is a regular appliance of the preventive measures based on the main factors for lameness including nutrition, feeding management, stress causing procedures, cow comfort, and hooftrimming.

Lameness prevention requires changes in herd management. Therefore, producers should be aware of the consequences and causes of lameness in cattle, as well as the benefits arising from the prevention of lameness: increasing of milk yield, cow welfare and quality of their life, and farm profitability.

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Oral presentation

TEAT-END HYPERKERATOSIS IN DAIRY COWS

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Abstract

Classification of teat tissue conditions in cows can be used in assessment of influences caused by milking management, milking equipment or the environment on the teat tissue and in assessment of risks of mammary glands infection. Influences of those factors cause short-term, medium and long-term consequences on the teat tissue. Short-term consequences refer to changes of teat color after milking, teat base swelling, swelling of teat end, and opening of the teat end (teat canal). Medium-term consequences refer to changes in teat skin condition, while long-term consequence is known as hyperkeratosis. From a physiological point of view, hyperkeratosis refers to excessive accumulation of keratin due to normal physiological response of the body to forces that press the teat skin during milking, either by a milking machine, by hand or by a sucking calf. There are many factors that can lead to hyperkeratosis, the most common of which are: shape of teat end, amount of milk, maximum milk flow, duration of milking and post-milking, order and stage of lactation, appearance of teat tissue and the relations between milking management and milking machine. Total time per day with a milk flow lower than 1 kg/min seems to influence the level of hyperkeratosis, which is connected with the ascending phase of milking, i.e. with the release rate of milk (after preparation of a cow and adjusting of a milking machine) and the milk flow rate and milking duration. Increased hyperkeratosis occurs with the appearance of bimodal release of milk or with too long subsequent milking. Increased thickening or roughness of teat end makes cleaning of teats more difficult and enables occurrence of lesions in this area, which increases the risk of mastitis. There are several methods to classify teat end and teat skin condition, one of which is determined according to Britt and Farnsworth, 2005.

It is necessary to maintain good condition of cow teats, especially the skin moisture that provides natural elasticity and greater resistance to occurrence of thickening. This can be achieved by good milking management, by hygiene milking, by quality preparation of cows for milking, as well as by good protection of teats at the end of milking.

Keywords: dairy cows, milking, teat-end hyperkeratosis, assessment of teat-end hyperkeratosis, mastitis

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Introduction

Introduction of machine milking into cattle production has been aimed to speed up and to simplify the milking process, as well as to increase the hygienic quality of milk. However, certain inconsistency is noticed between functioning of milking machines and cows' udder, so that udder health is greatly deteriorated. Mastitis remains the most common disease that affects dairy cows worldwide. At the same time, it is the most expensive disease for farmers (Miller et al., 1993, Hillerton, 1996, Carlen et al., 2004). One of the factors that cause occurrence of mastitis is associated with inadequate levels of milk flow during milking (Vági 2002, Húth 2004), which happens because of poor milking capability of dairy cows and their poorer adaptation to machine milking (uneven udder quarter, too slow or too fast milk flow, bad milk flow curves, bimodality, etc.). Moreover, occurrence of mastitis is also related to bad management of milking, poor preparation of cows for milking, aggressive disinfectants, bad climatic conditions and some other factors. Dairy cows are often exposed to a threefold influence of: people, milking machine and the surroundings. These factors influence the cows separately or in interaction. The consequence of one factor also entails the other two, and all together affect the animal itself and its susceptibility to mastitis. Selection trends are often aimed towards increasing the speed of milking, however such trend consequently influences the increase of teat tissue burden and reduce the defense mechanism of teat canal (Reinemann et al., 2008). The pressure on teat tissue during milking leads to development of edema, which then causes slower closing of teat canal and leads to hyperkeratosis, thus increasing the risk of mastitis occurrence.

Interaction of Teat Tissue and Milking Machine
The process of cows' milking should be quick, clean, complete and carefully performed (Neijenhuis, 2004). During the entire milking process, cup rubber liner is in constant contact with the teat tissue. Changes in over-pressure and the entry of atmospheric air cause compression of cup rubber liner below the teat end (the phase of resting) and the phase of milking (when the milk is extracted from teat canal). According to Mein et al. (2003), optimal pressure of cup rubber liner on the teat tissue is 8-12 kPa (Figure 1), which is close to normal arterial pressure of 10 kPa. The level of over-pressure and duration of liner opening during one pulsation cycle are the main factors that influence the maximum milk flow and milking speed during milking (Spencer et al. 2007). The increase in over-pressure and the D-phase of milking affect the increase of teat tissue congestion, which is seen as thickening of teat wall (Gleeson et al. 2004), and as slower closing of teat canal (Neijenhuis et al., 2001).
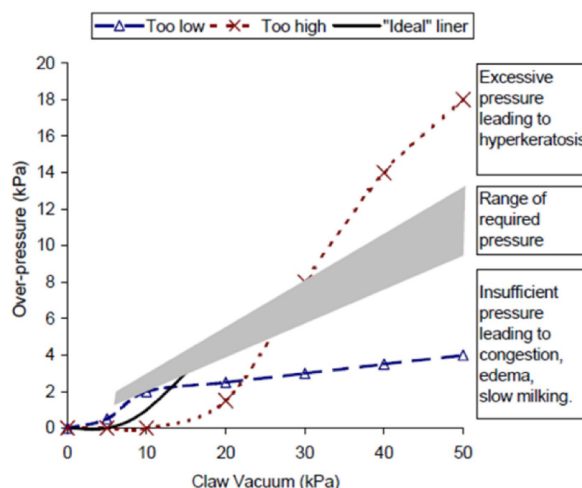
Figure 1. Relationship between over-pressure applied by different liners, and the pressure required to relieve congestion and edema, with increasing milking vacuum level (Mein et al., 2003). Prolonged duration of low milk flow can lead to increased thickening of teat wall and should be therefore avoided (Mein et al., 1973). Mein et al. (1993) determined a significantly ($p<0.05$) greater thickening of teats (7 - 25%), shortening (3 - 6 mm) and compression after milking with higher values of over-pressure (≥ 40 kPa), in comparison to milking of cows with lower values of over-pressure (25 and 30 kPa). The level of liner pressure is a key factor to reduce congestion of teat tissue and to increase the maximum milk flow (Capuco et al., 1994). Teat tissue takes a few hours to fully recover after milking (Gleeson et al., 2002). There is a greater risk of infection when tissue thickness changes by more than 5% (Zecconi et al., 1992).

Changes in Teat Tissue Induced by Machine Milking
Appearance of teat tissue of dairy cows is a good indicator of quality surroundings in which animals are kept, of milking management and safety of milking machines. Appearance of teat tissue can be also taken as an indicator for mastitis risk. From a physiological point of view, machine milking should not cause more than 5% change in the teat tissue, because this is a limit within which the tissue can recover. Any change above that limit increases the risk of mastitis. Cows' udder are in contact with milking device from 50 to 100 hours per lactation (Technote, 2012), and teats (sphincter and teat canal) are the first line of defense against diseases. Any injury, change in teat tissue or in teat orifice increases the risk of a larger number of microorganisms penetrating into the tissue, thus causing occurrence of udder diseases. Changes that are induced by machine milking can be divided into: short-term, medium and long-term changes. Short-term consequences refer to changes in teat color after milking, swelling of teat base and nearby area, swelling of the teat end and nearby area, and open teat orifice (Table 1). Medium-term consequence refers to changes of udder skin, and the long-term consequence is hyperkeratosis.

Table 1. Some of the common primary causes of machine-induced teat condition (Ohnstad et al., 2007)

	Teat color	Swelling at the base	Firmness/hardness of the teat end		Open orifice
Observation	Red/blue	Ringing	Hard	Wedge	Diameter
Machine factors					
High milking vacuum	X	X	X		X
Faulty pulsation	X		X	X	
Short D-phase	X		X		
Long D-phase				X	
Liners					
✓ wide bore	X				
✓ aged	X	X			
✓ high tension	X			X	X
Mouthpiece					
✓ large chamber	X	X			
✓ small lip diameter	X	X			
✓ stiff lip		X	X		
Mismatch of liner and teats	X	X			X
Milking management					
Long dribble times	X	X	X		
Over-milking	X	X	X		X
Teat cup crawling		X			

When cows' teat is flattened after milking, defense mechanism of the teat canal is weakened, so the penetration of microorganisms into the udder is facilitated, thus increasing the risk of mastitis (Zecconi et al., 1992; Gleeson et al., 2004). The research into influences of subsequent milking on health and condition of teat end, performed by Natzke et al. (1982), showed that there was a greater risk of mastitis because of subsequent milking, but there were no significant changes occurring in appearance and condition the teat ends. Sieber and Farnsworth (1984) pointed out the importance of monitoring the milking machines (pulsators, vacuum regulator, pump, etc.) in order to prevent injuries of teats. Hamman and Mein (1990) observed the changes in teat end tissue induced by machine milking, and they determined that the increase in average changes at the teat end varied from 10 to 20% or more, which depended on the milking method. Prolonged exposure of teats to over-pressure, prolonged duration of milking and longer period of low milk flow during milking result in occurrence of rough rings on teat ends (Rasmussen, 1993). Although the accumulation of keratin is a normal physiological response of a body to forces that press teat tissue during milking, some factors still negatively affect that mechanism, causing excessive amount of keratin to accumulate, thus causing hyperkeratosis.

Hyperkeratosis
Due to excessive accumulation of keratin, there are more rough tissue created on teat ends, which deteriorates the appearance and condition of teat tissue. Such formation is called: liner rings, teat flowers, teat erosion, thickening, cornification or teat-end roughness. This formation is greatly influenced by position of teats, teat-end form, amount of milk, maximum milk flow, duration of milking and subsequent milking, lactation stage, number of lactation and milking management (Bakken, 1981, Johansson, 1957, Neijenhuis et al., 2000, Sieber and Farnsworth, 1981). Thickening of teat ends increases after about the fourth month of lactation and decreases afterwards (Neijenhuis et al., 2000). Stronger

hyperkeratosis occurs in high production cows, especially during the colder periods of the year. Total time per day with the milk flow being less than 1 kg/min (Mein et al., 2001) seems to affect the level of hyperkeratosis, which is connected with the ascending phase of milking, i.e. the release of milk (after preparation of a cow and after assembling of milking device), and the milk flow and duration of milking. The Table 2 overviews the risk factors connected with development of hyperkeratosis.

Table 2. Overview the risk factors connected with development of cow teat-end hyperkeratosis (Mein et al., 2001)

Risk Factor	Reason for Increased Likelihood of Teat-end Hyperkeratosis
Pointed Teats	The load applied by the closing liner acts on a smaller area of teat surface
Increasing age	The "wrinkle" factor in all species
Higher production	Cups are on for longer
Peak lactation	Cups are on for longer
Cups on before milk let down	Increased period of milk flow <1.0 kg/min (<2.2 lb/min)
Low threshold for ATOs	Increased period of milk flow <1.0 kg/min
Over-milking	Increased period of milk flow <1.0 kg/min
High vacuum	Increased stress on teat tissues

More expressed symptoms of hyperkeratosis occur due to bimodal release of milk or too long subsequent milking. Increased thickening or roughness of the teat ends disables proper cleaning of teats, thus facilitating lesions in this area, and leading to increased risk of mastitis. In their research, Sieber and Farnsworth (1984) pointed out great influences of environmental factors on teat condition and on accumulation of bacteria or occurrence of lesions. Increased risk of udder infection and lesions happen because of influences of viruses, chemicals, environmental conditions and milking machines. Occurrence of greater lesions on teats is of seasonal character, being reduced in spring and enhanced in mid-autumn. The research of Rønningen and Reitan (1990) reported significant (p<0.01) relation between the depth of teat penetration into the teat cup during milking and udder health. It was proven that deeper penetration of teats into teat cup during milking was in positive relation with teat end roughness (p<0.001) and occurrence of clinical mastitis (p<0.01). Rasmussen and Larsen (1998) reported a significant (p<0.01) impact of teat protective ointment (based on 10% glycerol) on teat condition, and noted that appearance of teat skin was improved in cows treated for three weeks with a protective spray, when compared to teats of cows that were not treated. Neijenhuis et al. (2001) stated that small keratinous ring at the teat end did not increase the risk of mastitis in lactating cows, thus it could be considered as a normal physiological response of an organism (teat) to machine milking. Greater thickening (hyperkeratosis) and teat-end roughness is connected with greater probability of new udder infection. Reinemann et al. (2008) determined that there was a relation between hyperkeratosis (teat end assessed as rough and very rough) and prolonged duration of milking with thick liners, while smooth keratinous ring at teat end was in relation with shorter duration of milking and silicon liner. Increased keratinization at teat end is affected by duration of milking, which is a consequence of amount of milk and other milking properties. Cows, i.e. their teats, that undergone milking in duration of <4.3 minutes are less probable to develop thickening of teat end if compared to cows that undergone milking in duration of >5.3 minutes. The reason for such occurrence is found in the fact that pressure of liner on teat tissue lasts shorter. Hyperkeratosis is undesirable for animal well-being because it causes discomfort and disturbs the milking process (Hamann, 2000).

Assessment of Teat End Appearance

In order to assess thickening of teat ends, it is necessary to take photo of teats once a week from below and from the side immediately after milking, i.e. after removing the milking unit, and before applying a disinfectant. For better assessment of the teat orifice, it is necessary to wipe the remains of milk properly with a paper towel. If the milking site is dark, it is necessary to use a flashlight. There are several methods and ways to assess the appearance of teat ends and condition of tissue, and one of them is a method of Mein et al. (2001) (Figure 1.a), that marks teats as follows: without keratinous ring (N), with partial ring (S), with a rough ring (R), and with very rough ring (VR). Another way is visual and palpation assessment according to Britt and Farnsworth (2005). The assessment is done in a way to distribute teats in five classes with three subclasses. For example, teats of excellent condition belong to the first class with three possible subclasses, which refer to the expression of keratinous ring on teat end (small, medium, high), as shown in the Figure 1.b. In a herd of 80 cows, it is necessary to examine all teats of all cows in order to obtain a realistic assessment. In herds of 80 - 400 cows, it is necessary to randomly examine 80 cows, and in herds with more than 400 cows, the assessment is done on at least 20% of cows. It is necessary to undertake certain measures to improve the condition of teats in cows' herd if the following occurs (Mein et al., 2001):

- ✓ Condition of teat tissue: more than 5% of cows has open lesions (including wounds and cracks) on one or more teats.
- ✓ Teat-end hyperkeratosis: more than 20% of cows has one or more teats with rough or very rough ring, or more than 10% of cows with very rough ring

These changes are primarily caused by poor surroundings, mechanical and chemical irritations or because of cows themselves (shape of teats, amount of milk, genetics). All of those causes can be deteriorated by machine milking, especially if managed badly (subsequent milking, prolonged milking because of low milk flow) and by malfunctioning milking machine parts.

Score	Description	Illustration
1	**No Ring** The teat-end is smooth with a small, even orifice. This is a typical status for many teats soon after the start of lactation.	
2	**Smooth or Slightly Rough Ring** A raised ring encircles the orifice. The surface of the ring is smooth or it may feel slightly rough but no fronds of old keratin are evident.	
3	**Rough Ring** A raised, roughened ring with isolated fronds or mounds of old keratin extending 1 to 3 mm from the orifice.	
4	**Very Rough Ring** A raised ring with rough fronds or mounds of old keratin extending 4 mm or more from the orifice. The rim of the ring is rough and cracked, often giving the teat-end a "flowered" appearance.	
5	**Open Lesions or Scabs**	Not pictured

      a   b

Figure 1. a, b. Classification of teat end condition (Mein et al., 2001; Britt and Farnsworth, 2005)

Conclusion

Daily milking of cows can cause long-term changes in teat tissue. The type and intensity of such changes depend on several factors, some of which refer to the animal itself (teat position, teat-end form), and some refer to the milking machine functionality (pulsators, vacuum regulator, pump, type of liners, etc.), as well as to environmental influences (climate, season, disinfectants, etc.). Certain discrepancies between milking machine and cows' udder cause excessive accumulation of keratin on teat ends (hyperkeratosis), which causes creation of rough tissue to negatively affect the appearance and condition of teat tissue and consequently to increase the risk of mastitis. It is necessary to maintain good condition of cow teats, especially the skin moisture that provides natural elasticity and greater resistance to occurrence of thickening. This can be achieved by good milking management, by hygiene milking, by quality preparation of cows for milking, as well as by good protection of teats at the end of milking.

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Oral presentation

RELATIONSHIP BETWEEN CLAWS TREATMENT AND SOMATIC CELL COUNT IN MILK OF SIMMENTAL DAIRY COWS

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Abstract

The relationship between claws treatment and somatic cell count in milk on 45 Simmental cows in age of 3 and 4 years (in the first and the second lactation) with observed claw changes in loose system of rearing and intensive production was analyzed in this paper. The somatic cell count (SCC) was determined by IDF staining and counting method on the day of claws correction and seven and fourteen days afterwards. All statistical analyzes were performed using the statistical program Statgraphics Centurion XV.

The results of investigation pointed out that there was very significant difference ($P<0.01$) regarding SCC values on the day of claw treatment (180 399 cells/ml) and seven days after treatment (87 071 cells/ml), as well as significant difference ($P<0.05$) between values on days 7 and 14 (162 049 cells/ml) after claws treatment. Value of SCC in milk decreased significantly seven days after claws treatment ($P<0.01$), but two weeks after treatment it significantly increased ($P<0.05$).

Keywords: dairy cows, Simmental breed, claws correction, somatic cell count

Introduction

Good health is "*conditio sine qua non*" for maximum production of dairy cows. One of the key health aspects is limbs condition, especially claws, enabling ease of movement for the cows, their good welfare and maximal production.

Research data (Zlatanović, 2016). revealed that lameness occurrence in dairy farms continuously increase worldwide in last twenty years, even up to more than 50% of the herd in certain farms, at least once a year.

Mostly, lameness is caused by disturbed morphological and functional integrity of muscle and bone system of the limbs. The other causes of lameness are mechanical insults in combination with predisposing factors acting for a long time. Concerning lameness occurrence rate, economic losses caused by diverse pathology of the locomotor apparatus

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are considerable which are reflected in culling cows, decrease of milk production and treatment fees (Clarkson et al., 1996; Shearer and Van Amstel, 2011).

The interaction between lameness, lying times, hygiene and the type of surface that the foot is exposed to when cow is standing, is a complex one. Current theories on the pathogenesis of laminitis clearly demonstrate an interaction between events around parturition, diet and the environment (Livesey et al., 1998; Webster, 2001). For instance, there is a growing body of evidence that increased lying times has a beneficial effect on lameness prevalence and claw health. Regarding this, excessive time spent weight bearing may facilitate the breakdown of the dermal-epidermal lamellar connection, initially triggered by the activation of metalloproteinases and other similar enzymes from either hormonal events around calving time (Tarlton et al., 2002), or from the action of *Streptococcus bovis* exotoxin (Mungall et al., 2001) released during an acidotic event. Increased duration of weight bearing may facilitate the transport of the exotoxin to the capillary beds of the dermal tissues, and also stress the connections between the dermis and epidermis, facilitating sinking of the pedal bone within the horn capsule, subsequently producing clinical signs of laminitis at the sole surface. In addition, rough walking surfaces have been shown to increase lameness prevalence and excessive exposure to concrete may result in excessive wear of the claws (Faye and Lescourret, 1989.).

Besides constitutional compounds, important milk constituents are leucocytes originating from blood and epithelial cells from udder, known as Somatic Cell Count (SCC), whose increased number and changes in composition indices inflammatory tissue reaction (Bramley et al., 1984; Harmon, 1994; Hamman, 1996; Barkema et al., 1999). Quantitative presence of SCC is used to estimate udder health as well as milk yield loses, milk processing traits (Politis and Ng-Kwai-Hang, 1988) and economic loses which are consequence of mastitis (Jones et al., 1984; Brown et al., 1986).

The findings of Warnick et al. (2001) revealed decrease in milk production associated with lameness in cows in second or greater lactation and for more severe cases. In their study, in one herd, the decrease in milk production was greater for cows with sole ulcers or foot abscesses than for foot rot or foot warts. Cows with abscesses or foot rot tended to have larger decreases in milk production in the other herd. The authors concluded that inconsistent results between farms may have resulted from differences in the relative frequencies of specific causes of lameness in the two herds and in the way lame cows were identified and defined for the study. Great attention was paid to the causes of lameness, especially in intensive systems of rearing (Green et al., 2002).

The aim of this paper is to investigate impact of the lameness on SCC of dairy cows.

Material and Methods

The relationship between claws treatment and somatic cell count in milk on 45 Simmental cows in age of 3 and 4 years (in the first and the second lactation) with observed claw changes in loose system of rearing and intensive production was analyzed in this paper. The average amount of milk in the first and second lactation was 4900 and 5300 kg of milk per cow, respectively. Cows consumed water *ad libitum* and were fed with total mix ration regarding milk production.

The samples for somatic cell count were taken at the time of milking on the day of correction of claws, and then after seven and fourteen days from the correction for determination of somatic cells count.

The number of somatic cells in 1 mL of milk was determined IDF staining and counting method. The method for determining the number of somatic cells in milk is given in the Rulebook on methods of microbiological analyzes and super analyzes of foodstuffs (Official gazette SFRJ, no. 25/80). Counting was performed on the day of claws correction and seven and fourteen days afterwards. All statistical analyzes were performed using the statistical program Statgraphics Centurion XV.

Results and Discussion

Results related to SCC and statistical parameters were presented in table 1.

Table 1 Descriptive statistic parameters regarding SCC in cow's milk

	Number of cows	X	SD	CV (%)	X_{min}	X_{max}	Range	Standard asymmetry
On correction day	45	180,399	125,983	69.83	27,550	633,650	606,100	5.28
7 days after correction	45	87,071	78,749	90.44	18,367	428,000	409,633	7.87
14 days after correction	45	162,049	203,624	125.65	27,550	826,500	798,950	7.06

According data in table 1, it could be observed that SCC was decreased from 180399 to 87071 cells/mL on day 7th after correction, but increased to 162049 cells 14 days after correction.

Standard deviation was large and in range from 78,749 on 7th day to 203,624 on day 14th after correction. The coefficient of variation was the lowest on the day of correction and amounted to 69.83%. Seven days later its value was 90.44%, and 14 days after treatment amounted to 125.65%.

Somatic cell count ranged from a minimum of 18,367 seven days after correction, to a maximum of 826,500 fourteen days after correction of claws. The range of values was 606,100 on the day of correction, after seven days had a value of 409,633, and fourteen days later, it was 798,950.

In table 2 were presented results of statistical analysis of significance of SCC differences on correction day, 7 days after correction and 14 days after correction.

Table 2 Significance of SCC differences on correction day, 7 days after correction and 14 days after correction

Source of variation	Σx^2	Degrees of freedom	Mean square	F-value	P-value
Between treatment	2.20028E11	2	1.10014E11	5.19	0.0067**
Inside the treatment	2.79558E12	132	2.11787E10	-	-
Σ	3.01561E12	134	-	-	-

**P<0.01

According table 2, it could be noticed that there were very significant difference between SCC of treatments - periods of SCC evaluation.

In table 3 were presented results of homogeneity of treatments testing and analyzed possible influence of extreme values on statistical significance of the results. Testing revealed the homogeneity of the examined groups, so nonparametric tests were not performed. The drastic drop of somatic cell count seven days after claws treatment might be understand through decrease of the stress and pain relief, while fourteen days after treatment numerous previously mentioned factors took over (Faye and Lescourret, 1989; Livesey et al., 1998; Webster, 2001; Mungall et al., 2001; Tarlton et al., 2002).

Table 3 Test of homogeneity of treatments

	Number	Mean value	Homogeneity of treatments
On correction day	45	180.399	Homogeneous
7 days after correction	45	87.071	Homogeneous
14 days after correction	45	162.049	Homogeneous

In table 4 were presented significances of SCC differences between treatments using LSD test.

Table 4 Significance of differences of SCC between treatments

Tested treatments	Difference	+/- Limit (range)	Significance
Correction day – 7 days after correction	93.328,0	60688,5	*
Correction day – 14 days after correction	18.350,0	60688,5	-
7 days after correction - 14 days after correction	-74.978,0	60688,5	*

ns = p>0,05; * = p<0,05; ** = p<0,01

SCC evaluation in milk was carried out on the day of correction of claws, seven and fourteen days after the correction.

Mean values of SCC in 1 mL of milk in three periods studied had the following results: 180,399 on the day of correction, after seven days 87,071 and 14 days after correction 162,049. Large standard deviation which had a value of 78,749 seventh day after correction to 203,624 (125.65% CV) on the fourteenth day after the correction of claws was noticeable.

Analysis of variance confirmed the significance of differences in SCC between the values obtained at three time periods studied (P<0.01; F=5.19). Homogeneity tests confirmed the homogeneity of the group. Significant differences in SCC were between the values at the correction claw and seven days later, as well as between the values of the seventh and fourteenth days. Average somatic cell count was significantly lower for seven days after the correction of the feet, and later rose again.

Average SCC in 1 mL milk healthy udder amounts 70 000 – 90 000, and in the most cases it is smaller than 150 000 in 1 mL. The count and population of somatic cells composition depends on many factors, such as season, parity, lactation phase, external factors and technological procedures on farm, udder health and activity (Bodoh et al., 1976; Kennedy et al, 1982; Barkema et al., 1998, 1998a; Hristov, 2002a; Hristov et al., 2002b;   Schukken

et al., 2003;). Increase of SCC values is related to the type of pathogen as well (Schepers et al., 1997). The value of SCC is significantly higher in colostrum than in milk. In the first days after calving in first lactation, SCC is more than 1000000 in 1 mL of milk, and after two weeks, about 500000 in 1 mL (Boboš and Vidić, 2005). At the end of lactation SCC again increases. It must be taken into account the variation in SCC in the morning or evening milking as well as during the same milking. Generally, the SCC is the greatest at the end of milking, and the lowest immediately before milking. It was found that SCC increases in the elderly cow's milk, as well as with the increase in days of the milking. The increase in SCC and increasing the number of lactation animals can be explained by the fact that the risk of infection increases with age, probably because the immune system of older cows is not efficient enough or because the udder is already exhibited many unfavorable factors in early lactation, which facilitates the penetration of bacteria in mammary gland (Detilleux et al., 1997). In addition, SCC can also be a characteristic of the breed, which may be used in the selection with the aim of reducing the incidence of subclinical and clinical mastitis (Mrod and Swanson, 1996). Morphological characteristics of the udder as a hereditary trait influence the SCC (Boettcher et al., 1998). Different stressors also influence SCC (Dohoo and Meek, 1982). The effect of season on the SCC has also been the subject of numerous studies. Besides a significant increase in SCC in milk, heat stress reduces milk production up to 10-20%. The number of somatic cells is the lowest during winter and highest during summer (Dohoo and Meek, 1982; Wells and Ott, 1998), which is in accordance with high occurring rate of cow's mastitis during hot season and high rate of infection in summer caused by environmental pathogens (Smith et al., 1985). In addition to all the above factors, the most significant factor influencing the increase of SCC in milk is an infection of the mammary gland (Dohoo and Meek, 1982; Djabri et al., 2002). This refers to the SCC in milk of individual udder quarter, milk cows in general and milk in a pooled sample of the herd.

Generally, SCC in aggregate herd milk should be determined after milking, while in particular quarters on high-capacity farms in countries with developed dairy production it is done usually once a month in different ways. This is in accordance with results of Warnick et al. (2001) and Green et al. (2002), who concluded that type and severity of clinical lameness has a significant impact on milk production and important information for assessing the economic impact of clinical lameness and its impact on cow health, pointing out the importance of early identification of clinical lameness and the urgency of techniques to improve the definition of this highly subjective diagnosis.

Accurate determination of SCC, mostly in bulk milk samples, is performed by Fossomatic which use a method of automatic counting cell nuclei on the principle of fluorescence microscopy (Sandholm et al., 1995). Considering that for subclinical mastitis there are no visible changes in the udder and the milk SCC determining the aggregate sample of milk used to assess the health status of the herd, and possible losses in production. Maximum limit of SCC is being used as a factor to determine the suitability of milk for processing, whereby the value of the upper limit of SCC varies in different countries (for us is 400,000 cells/mL pooled sample of milk). It is also accepted as an international standard for determining the quality of milk, both in developed and in developing countries.

These results indicate that the positive effect of the correction claw reflected in the reduction of the number of somatic cells in milk, even though many factors, such as breakdown of the dermal-epidermal lamellar connection, acidosis, rough walking surfaces,

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took over afterwards. Nevertheless, for more specific results and conclusions further and much more extensive investigations should be performed.

Conclusions

According presented data, it could be concluded that there was very significant difference ($P<0.01$) between SCC values on day of correction and 7 days after correction, as well as significant difference ($P<0.05$) between SCC values seven and fourteen days after claws correction. The number of somatic cells in milk was significantly reduced week after correction of claws, then, fourteen days after the correction claw increased significantly ($P<0.05$). This indicates that the positive effect of the correction claw reflected in the reduction of the number of somatic cells in milk, even though numerous factors, such as breakdown of the dermal-epidermal lamellar connection, acidosis, rough walking surfaces, took over later. Even if it is so, for more specific results and conclusions further and much more extensive investigations should be performed.

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Oral presentation

BOVINE SUBCLINICAL MASTITIS ASSOCIATED WITH *PROTOTHECA SPP.*

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Abstract

Mastitis is the most common disease in dairy cattle and presents the most costly disorder to the dairy industry. Subclinical mastitis is inflammation of the mammary gland that does not create visible changes in the milk or the udder, thus, subclinically infected cows will produce less milk, and the quality of the milk will be reduced. In addition, infected cows can be a source of infection to other animals in the herd. Among other unspecified causative agents of mastitis, algae *Prototheca spp.* is not included in routine diagnosis in laboratories. However, *Prototheca spp* infections usually result in a chronic subclinical or mild clinical, inflammatory process in the udder, and were followed by a dramatic loss in milk production and a permanent increase in somatic cell count. This type of infection is rare, but the incidence of reported cases is increasing worldwide. In our investigation, 605 milk samples were obtained from dairy cows in a total of 5 herds with a history of increasing somatic cell counts, subclinical and mild clinical signs of udder infection, and/or unsuccessful response to the usual therapy. Microbiological isolation of algae on Blood agar and Sabouraud agar at 25°C and 37°C, respectively, showed that 39 (6.45%) samples were positive on *Prototheca spp.* which was confirmed microscopically. The SCC of *Prototheca spp.* positive milk samples were from 5×10^5/mL up to 13×10^6/mL. Considering the wide distribution of these algae as saprophytes in the environment, further investigation should include detail microbiological and molecular techniques in order to reveal genotypes involved prevent infections and decrease economic losses.

Keywords: *Prototheca spp.,* mastitis, SCC, dairy cattle

Introduction

Mastitis remains a major challenge to the worldwide dairy industry despite the widespread implementation of mastitis control strategies (Bradley, 2002). In addition to economic losses, mastitis milk is a source of infection for humans and animals, while residues of antibiotics, which are used in local and parenteral therapy, can be dangerous to human health. The most common causative agents of mastitis are bacteria and fungi. Recently, more attention is given to algae as possible causative agents of clinical and subclinical mastitis. Unicellular algae of the genus *Prototheca,* for now are considered as the only algae with pathogenic potential that can cause infections in animals and humans (Tsuji et al., 2006; Lass-Flörl and Mayr, 2007; Marques et al., 2008; Thompson et al., 2009; Ahrholdt et al., 2012; Min et al., 2013; Nguyen and Rosen, 2015). Chronic subclinical mastitis in dairy cattle is the most common clinical manifestation of udder infection caused by algae *Prototheca spp.* and was recorded worldwide (Spalton et al., 1985; Costa et al., 1996; Baumgärtner, 1997; Jensen et al., 1998; Buzzini et al., 2004; Bueno et al., 2006; Roesler et al., 2006; Milanov et al., 2006; Osumi et al. 2008; Ahrholdt et al., 2012). Bovine prototheca mastitis is serious economic problem, primarily because the treatment is uncertain, difficult

and time consuming. The incidence of prototheca mastitis is constantly increasing, mostly from geographic areas with relatively high humidity and/or temperature, e.g. from climatic conditions which are known to be advantageous for the rapid multiplication of this pathogen in the environment. Today, bovine mastitis with this etiology gets more attention, not only because of the economic losses, but also because of the impact on public health (Jánosi et al., 2001; Roesler and Hensel, 2003). The genus *Prototheca* consists of five accepted species: *Prototheca zopfii, Prototheca wickerhamii, Prototheca stagnora, Prototheca ulmea* and *Prototheca blaschkeae* (Roesler et al., 2006; Ricchi et al., 2010). The first case of *Prototheca spp.* udder infection was described in 1952 (Lerche, 1952). Thereafter, for many years in all the cases of prototheca mastitis was identified only *P. zopfii* (Jensen et al., 1998; Jánosi et al., 2001; Roesler et al., 2003; Möller et al., 2007; Osumi al., 2008) which makes the *P. zopfii* the most common cause of prototheca mastitis (Ahrholdt et al., 2012). Today, advanced monitoring and microbiological surveillance techniques revealed that mastitis in cows can be also caused by other species of the genus *Prototheca*. As a causative agent of mastitis, *P. blaschkeae* was first established in Portugal (Thompson et al., 2009), then in Poland (Jagielski et al., 2011), Germany (Ahrholdt and Roesler, 2011; Ahrholdt et al., 2012) and Italy (Capra et al., 2014), while *P. wickerhamii* was proven as a cause of mastitis in buffaloes (Capra et al., 2014). In most cases of prototheca mastitis in cattle (over 90% of cases), were isolated *P. zopfii* genotype 2 (Jagielski et al., 2007; Roesler et al., 2006; Möller and al., 2007; Kishimoto et al., 2010; Gao et al., 2012; Onozaki et al., 2013). Because bovine mastitis caused by *P. zopfii* is unaffected by conventional antimicrobial therapy, the best method for controlling this type of mastitis is through early diagnosis and elimination of the infected cows (Tortorano et al., 2008).

Material and Methods

In accordance with CMT (California mastitis test) carried out on 5 farms (A, B, C, D, E) in Serbia, there were collected 605 positive milk samples from Holstein-Friesian cows. Survey was conducted in period from September 2015 to February 2016. All cows were kept in similar zootechnical and hygiene conditions in tied breeding systems. In addition, all the cows were fed with appropriate diets depending on the breeding categories, with minimal fluctuation in raw material composition. On farms, milking was performed twice per day. Samples of milk were collected from each quarter before morning milking and stored in sterile vials. After teat and cleaning (with 70% ethanol), first streams of foremilk were discharged and then 10 ml of milk was collected aseptically from each teat into sterile vials. Milk samples were stored in a refrigerator at 4°C until microbiological analysis and microscopic somatic cell count (SCC) in laboratory (within 2 hours). SCC is performed according to standard method SRPS EN ISO 13366-2:2008. To isolate *Prototheca spp.* from each intense homogenized sample, one microbiological loop (0,1mL) was sowed on Sabouraud dextrose agar (Torlak, Serbia) and 8% sheep blood agar medium (Torlak, Serbia), incubated at 37ºC for 24-48h in aerobic conditions. Identification of *Prototheca spp.* was made after examination of cultural features on Sabouraud dextrose (white to yellowish matte colonies, Figure 1) and sheep blood agar medium (small, grey, opaque, non-hemolytic colonies up to 1 mm in diameter). Morphological features (spores and sporangia) were examined using Gimza stain and light microscopy (Figure 2).

Results and Discussion

Few hours after the infection of the udder with pathogenic microorganisms, the number of SCC in milk increases in response to activation of inflammatory processes. The International Dairy Federation recommended classification of cow milk as subclinical mastitis or non-mastitis (normal) using a SCC threshold of 500.000 cells/mL. In Europe, the ECC directive 92/46 (1992) stated that milk with SCC over 400,000 cells/mL cannot be used for human consumption. Starting from this recommendation, milk samples are classified in two categories, normal (values below 400.000 cells/mL) and milk originating from cows with subclinical mastitis (values above the limit of 400.000 cells/mL).

From the 605 milk samples selected on the basis of a positive CMT, increased number of SCC (above 500,000 cells/mL) were observed in 420 samples. *Prototheca spp.* was isolated in 39 samples based on the macroscopic appearances of colonies on Sabouraud dextrose and sheep blood agar and morphological features. The number of SCC in protothecal milk of cows with subclinical mastitis ranged from 500.000 to over 5.000.000 cells/mL, while in clinical mastitis cases obtained values of SCC were between 521.000 and 13.367.000 cells/mL. Cows with clinically diagnosed prototheca mastitis had altered milk with flakes or clots, but without disturbance of the general condition.
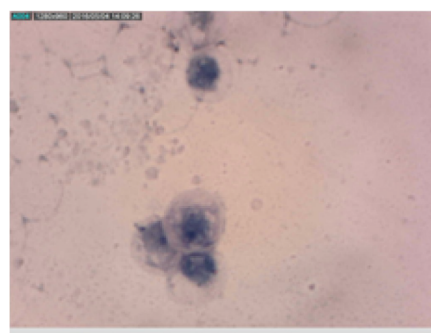
Figure 1. Cultural features of *Prototheca spp.* on Sabouraud dextrose agar

Figure 2. Morphological features of *Prototheca spp.* (1000x) on microscopic slide Gimza stain

Table 1. Number of clinical and subclinical mastitis caused by *Prototheca spp.* in investigated farms in Serbia

Farms	SCC>500.000	No. of isolated *Prototheca* spp.	Subclinical mastitis	Clinical mastitis
A	70	-	-	-
B	98	17	13	4
C	128	12	10	2
D	83	10	9	1
E	41	-	-	-
Total	420	39	32	7

All samples of milk (70) originating from farm A and samples of milk (41) originating from farm E with positive CMT, after microbiological processing there were no *Prototheca* spp. isolated. From farm B, in 98 of 148 milk samples, somatic cell count were over 500,000 cells/ml, of which *Prototheca* spp. were found in 4 and 13 milk samples from cows with clinical and subclinical mastitis, respectively. From farm C, in 128 of 142 milk samples,

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somatic cell count were over 500,000 cells/ml, of which *Prototheca* spp. were found in 2 and 10 milk samples from cows with clinical and subclinical mastitis, respectively. From farm D, in 83 of 93 milk samples, somatic cell count were over 500,000 cells/ml, of which *Prototheca* spp. were found in 1 and 9 milk samples from cows with clinical and subclinical mastitis, respectively.

Among the various mastitis types, prototheca mastitis is rapidly becoming a global problem (Jánosi et al., 2001). Although prototheca mastitis is often recognized as a symptomless infection that progresses slowly, acute clinical mastitis associated with reduced milk production, thin watery secretion with white flakes and resistant to conventional therapy may occur in outbreaks (Jánosi et al., 2001; Roesler and Hensel, 2003). In the literature there are more data on the medium and temperature conditions for the *Prototheca* spp. isolation. The majority of researchers agree that algae of the genus Prototheca can be successfully isolated on Sabouraud dextrose agar at 37°C during incubation time of 48-72 hours (Roesler et al., 2001; Janos et al., 2001; Marques, 2010b; Gao et al., 2012). However, Onozaki et al. (2013) suggest that the temperature of 25°C is more efficient for growth of *Prototheca* spp. while Zaini et al. (2012) suggest that the incubation period of 48-72h at a temperature of 27ºC is most suitable for the isolation of the algae. The results obtained in our investigation are consistent with the results of several studies (Roesler et al., 2001; Janos et al., 2001; Marques, 2010a; Gao et al., 2012) where the temperature and incubation conditions were equivalent (Sabouraud dextrose agar, 48-72h/37°C).

Milk samples from cows with prototheca subclinical mastitis had significantly increased SCC (up to 5.000.000/ml). Increased SCC in the cumulative milk samples from the dairy farms have negative effects on the price of milk and usage of milk for human consumption. In our survey, the SCC in milk samples taken from udder quarters with prototheca subclinical mastitis ranged from 500.000 to over 5.000.000 cells/mL. The obtained data of our examination are in accordance with the results of Malinowski et al. (2002) and Jagielski et al. (2011), where in cases of prototheca subclinical mastitis determined SCC ranged from 591.000 to 3.072.000 cells/mL and 434.000 to 1.325.000 cells/ml, respectively. In the cases of prototheca clinical mastitis, SCC in milk obtained in our study were between 521.000 to 13.367.0   cells/mL, while in most of the milk samples SCC were above 7.118.000 cells/ml, which were in accordance with previous investigations (Malinowski et al., 2002; Jagielski et al., 2011).

Conclusion

Due to the potentially pathogenic effects of *Prototheca* spp. on human and animal health, economic losses and ineffective therapy treatment, these microorganisms should be routinely monitored and included in a regular control of mastitis on dairy farms.

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Oral presentation

INFERIOR REARING CONDITIONS CAN LEAD TO HIGH SKATOLE LEVEL IN PRE-PUBERTAL ENTIRE MALE PIGS

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Abstract

Effect of rearing conditions on the level of boar taint in entire male (EM) pigs was studied. EM assigned to two trials (with individual or group housing) were of the same crossbreed (Landrace × Large White) and originated from one nucleus herd (>20 litters). Pigs received similar (corn based) commercial diet. In the first trial EM (n=29) were individually housed and slaughtered at the age of 193 days and ~122 kg live body weight (BW). In the second trial, EM were reared in groups (four pens) with initial stocking density 0.7 m^2/pig. Slow growing or injured EM (tail biting) were slaughtered earlier, at the age of 169 days and ~74 kg live weight (n=22). The remaining pigs (n=51) were kept at stocking density of 1.0 m^2/pig and were slaughtered 35 days later at ~127 kg BW. Androstenone (A) and skatole (S) in subcutaneous fat were determined by HPLC. Analysis of variance was performed to assess differences due to housing (individual or group) or conditions in group housing. Individually housed EM had lower concentrations of S than group housed (0.111 vs. 0.170 μg/g fat, P=0.02) despite similar A level (0.533 vs. 0.588 μg/g fat, P=0.65). EM that were sent to slaughter earlier (younger and sexually immature) had almost 2-fold higher levels of S (0.298 vs. 0.170 μg/g fat, P<0.0001) despite 3-fold lower A (0.188 vs. 0.588 μg/g fat; P=0.002) indicating that non-optimal rearing conditions associated with poorer welfare may present a significant issue in boar taint development.

Keywords: entire males, boar taint, rearing conditions

Introduction

Surgical castration of male piglets intended for fattening is currently the most common practice in the majority of developed pig breeding countries. The main reason for conducting this procedure is the prevention of boar taint, while reducing aggressive behavior and increasing meat quality is also achieved (Babol and Squires, 1995). Due to the strong public initiative and a foreseen ban on surgical castration in the EU by the end of the year 2018 (European Declaration on Alternatives to Surgical Castration of Pigs, 2010), rearing uncastrated i.e. entire males (EM) might become a predominant practice, raising the issue of boar taint. Boar taint, defined as urine- or fecal-like odor, disfavored by most of the consumers, is ascribed to the accumulation of substances skatole (S) and androstenone (A) in the fat tissue (Lundström et al., 2009). Whereas androstenone is a testicular steroid, largely under genetic control (Robic et al., 2008), the accumulation of

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skatole (a product of microbial degradation of tryptophan in the intestine) depends on numerous influences that affect its intestinal production, absorption and hepatic degradation. Steroid hormones (including androstenone) are known to inhibit skatole metabolism in the liver, being the main reason for boar taint occurrence after sexual maturation of the EM. Among factors that influence the level of skatole, pig nutrition has been indicated as the most influential one (Zamaratskaia and Squires, 2009), whereas stress- and health-related factors may be important as well. For example, our study on pigs with severe intestinal infection (Škrlep et al., 2012) revealed unusually high skatole concentrations in fat tissue not only of EM but also surgical castrates and immunocastrates, implying that boar taint might also be associated with health status. Aiming to investigate this hypothesis further, the present study was conducted to compare different housing regimes and rearing conditions.

Material and Methods

The study was performed with young EM assigned to a trail with either individual or group housing. All animals were of the same crossbreed (Landrace × Large White) originating from the same nucleus herd. Pigs were fed corn based commercial diet (Jata Emona d.o.o., Ljubljana, Slovenia) containing 16% crude protein and 13.1 MJ ME/kg and had ad libitum access to feed and water. In the first trial (n=29), EM were housed in individual pens (2.7 m^2, slatted walls) and slaughtered at the age of 193 days and the average live body weight of 122 kg. In the second trial, pigs were raised in four pens with initial stocking density 0.7 m^2/pig. At the age of 169 days and the average live weight of 74 kg, 22 slow growing and injured pigs, with injuries mostly due to ear and tail biting and associated infections of the pelvic body region, were removed from the pens and sent to slaughter. The remaining animals (n=51) were then reared for another 35 days at lower stocking density (1.0 m^2/pig) and finally slaughtered at the average live weight of 127 kg. All animals were slaughtered in the same commercial abattoir; the duration of the transport was similar (~1h). Pigs were slaughtered according to standard procedure (CO_2 stunning, vertical exsanguination, vapor scalding and evisceration followed by veterinary inspection and carcass classification). After slaughter subcutaneous fat samples were taken at the level of last rib for subsequent S and A determination.

The concentration of both boar taint compounds was measured by HPLC (HP 1200, Agilent Technologies, Waldbronn, Germany) equipped with fluorescence detector according to the procedure described by Batorek et al. (2012). Concentrations were expressed as µg/g of liquid fat. The detection limits of the methods were 0.03 µg/g and 0.24 µg/g for skatole and androstenone, respectively. Inter- and intra-assay variation did not exceed 10%.

Data were analyzed using the General Linear Models (GLM) procedure of SAS statistical software (SAS Institute Inc., Cary, USA) testing differences between individual and group housing and between group of EM slaughtered younger and older. Significant differences (P<0.05) in least square means between the tested groups were compared using Tukey-Kramer adjustment.

Results and Discussion

Comparison of individual and group housing (Figure 1) revealed significantly lower S levels in individually housed EM (i.e. 0.111±0.019 vs. 0.170±0.017 µg/g fat respectively,

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or 35% difference), with similar A levels (0.522±0.096 vs. 0.588±0.073 µg/g fat, respectively) and slaughter weights (122.4±2.0 vs. 126.7±1.5 kg, respectively). The average S levels in the case of both groups were not exceeding thresholds of sensory perception (0.20 to 0.25 µg/g, respectively) according to Walstra et al. (1999), despite relatively high A concentrations (sensory threshold levels being set at 0.5 to 1.0 µg/g).
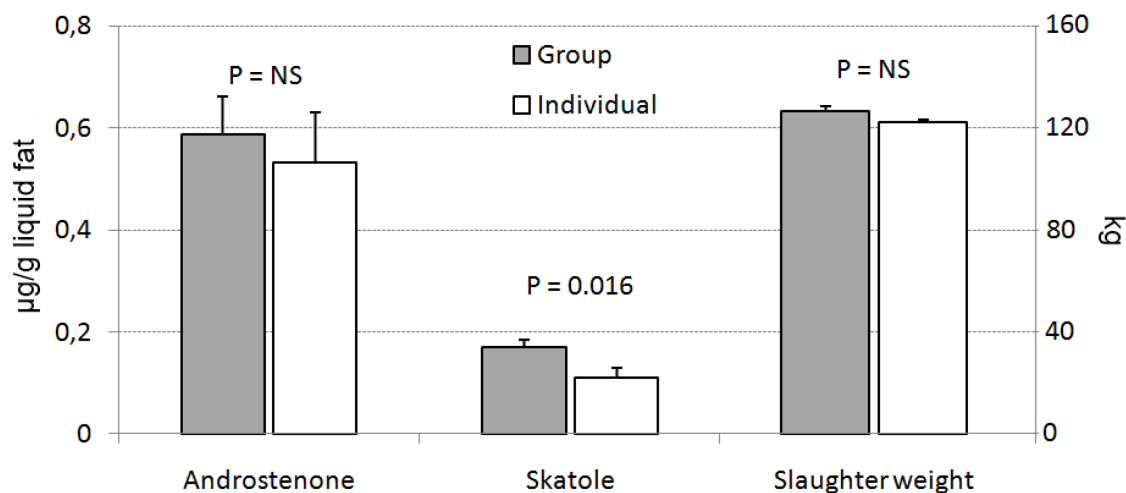
Figure 1. Effect of housing on androstenone, skatole (µg/g) and slaughter weight (kg).

Figure 2. Effect of rearing conditions on androstenone, skatole (µg/g) and slaughter weight (kg). Pigs slaughtered at younger age (early slaughter) were reared in inferior living conditions (high stocking density, slow growth, body lesions) compared to the ones slaughtered at normal slaughter age (late slaughter).

Difference in A, S between EM in early and late slaughter batch was even more pronounced than in the case of housing (Figure 2). Although higher age (and body weight) with associated sexual maturation usually leads to the increase of A and S (Zamaratskaia and Squires, 2009), this was not the case in the present study. EM pigs slaughtered earlier (due to illness, lesions and slow growth) i.e. at younger age and lower weight (74.5±2.3 vs. 126.7±1.5 kg) and reared at higher stocking density had 43% higher S level compared to their older peers (i.e. 0.298±0.025 vs. 0.170±0.017 µg/g fat) despite 68% lower A level (i.e. 0.188±0.104 vs. 0.588±0.069 µg/g fat).

As already mentioned, fat tissue S can be influenced by numerous factors of different etiology. It was shown in our earlier report (Škrlep et al., 2012) that intestinal infection

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can lead to higher levels of S. Illness could also hinder S degradation by suppressing P450 enzymes, responsible for its metabolism in the liver (Cheng and Morgan, 2011). Further on, high incidence of skin lesions (denoting lesser welfare) was associated to increased level of S in fat (Wesoly et al., 2015) or higher boar taint scores (Fàbrega et al., 2011). Hansen et al. (1994) showed higher S with higher stocking density. It may be assumed, that differences in S levels in our study are due to different levels of welfare and stress, associated with different housing conditions of EM. Claus et al. (1994) indicated that the level of stress experienced by pigs during rearing or slaughter affects S accumulation. This was clearly demonstrated by Wesoly et al. (2015) showing that even short exposure to stress (transport time and time spent on the vehicle prior to slaughter) significantly increases levels of S deposited in fat. As they explained, the most possible cause could be either a parallel rise of steroid hormones (known to inhibit skatole degradation) or stress-induced shift in microbial skatole production in the intestine. Thus there is growing body of evidence about the importance of stress and welfare on boar taint. In particular S, however the underlying mechanisms are far from being understood and need further substantiation and clarification.

Conclusion

Inferior living conditions like high stocking density, skin lesions and illness can be related to significant rise in the level of fat S independently of the levels of A. Our results imply that, even if EM pigs are to be slaughtered at younger age, welfare assuring measures should be considered, if boar taint is to be completely avoided.

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Oral presentation

HEALTH CONTROL OF PIGS ON COMMERCIAL FARM

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Abstract

In this paper we have presented the results of research on the health status of pigs of different age and production categories conducted on commercial farms. The concept of modern industrial pig production is based on the implementation of biosecurity measures and solving of problems on protecting and preserving the environment which greatly burden the production. The goal of intensive pig production on commercial farms is the production of large number of weaned pigs or fattening pigs per saw per year. It is well known that good health of pigs is a prerequisite for high reproductive performance and therefore successful profitable productions. The health status of the herd depends on many factors such as a technology of breeding care, nutrition, the organization of production, the level of training of personnel as well as continuous and proper implementation of health protection. Today we are witnessing the fact that a large number of diseases caused by bacteria and viruses as well as certain types of parasites seriously affect the production of pigs in intensive breeding. Technological disease can be kept under control by applying prophylactic and therapeutic measures as well as increased supervision of professional services. On the farms covered by our research projects the health control of boars, sows, weaned piglets and fatteners was done. In boars and sows the most common disease is the disease of locomotor system and in weaned piglets it is the respiratory system of multifactorial etiology. In suckling piglets it is hypoglycemia. In conclusion, high reproductive efficiency of breeding animals in industrial pig production is an attainable goal.

Keywords: pigs, commercial farms, health protection

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Health Control of Pigs

Production of pigs on commercial farms is greatly burdened by diseases of piglets. Pathology of piglets is a very dynamic discipline within the entire herd epizootiology in which the large agglomerations of animals in a confined space can easily come up with horizontal and vertical transmission of infection and certain microorganisms (Bojkovski etal.2012b, 2015b). Great importance is attached to the variations of pathogens in pigs, not only in showing resistance to drugs, but the occurrence of genetic recombination, which affect the clinical picture and the course of a disease.(Blackburn, 1995; Bojkovski et al., 1997, 2005, 2011).

In the processing line of a slaughterhouse we have the following gross pathology changes: esophageal surface changes, hyperkeratosis, nonstructural yellow surface. Results: in 37 animals out of a total of 103 animals the erosion of esophageal part of stomach was noticed. Ulcers of the esophageal part of the stomach which affect total thickness of mucosal membrane were present in 4 of 103 examined animals (Bojkovski et., al 2014b). Also, changes caused by ochratoxin were noticed in pigs' kidneys during routine examination in the abattoir (Bojkovski et al., 2013b).

At our pig farms the following is most frequently found in different categories: neonatal collibacillosis, endemic disease, necrotic enteritis, circoviral infections, colitis caused by spirochetes, enterohaemorrhagic syndrome, dysentery and respiratory disease complex. *Clostridia* infection of suckling pigs is also a serious health and economic problem. Clostridial infection is an etiology related to *Clostridia perfringens*-type C, *Cl. perfringens* type A and *Cl. difficile*. They cause special nosologic units also known as hemorrhagic necrotic enteritis of suckling pigs and *Clostridium difficile* (CDAAD) associated disease-d caused by *Cl.difficile* (Ivetić et.al 2006). In recent years, internationally and at our pig farms, there was a mass occurrence of respiratory disease complex (PRDC), which is becoming a serious health hazard in all technological stages of production. It is a respiratory disease of pigs characterized by simultaneous infection of lung tissue with more respiratory pathogens and this is a common term for pneumonia in pigs with multifactorial etiology. Isolated pathogens vary between and within production herds *(*Honnold, 1999, Gagrčin et.al. 2002, Ivetić et al., 2005; Golinar et al., 2006; Bojkovski et al., 2012*)*. Control of respiratory disease complex is difficult and complicated. The significance of respiratory diseases complex is based on the interaction of respiratory pathogens. Knowledge of the interaction of respiratory pathogens should be considered in order to implement effective control measures. Respiratory disease of pigs occur if the causative agents are present in the same habitat or if due to an unknown cause for us, the immune response mechanisms of respiratory system weakens *(*Ivetić et al*., 2005)*. As opposed to classical control of spreading of infectious diseases in pigs that persist in our country which is mandated by law, detection and suppression of technopathies are more of an economic need of the producers themselves.

Porcine circovirus type 2 (PCV2) and hepatitis E virus (HEV) are the most recently recognized causes of infectious hepatitis of pigs and may or may not act independently in the development of the disease **(**Savić et.al., 2012). Recently it has been suggested that swine torque teno viruses (TTVs) in co-infections with some viral pathogens may potentiate the severity of disease. In order to search for virus cofactors associated with infectious hepatitis in pigs, we investigated liver tissues to determine the presence of TTVs,

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PCV2 and HEV of naturally infected pigs and analyzed the prevalence of both geno groups of the TTVs in the hepatitis lesions. Histopathological techniques, nested polymerase chain reactions (nPCRs), polymerase chain reaction (PCR) and one-step reverse transcriptase polymerase chain reaction (RT-PCR) were applied to detect hepatitis lesions, TTVs genogroups 1 and 2, PCV2 and HEV infection. Of the livers examined 58% (29/50) had mild to moderate hepatitis and 74% (37/50), 56% (28/50) and 26% (13/50) samples were nPCR, PCR and RT-PCR positive for TTVs PCV2 and HEV respectively. TTVs were detected in 84% (16/19) of the samples which we found to be of mild severity, while in almost all (90% or 9 out of 10) samples we identified moderate hepatitis lesions. Additionally, the livers of 12 out of 21(57%) pigs without hepatitis lesions were positive for TTVs. These results demonstrate an association between TTVs and infections with hepatitis of pigs in concomitant infections with PCV2 and/or HEV and indicate that TTVs may play a role as a cofactor in the pathogenesis of disease (Savić et.al, 2010).

Health Control of Boars

Breeding and exploitation of boars on commercial farms is aimed at producing sperm doses for personal use. The lifetime of boars and therefore the length of their exploitation largely depend on their health. Therefore, control of the health status of the boars, quality control of the sperm for artificial insemination by overseeing the entire process of taking to sperm quality insemination dose optimization and comprehensive environmental conditions hold (accommodation, microclimate, food, power, attitude of employees), represent important parameters in terms of health control of boars themselves, health control of the entire herd or economic parameters and productivity of farms and the profitability of the entire production **(**Petrujkić et al., 2011; Rogožarski et al., 2014). Our results suggested that heavy metals were transported from the circulation into reproductive organs of boars, with lead quantity being the highest at 0.36 mg/kg, followed by cadmium with 0.013 mg/kg and mercury with 0.0021 mg/kg (Bojkovski et.al. 2010). At a commercial farm with a total of 28 boars, Landrace and Yorkshire breeds, which were in exploitation over the course of one calendar year, the following parameters were recorded: the number of inseminated gilts, the sows insemination number, repeated heats, the number of pollinated piglets, number of live births and the number of stillborn piglets. From all the 28 boars that were in exploitation the blood sample was taken. Blood sera of boars were used to study prevalence of porcine circovirus antibodies to type 2 (PCV2 by the ELISA test. We found that out of a total of 28, 21 samples were positive. We established the prevalence of antibodies to porcine circovirus type 2 (PCV2) in tested boars. We also established the prevalence of antibodies to porcine circovirus type 2 (PCV2) in boars from commercial farms in operation (Bojkovski et al., 2014a, 2015a). Besides infective agents, one of the most important health problems in boars in intensive farm production are locomotor disorders (Muirhead et al., 2002). In majority of cases lameness appears in smaller or higher degree (Sims and Glastonbury 1996; Bojkovski et al., 2012d). Lameness is also the cause of the culling of boars ahead of time due mainly to inability to mount phantom semen collection. The consequences are the increased costs of replacement and overwork of the remaining boars resulting in lower conception rates.

Health Control of Sows

In modern pig farming more and more attention is paid to the body condition score. At most commercial farms feeding of gilts and sows is based on their body condition. The goal is that sows do not gain or lose too much of a body weight between farrowing and

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insemination. Maintenance of body weight (condition) of sows within the optimum value (3 in the time of farrowing and 2.5 during matting) over their life time can result in optimal reproductive results. In contrast, in adequate control of condition of the sow may lead to difficulties in farrowing and occurrence of health problems. The aim of our study was to estimate the body condition on the 90th day of gestation at a farm of commercial type (Bojkovski et al. 2012d). It is not easy to evaluate the body condition in an objective way in practice. In many herds, body condition is evaluated by the pig producer by way of visual scoring on a sliding scale from 1 to 5. Although visual scoring systems may work well in some herds, e.g. in outdoor systems, they have several disadvantages. First, a sow that appears to be thin can still have a fairly high amount of back fat (Muirhead et al., 1997). Second, it is a subjective and inaccurate method that largely depends upon the scoring skills of a person in question. Finally, when visual scoring is performed by the pig producer in the same herd over time, it is likely that less attention will be paid to deviations from the optimal condition due to herd blindness. Determining the optimal body condition by visual scoring is particularly difficult in herds with sows of >1 type of breed because of the inherent variation in conformation existing between breeds (Whittemore and Schofield, 2000).

In our experiment, we found that 7 sows had a body condition score of 5. Farrowing sows with the body condition score of 5 may suffer from health-related and reproduction disorders. For these reasons, we must try not to turn to the farrowing sows with the body condition score of 5. There were 24 sows with a score of body condition 3. The body condition score of 3 is optimal. In our experiment, 16 sows had a body condition score of 4. This body condition score presents an opportunity for correction of the meal to farrowing as these are sows that have no such health/reproduction problems. Our recommendation for commercial farms is to introduce the body condition scoring in daily routine (Bojkovski et al., 2013a, 2014a).

Biosecurity on Pig Farms

Planned implementation of biosecurity measures is crucial in protecting the health of pigs and ensuring the production success. Required level of biosecurity in pig farms should stem from logical thinking and timely action taken, as well as the recognition of specific threats and weaknesses in the production process. The introduction of HACCP principles and sanitation protocols in the farm management may prevent the introduction and spreading of infectious agents in the herd. The aim of our study was to evaluate certain biosecurity measures at pig farm: farm isolation, quarantine for animals, herd health status control, relation of personnel to animals and equipment, between-farms and on-farm traffic control, food safety, manure management, removal of dead animals, and other animals, birds and rodents control. Our recommendation is that regular control of biosecurity level should become a routine at pig farms of industrial type ((Uhlepnhoop, 2007,Stanković and Hristov, 2009b, Bojkovski et al., 2012c).

We analyzed biosecurity measures at two commercial pig farms. Space for suckling piglets and weaning piglets as well as the space for finisher are in the same building at the tested farms. As of lately separate compartments are being introduced. There is a possibility of direct contact between newly purchased animals and the animals that have been on the farm for a while. There is a test area for quarantine at both farms. Diseases of the digestive tract and breathing problems in the weaning category are present at both farms (Bojkovski et al. 2012c). At swine farms in Serbia respiratory diseases complex was diagnosed. Studies were

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conducted to determine the prevalence of certain biological agents that participate in the development of respiratory diseases complex (Došen et al., 2007; Radanović et al., 2008, Žutić et al., 2009; Savić et al., 2011, Bojkovski et.al.2012a). This research was intended to identify and determine the prevalence of major pathogenic bacteria in lung tissue and carry out bacteriological examination of altered parts of the lung using standard methods and commercial microbiological diagnostics (Došen et al., 2007; Radanović et al 2008, Žutić et al. 2009), and some research has been focused on the identification of viral respiratory pathogens and *M.hyopneumoniae* in the pneumonic lesions (Savić et al. 2011). This is due to *in situ* hybridization (ISH), polymerase chain reaction (PCR), etc., where these causes may be shown *in situ* as they are not implemented in all laboratories. Commercial farms are making significant efforts to improve the health status of the herd. One of the main problems is that farms fail to educate their workers. Efforts have been made adjust workers' shifts and organize training courses for workers related to the technological stages of production. Biosecurity, welfare, good producers, clinicians and hazard analyses at critical points are very important issues in the intensive pig production. The biggest biosecurity failures on the surveyed farms tend to be related to the control of rodents, birds and removal of dead animals (Stanković et al., 2008;b ,2009 a, b).

Conclusion

Maintenance of good health is a prerequisite for successful pig reproduction and cost-effective production. To this end, it is necessary to provide farm holders with professional know-how, apply a number of biotechnical measures and focus on prevention of disease in pigs in order to enhance their good health.

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Oral presentation

POSSIBILITY USE ESSENTIAL OILS IN CONTROLLING INSECT VECTORS

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Abstract

Arthropods, i.e. insects, ticks, etc. are the principal vectors spreading the pathogens causing many human and animal diseases. Insect transmitted pathogens such as those causing malaria, dengue fever, Lyme disease, West Nile diseases, Rift Valley fever, chikungunya and leishmaniosis kill millions of people annually throughout the world. Nearly half of the world population is infected with at least one type of arthropod-borne pathogen. Standard practice in combating insects during the last few decades is use of synthetic insecticides. However, recent studies indicate that there are over 500 insect and mite species resistant to pesticides, presently. Incidence of multiple resistance (resistance to more than one pesticide and to pesticides in more than one chemical class) is increasing rapidly. Management of insects through the use of naturally produced pesticides is a highly desirable component of pest management strategies which provide useful alternative or supplementation to conventional insecticides. The traditional sources of natural pesticides have been plants. These chemicals are more desirable than conventional insecticides due to their rapid biodegradation in environmental and low toxicity to non-target organisms.

Essential oils and their derivatives can be an alternative means in control of many harmful insects. Essential oils are volatile, natural complexes characterized by strong odor emerging from metabolism of aromatic plants. They are lipophilic in nature and interfere with basic metabolic, biochemical and physiological and behavioral functions of insects. Toxic, repellent, ovicidal and/or growth suppressing/retarding action of many essential oils on many hematophagous insects, fleas, lice, ticks and mites have been demonstrated. However, the bioactivity of essential oils on arthropods has been evaluated primarily against mosquitoes and to a lesser extent on other arthropods, perhaps due to the greater significance of mosquitoes in transmission of pathogens.

This paper will provide an overview of the compounds isolated from plants that have been evaluated for control of insect vectors of human and animal pathogens.

Keywords: arthropod, control, essential oils

Introduction

Insects are main vectors, carrying pathogens which cause many human and animal diseases. Insects transmit pathogens which cause West Nile disease, dengue, malaria, Lyme disease, yellow fever, Rift Valley fever and leishmaniosis thus killing millions people around the world every year. Nearly half of the human population is infected with at least one type of the insect-borne pathogen. Malaria is still considered among the three main causes of infection in the world. The yellow fever mosquito (*Aedes aegypti)* has returned to the

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regions where it was once eliminated, so that dengue and related severe form of dengue hemorrhagic fever have expanded geographically in recent decades (*WHO*, 2011).

Synthetic insecticides have been used in the last few decades in order to destroy insects more efficiently. Recent studies have shown that there are over 500 species of insects and mites resistant to pesticides. The incidence of multiple resistance (resistance to more than one pesticide) is increasing rapidly (*WHO,* 1992; Hemingway *et al.,* 2002). Conventional insecticides exhibit inherent toxicities that endanger the health of the farm operators, consumers and the environment. Negative effects on human health led to a resurgence in interest in botanical insecticides because of their minimal costs and ecological side effects (Khater, 2012). Therefore, insect control by using natural pesticides is an appropriate component of pest management strategy which can provide useful alternatives or supplement to conventional insecticides. Natural insecticides are more desirable than conventional insecticides due to its quick biodegradation in the environment, and low toxicity to non-target organisms (Isman, 2006). Plants are common resource of natural pesticides due to evolutively developed adaptabilities allowing them to enhance their survival and reproductive performance while diminishing the harm inflicted by herbivorous pests. Usual resources of natural pesticides are plants because they have developed adaptive skills allowing the improvement of their survival and reproduction and diminishing the harm done by herbivorous pests (Mann and Kaufman., 2012). Plants defend from pests by creating secondary metabolites which have numerous activities like insecticide effect, repellency, growth depleting, etc.

Use of natural insecticides has become important procedure for elimination of insects in public health care and in community hygiene, as well as in plant protection and production developed for protection of environment from contamination with conventional pesticides, which is a global problem nowadays. Natural insecticides decompose significantly faster (in a course of a few days, or in a few hours) than conventional pesticides and therefore, they are less dangerous for useful insects and mites. These insecticides should be used more frequently. However, due to their production processes, they are significantly more expensive than many conventional pesticides. Although there is a lot of information on their insecticidal properties, only a small number of natural insecticides are used today because there are numerous reasons and obstacles for large commercialization of natural pesticides. Isolation from plants and chemical characterization of active compound can be a demanding task due to the small amounts of natural compounds extracted. Researches have revealed that many plant species produce extremely efficient substances that control of many insect pests (Korunić et al., 2004).

Essential oils and their constituents
Essential oils are complex mixtures of evaporative organic compounds. They are created by aromatic plants as secondary metabolites which protect plants from insects and microorganisms. Essential oils are characterized by a strong odor and have a generally lower density than the water. These oils are considered to be alternate mean used to control many harmful insects because they interfere with many biochemical, physiological and behavioral functions of insects. Nearly 3,000 essential oils are known from which 300 are commercially important for pharmaceutical and pesticide industries (Raja, 2014; Bruneton 1999).

Majority of essential oils originate in the plant families: *Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zingiberaceae* and *Piperaceae*. Composition, quality, and quantity depend on several factors like extraction methods, source, plant growth, climate, geographic region, plant structure and vegetative stage of the source plant (Bakkali *et al*., 2008). It is presumed that essential oils interfere with basic metabolic, biochemical, physiological and behavioral functions of insects, but the basic routes of activity allowing effect of essential oils on insects is mostly unclear. The rapid onset of toxic signs suggests a neurotoxic activity which may involve competitive inhibition of acetylcholinesterase, competitive activation of octopaminergic receptors or GABA receptors.

A large number of essential oils or their components have shown toxic, repellent or ovicidal or growth retardant activity affecting large number of hematophagous insects, like mosquitoes, fleas, lice, flies, ticks and mites. The bioactivity of essential oils have been estimated primarily against mosquitoes, and to a lesser extent on other insect vectors, probably due to the recognized significance of the species in pathogen transmission.

Toxic activity of essential oils on mosquitoes

Essential oils interfere with basic metabolic, biochemical, physiological and behavioral functions of insects. Insects inhale, ingest or absorb essential oils from the surface of their cuticula. A survey of literature concerning the insecticidal properties of essential oils indicated that essential oils obtained from around 90 plant genera belonging to 38 families, exhibited larvicidal effects against mosquitoes (Shallan *et al*., 2005). Although the majority of essential oils are less toxic than synthetic insecticides, LC_{50} values as low as 0,004 mg L^{-1} from pipercide extracted from Black pepper (*Piper nigrum)* against *C.. pipiens pallens* larvae (Park *et al*., 2002) were confirmed. It has been found that 84 plant species can be used against *A aegypti, C. quinquefasciatus, Anopheles dirus* and *Mansonia uniformis* larvae, out of which *Rhinacanthus nasutus* extract exhibited strongest larvicidal activity with LC_{50} values ranging between 3,9 and 11,5 mg L^{-}.Pulegone, thymol and eugenol extracted from rosemary oil showed high larvicidal activity against multiple larval stages of *A.. aegypti.* The LC_{50} values for these compounds ranged from 10,3 - 40,8 mg L^{-1}. Piperitenone oxide which is isolated from *Mentha spicata viridis* showed high larvicidal and adulticidal activity against *A. stephensi*. Essential oils from *Citrus hystrix, C. reticulata, Zingiber zerumbet, Kaempferia galanga* and *Syzygium aromaticum* showed toxicity to permethrin resistant *A. aegypti*. Pellitorine, a chemical isolated from *Asarum heterotropoides* roots*,* showed toxicity to *C pipiens*, *A. aegypti* and *O. Togoi,* mosquitoes resistant to fenthion, chlorpyriphos, fenitrothion, deltamethrin, chlorfenapyr and α-cypermethrin in laboratory conditions.

Essential oils from Indian birthwort (*Aristolochia indica),* Alexandran senna *(Cassia angustifolia),* East Indian ebony *(Diospyros melanoxylon),* Catjang cowpea (*Dolichos biflorus),* Australian cowplant *(Gymnema sylvestre),* Water willow *(Justicia procumbens),* touch – me-not plant *(Mimosa pudica)* and of bitter ginger *(Zingiber zerumbet*), have shown good activity against adults of *Cx. gelidus* and *Cx. Quinquefasciatus adults*. Toxicity doses are comparable to many synthetic insecticides including permethrin and imidacloprid (Chaiyasit *et al.,* 2006). Terpenoid compounds from clove, coriander, thyme, parsley and anise oils have high larvicidal activity against *Ochleotatus caspius* with LC_{50} values ranging from 7,5 mg L^{-1} to 156 mg L^{-1}. Anthraquinone compound, emolien isolated from Burkina Faso bark (*Cassia nigricans)* has had LC_{50} values as low as 2,4 mg L^{-1} against *Anopheles gambiea* larvae. Similarly, piperolein-A and piperine extracted from *Piper nigrum* exhibited LC_{50} values as low as 1,46 and 1,53 mg L^{-1} , against *A. Aegypti* (Simas

et al., 2007).

Essential oils and their components are very efficient and do not have negative effects on the environment most of essential oil are less effective than synthetic insecticides. Therefore, it is considered that essential oil products should better be used in combination with synthetic insecticides. It is recommended to apply essential oils in rotation with synthetic insecticides for vector control strategies, especially in documented cases of multiple insecticide resistance on several active ingredients used against hematophagous insects (Mann and Kaufman., 2012).

Repellent activity of essential oils on mosquitoes
Repellents are substances that act locally or at a distance, usually by providing a vapor barrier deterring the arthropod from coming into contact with the surface. Global warming has allowed spreading of the mosquitoes that transmit malaria, yellow fever and dengue into some temperate region and higher altitudes, affecting people who are vulnerable to these diseases (EPA, 1997). Synthetic chemicals have been developed in order to protect humans from mosquito bites. Unfortunately, it may cause environmental and human health risks For that reason, search efforts were directed towards production of natural repellents. (Pitasawat *et al*., 2003).

Surveys have shown that essential oils of great number of plants can have repellent activity against various hematophagous arthropods. Oils containing repellent substances for insects are, among others, citronella, cedar, vrbena, pennyroyal, geranium, cajeput, cinnamon, rosemary, basil, thyme, allspice, garlic and peppermint. Lemongrass, *Cymbopogon* spp. produces the most used natural repellent in the world. Essential oils from *C. excavatus* provided 100% repellent activity for 2 hours against *A. arabiensis* and, for the equal amount of time. Essential oils of *Syzygium aromatica* have shown repellent activity against *A. aegypti, Cx. quinquefasciatus* and *Anopheles dirus.* An outdoor testing proved that the oil from *C. martinii* provided 100% repellency for 12 h against mosquitoes of genus *Anopheles*. Essential oil of *Cymbopogon winterianus* mixed with 5% of vanillin have given 100% protection for 6 h against *Ae. aegypti, C. quinquefasciatus* and *Anopheles dirus*. It has also been found that essential oils derived from Eucalyptus produced high repellency against the mosquitoes *A. albopictus* and *Mansonia* spp. while piperitenone oxide isolated from *Menta spicata viridis* was actively repellent against *A. spethensi.* The extract of *Maelaleuca ericifolia* repelled mosquitoes *A. vigilax* and *Verrallina carmenti*. Essential oils from catmint, *Nepeta cataria* at concentration of 15% of active ingredient conferred complete protection for 7.5 hours from *A. intrudens* under field conditions (Tawatsin *et al.,* 2001; Nerio *et al*., 2010).

Recently, several new essential oil-based chemicals have been commercialized as mosquito repellents. Chemical substances included in commercialized products mostly contain citronella oil alone, or in combination with cedar or lavender peppermint, clove, Eucalyptus and garlic . Commercial repellent substances (Swamp Buddy, Bug Chaser, All sport, Neem Aura, GONE, Sun Swat, Bite blocker, Green Ban), based on natural chemicals are used against several species of mosquitoes. Qualls and Xue (2009) reported that the commercial product of "Gerraniol" provided protection for 4 hours, "All Sport", for 1.5 hours "Swamp Buddy Bug Chaser" for 1 hour against *Psorophora ferox, A. atlanticus* and *A. mitchelle* bites. which means that their protective activity is not long acting,, while "Bite Blocker" provided protection for 7.2 and 3.5 hours under laboratory and field conditions against *A.. aegypti, A. canadensis, A. udes* and *A. fitchii.* The field of effectiveness of these

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commercial products is very variable and it depends on the species of the insect, product formula and the method of evaluation. Biological properties of many essential oils have been observed by *in vitro* testing (using canvas, filter paper, animal membrane and olfactometer). Rarely the results of repellent activity have been obtained by *in vivo* examinations using animal and human subjects.

Repellent activity on the hematophagous arthropods

A lemon eucalyptus extract from *E. Maculata citriodon* showed good repellent activity against flies, mosquitoes, ticks and stable flies. Essential oil from *Pogostemon cablin* provided protection against *S. Calcitrans* up to 3.7 hours. Essential oils from *Citrus hystrix* provided up to 100% repellency against *Periplaneta americana* and *Blattella germanica,* and 88% against *Neostylopyga rhombifolia* in laboratory conditions. Essential oils obtained from eucalyptus have high repellent activity on human head lice *P. humanus capitis.* Essential oils have repellent activities on many arthropods which are not insects, like ticks and mites. Oils from *Amirys balsamifera* and *Maclura pomifera* successfully, in a period of 4 hours, repelled the black tick *Ixodes scapularis* and the tick *Amblyomna americanum.* Essential oil obtained from eucalyptus is highly repellent and protects from the *Ixodes* ticks (Caroll et al., 2010; 2004).

Conclusion

Insect control by using natural pesticides is a desirable component of pest management strategy which can provide useful alternatives or supplement to conventional insecticides. Natural insecticides are more desirable than conventional insecticides because of their quick biodegradation in the environment and low toxicity towards non-target organisms. After identification and isolation of sustainable natural insecticides which aggressively interfere with the target pest population, an evaluation should be done on how safe these compounds are for people and organisms which are not included in the target group. Even though natural pesticides, in this case essential oils, do not pose a prominent threat regarding general toxicity of pesticides, they are also toxic, and as all other toxins used for pest control can act harmfully both on the user and on the environment. In spite of their large usage in the fragrance and flavor industries, there is not enough information on safety of these chemicals for people and other animals in the habitat. Therefore, it is necessary to recognize that natural pesticides or their derivatives are a risk and that further field research is required in aim to clarify the effect of these products on environment and living organisms. However, in some parts of the world the danger of arthropod transmission of disease poses by far greater threat than the danger imposed by natural pesticides.

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Poster presentation

ASSESSMENT OF SOME WELFARE PARAMETERS IN LACTATING SOWS

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Abstract

In this paper, some welfare problems of sows during period of the lactation are considered. The study was conducted on 40 randomly selected sows in 2 to 4 weeks of lactation, housed in separate facility at the commercial pig farm and in common farrowing pens. Using appropriate protocols, the following parameters have been assessed: body condition, cleanliness, skin condition, body lesions in general and in different body regions, and claws condition. According to the results, body condition of the sows was significantly influenced by period i.e. week of the lactation ($p<0.001$), and 20% sows were too thin. Skin was not clean enough in 15% of the animals, 30% had some signs of skin inflammation and in 42.5% claws lesions have been recorded. The strongest positive correlations between skin condition and total surface under lesions ($p<0.001$), and between total lesions and hindquarters lesions (both $p<0.01$) were found. The results show that welfare of lactating sows had been impaired in all of observed aspects. More attention should be given to monitoring of sows' condition, to minimize the occurrence of welfare problems and to reduce production losses.

Keywords: lactating sows, body condition, cleanliness, skin, claws, lesions

Introduction

Commercial pig farming faces numerous animal welfare problems in all production categories. Animal welfare of sows during the lactation period is an increasing concern and the widely used farrowing crates are under discussion (Lambertz et al., 2015).

In last few decades, different welfare assessment protocols for pigs have been developed. They are based on various combinations of welfare indicators related to the production system, husbandry routines, and animal responses such as behavior and health. Special attention is paid to animal-based indicators, such as body condition, body marks, lameness, cleanliness etc. Regularly scoring appropriate outcome measures can identify welfare problems and be used to set targets or benchmark for improvements through an active program. Actually, in a broader sense, the goal of a welfare assessment system may be to certify the level of welfare on specific farms, compare the welfare in different production systems, or serve as an advisory tool that allows the farmer to identify, prevent or rectify

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welfare problems on the farm (Johnsen et al., 2001; Bonde, 2003; Welfare Quality, 2009; Sommerville, 2016).

Data on the extent of welfare problems at Serbian pig farms are limited, considering number of the study in this field is not large. The aim of this paper is to give an overview of general condition of sows in lactation and to point on the presence of some welfare problems at one typical commercial farm.

Material and Methods

The study was conducted on total 40 sows in a farrowing facility of the commercial pig farm. The sows were randomly selected (every third box); selected animals have been in second to fourth week of lactation (17 in second, 3 in third and 20 in fourth week). Dimensions of single farrowing pen with semi-slated floor were 3 x 2.5 m, with area for the sow inside the pen of 0.65 x 2.20 m (1.43 m²).

In the sows, several welfare parameters have been visually assessed using following protocols: body condition, cleanliness, skin condition, lesions in general and in specific body regions - shoulder, hindquarters, flanks, legs, ears and head (AssureWel, 2015), and claws condition - toes length and dew claw condition (according to ZinPro classification; Feet First Team, 2010).

For assessment of the most of the parameters, three levels scale (from 0 to 2) is used: (0) refers to normal (or desirable) condition or mild changes, (1) is worse and (2) is the worst condition of the parameter which is observed (in the protocols, a detailed description for the each score of single parameter is given). However, the protocols for body and claws condition assessment have different scales. For performing statistical analyses, their scales were adjusted using description in their protocols, what is shown below the tables with results.

Data were processed by SAS® 9.3 Software and Microsoft Office EXCEL 2010.

Results and Discussion

In table 1 the average scores for body condition of lactating sows in different weeks of lactation are presented.

Table 1. Body condition of the sows in lactation

BODY CONDITION		Sows in different weeks of lactation (%)		
score*	description in the protocol	2nd	3rd	4th
1	thin	/	/	20.0
2	thin	29.4	100	80.0
3	moderate	70.6	/	/
4	fat	/	/	/
5	fat	/	/	/

* adjustment from 1-5 scale to **0-2** scale: 1 = **2**, 2 = **1**, 3 = **0**

Results show that body condition of the sows decreased with the length of lactation. One-way ANOVA and LSD test confirmed that week of the lactation significantly influenced sows' body condition (p<0.001), with significant difference between 2nd and 4th week (p<0.001).

Sows should not enter the farrowing house with a condition score of less than 3. Body condition should not drop more than 1 point during lactation (Sommerville, 2016). The condition of the sow may reduce to score 2 during the lactation period but it is unacceptable for any sow to have a condition score of less than this (Anon., 1998). In our study, 20% of sows in the fourth week of lactation were in poor condition.

Table 2 shows results for skin and claws condition parameters assessment.

Table 2. Skin and claws condition of the sows in lactation

Score	Sows (%)			
	Cleanliness	Skin condition	Body lesions	Claws condition*
0	85.0	70.0	70.0	57.5
1	15.0	27.5	25.0	37.5
2	/	2.5	5.0	5.0

* adjustment from 1-3 scale to **0-2** scale: 1 = **0**, 2 = **1**, 3 = **2**

According to AssureWel protocol (2015), it is preferred to have >80% pigs with score 0 and <5% with score 2. In our study, skin of the majority of sows (85%) was clean. It means that <20% of their body was soiled with slurry/urine/feces (protocol description). In other animals (15%), body surface was dirty between 20 and 50% (almost all in the second week of lactation), and there were no animals whose body is ≥50% soiled.

In 70% of the sows skin was without signs of diffuse inflammation or discoloration. Mild changes were noticed in 27.5% animals (mostly in the second week of lactation), and in 2.5% (in second week of lactation) more than 10% of the skin had an abnormal color or texture.

Similar result is for skin condition regarding the existence of different lesions (grazed/broken skin, fresh i.e. bleeding wounds and healing lesions i.e. scabs): the majority was without lesions or they were less severe than described as mild changes (70%), 25% had mild changes (linear lesion longer than 10 cm, or ≥3 linear lesions, or a circular area 1-5 cm diameter), and lesions in 5% of sows were assessed as severe (circular lesion or area of lesions ≥ 5cm diameter, or lesion extends into deeper layers of skin, or the lesions cover >25% of the skin).

Single mild lesions probably have little impact on the animal, whereas increased numbers of lesions or more severe lesions are likely to be painful and cause distress. Anyhow, skin lesions provide a route for infection into the body. A high percentage of pigs with mild lesions is an evidence of unrest within the pigs or poor housing maintenance. In lactating sows, wounds and lesions can be consequences of poorly designed pens with rough flooring or sharp edges. Also, they may occur earlier, as a result of agonistic interactions among dry sows in group pens.

Claw lesions are very common problem in sows, associated with lameness, poor reproduction results and a high risk of early culling (**Pluym** et al., 2012; Lisgara et al.,

2015). Among all common claws' problems, we paid attention on toe and dew claws length: there were 57.5% animals with one or more toes and/or dew claws slightly longer than normal, and 37.5% with one or more toes significantly longer than normal or dew claws extend to floor surface when the pig is standing. The other 5% have had very long toes or dew claw is torn and/or partially or completely missing.

The next table shows the incidence of lesions in certain body regions of the sows.

Table 3. Lesions in different body regions of the sows

Score	Sows with lesions (%)				
	Shoulder	Flank	Hindquarters	Legs	Ears and head
0	70.0	92.5	92.5	95.0	100
1	27.5	5.0	7.5	5.0	/
2	2.5	2.5	/	/	/

Lesions on the shoulder are typically round and likely to be very painful. They are usually present long periods of time, and they are also often reoccurring. They indicate inappropriate housing, feeding or management. Marks on the hindquarters are likely to indicate competition around the feeders – but can also be the result of social fighting where the defeated pig is unable to escape the aggressor. Marks on the back and flanks (especially parallel scrape marks) indicate mounting behavior (which can indicate disturbance or excitement in the pen, as well as increased sexual maturity). Marks on the legs are likely to indicate an issue with flooring, e.g. abraded bursae are associated with rough/uneven surfaces. Marks on the head and ears often indicate bite marks from fighting to establish or maintain social rank. The goal in the heard is to have minimal number of minor lesions and no severe lesions across all individuals (Mullan, 2013; Sommerville, 2016).

According to the results, in 70% sows were no skin damage on the shoulder; 27.5% had mild changes (grazed or broken skin or swelling), and in the rest (2.5%) lesions were severe (>5x5cm or deep tissue injury). Lambertz et al. (2015) have had better results in their study: they have also found shoulders lesions as a dominant type of injuries, but only in 14% of lactating sows.

The most of the sows have had no lesions in flank and hindquarter region (both 92.5%), and no lesions or swellings in the region of legs (95%). Mild lesions were found in all three regions (5%, 7.7% and 5%, respectively), but severe lesions were found only in the flank region in 2.5% of the animals. The sows have had no lesions at their ears and/or head.

In table 4, only significant correlation found between observed welfare parameters are presented.

Table 4. Significant correlations between welfare parameters in lactating sows

Parameters	r_s*	p-level
Cleanliness & Skin Condition	0.365	0.021
Cleanliness & Lesions Total	0.348	0.028
Skin Condition & Lesions Total	0.886	0.000
Skin Condition & Shoulder Lesions	0.328	0.039
Skin Condition & Claws Condition	0.339	0.032
Lesions Total & Hindquarters Lesions	0.462	0.003
Leg swellings & Shoulder lesions	0.336	0.034
Leg swellings & Hindquarters lesions	0.370	0.019

* r_s – Spearman's rank correlation coefficient

According to the results, there were significant positive correlations between following parameters: cleanliness and skin condition, and cleanliness and body lesions total (both $p<0.05$); skin condition and lesions total ($p<0.001$), skin condition and shoulder lesions, and skin condition and claws condition (both $p<0.05$); lesions total and hindquarters lesions ($p<0.01$); leg swellings and shoulder lesions, and leg swellings and hindquarters lesions ($p<0.05$). Body condition and flank lesions were not correlated with any other parameter ($p>0.05$).

Conclusion

The results showed that welfare of selected lactating sows had been impaired in all of observed aspects. It is particularly worrying bad condition of sows in the fourth week of lactation, as well as claws condition in general and number of animals with lesions in the area of shoulders. Applied methods proved to be useful in obtaining quick insight into some welfare aspects of sows. Deeper analysis of the causes and age of the processes on animals' body is subject of other types of research.

We can recommend that more attention should be given to monitoring of sows' condition in all segments of production cycle, to minimize the occurrence of welfare problems and to reduce production losses.

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Poster presentation

DIAGNOSTICS AND CONTROL OF MYCOPLASMAL PNEUMONIA (MYCOPLASMA HYOPNEUMONIAE) IN FARROW-TO-FINISH SWINE HERDS

Prodanov-Radulovic J.[1], Petrovic T.[1], Stojanov I.[1], Lupulovic D.[1], Marcic D.[1], Petrovic J.[1], Bojkovski J.[2]

Abstract

Mycoplasmal pneumonia caused by *Mycoplasma hyopneumoniae* (Mhyo) is an important respiratory pig disease that causes severe economic losses to swine industry. It is considered to be most common porcine respiratory disease in commercial swine farms, with low mortality but high morbidity. The aim of research was to evaluate the diagnostic process of mycoplasmal pleumonia caused by Mhyo and implementation control measures at a herd level. The research methods included clinical and gross pathological examination of diseased and dead pigs, and control of respiratory organs at the slaughterhouse. The material for examination included lungs, tonsils and lymph node samples of dead animals and fatteners obtained at slaughter line (360 samples) and blood samples from selected animals (75 samples). The following research laboratory methods were applied: standard bacteriological diagnostic methods (aerobic and microaerophilic cultivation) and molecular diagnostic methods (RT-PCR) for Mhyo. Serum samples were examined using immunoenzyme (ELISA) test for Mhyo (IDEXX M. hyo). By examination of respiratory organs at the slaughter line, no visible changes in lung tissue were observed only in 19.44% of examined pigs. In others, examination of respiratory organs frequently revealed pathological changes indicative for Mhyo infection. Serological examination revealed presence of specific antibodies against Mhyo in 88% of tested sera samples. By bacteriological examination the following bacteria was detected: *Haemophilus parasuis, Pasteurella multocida, Staphylococcus aureus, Mannheimia haemolytica.* The obtained results suggest the necessity of implementation of an updated medication and vaccination program, taking into consideration the organization structure of farrow-to-finish herds in the region.

Keywords: swine, mycoplasmal pneumonia, control

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Introduction

Mycoplasmal (enzootic) pneumonia caused by *Mycoplasma hyopneumoniae* (Mhyo) is an important respiratory pig disease that causes severe economic losses to swine industry (Maes et al., 2008). It is considered to be most common porcine respiratory disease in commercial swine farms, with low mortality but high morbidity (Jackson and Cockcroft, 2007; Hillen et al., 2014). Moreover, Mhyo is also considered to be one of the primary agents involved in the porcine respiratory disease complex (PRDC) (Savić et al., 2015). The organism is primarily found on the mucosal surface of the trachea, bronchi, and bronchioles, and adherence of Mhyo to the ciliated epithelium is a prerequisite for initiation of the infection (Maes et al., 2008). The loss of cilia is thought to be important in the increased incidence of secondary bacterial infections associated with Mhyo (Opriessnig et al., 2011; Simionatto et al., 2013). Therefore, Mhyo predisposes animals to concurrent infections with other respiratory pathogens including bacteria and viruses (Maes et al., 2008; Thacker and Minion, 2012).

Clinically, the disease in pigs is characterized by a chronic, nonproductive cough, and decreased growth rate and feed conversion ratio (Sibila et al., 2009). However, frequently the severity of clinical symptoms increases after infection with secondary bacteria such as *Pasteurella multocida (P. multocida), Haemophilus parasuis (H. parasuis), Streptococcus suis (S. suis) or Actinobacillus pleuropneumoniae (A. pleuropneumoniae)* (Savic et al., 2015; Thacker and Minion, 2012). It is considered that Mhyo is ubiquitously distributed across most swine producing countries (Maes et al., 2008). Although pigs of all ages are susceptible, the animals in the growing to finishing phase are most affected. However, in herds without immunity, the disease can affect pigs from all age groups, including suckling pigs and breeding animals (Sibila et al, 2009; Simionatto et al., 2013). Persistently infected pigs typically have subclinical disease, are difficult to detect using currently available diagnostic tools and remain carriers, capable of transmitting the pathogen to susceptible animals (Maes et al., 2008; Sibila et al., 2009). The assessment of respiratory disease within a pig herd by lung 'lesion control' at abattoir inspection is frequently used to estimate the incidence and its impact on carcass market price (Dosen et al., 2014a; Prodanov-Radulovic et al., 2015). Such surveillance may also be useful in detecting subclinical disease which can adversely affect production during the fattening period (Christensen et al., 1999; Sibila et al., 2009).The aim of the research was to evaluate the diagnostic process of mycoplasmal pleumonia caused by Mhyo and possible implementation control measures on a herd level in farrow-to-finish swine herds.

Material and Methods

The applied research methods included history data and clinical evaluation, gross pathological examination of diseased and/or dead pigs. In the case of observation of gross pathology lesions indicative for respiratory disease, the tissue samples (lungs, mediastinal lymph nodes) deriving from diseased, dead pigs on the farm was sampled for further laboratory examination.

History of pig herds
This study was conducted on two conventional farrow-to-finish herds located in Vojvodina Province. The number of sows in the examined farms was 850 and 1200 sows, respectively. The selected herds had a history of health respiratory problems in fatteners. On the each

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farm, the piglets are weaned at the age of 28-35 days, when they are transferred to a weaning and after that growing unit with pens. Pens are cleaned and disinfected each time before a new group enter the unit, but nose-to-nose contact between different age groups of pigs housed in adjacent pens is possible. In the time of examination, vaccination against Mhyo was not carried out in the examined herds.

Slaughterhouse control
At the slaughterhouse, thoracic cavity organs from in total 360 fatteners that have reached the slaughter body mass were examined and observed gross pathology changes indicative for respiratory infections were recorded. Pigs were delivered to slaughterhouse in which the carcasses were not submerged in billing water, but steamed, which decreased the risk for contamination with respiratory pathogens during slaughter process, and allowed us to microbiologically examine the lungs. Stage (acute, chronic), location (cranial, middle, caudal or intermediate lobe), and extent of lung lesions were classified and recorded, according to Christensen et al., (1999).

Laboratory testing
From fatteners at the slaughter line, in total 75 blood samples were taken in order to perform serological investigation on the presence of specific antibodies against Mhyo by immunoenzyme (ELISA test) according to the manufacturers instruction (IDEXX Mhyo Ab test). Isolation of bacteria from tissue samples deriving from diseased, dead pigs was performed by standard aerobic and microaerophilic cultivation. Microscopic examination determined whether the isolated bacteria were Gram positive or not and whether it is a coccoid or rod-like organism. The determination was carried out by determining the biochemical characteristics of the isolated bacteria (Quinn et al., 2011). In the slaughterhouse, the tissue samples (lungs, mediastinal lymph node) of some numbers of fatteners (in total 150) with altered respiratory organs were also collected for bacterial examination. Beside this, in some number of tissue samples, the molecular diagnostic method, real time reverse transcriptase - polymerase chain reaction (RT-PCR) for detection of Mhyo (Strait et al., 2008) was applied.

Results and Discussion

In the investigated swine farms, by clinical examination symptoms of respiratory disease in growing pigs and fatteners were detected. The main sign was prolonged non-productive coughing, usually worsened by exercise. Beside this, individual animals showed the signs of severe respiratory distress, with more productive cough and open-mouthed breathing. In severely diseased animals depression was noted together with the respiratory distress and growth disorders. Outbreaks of respiratory disease were frequently observed shortly after growing pigs entered the fattening units and when some of the feed components where added and /or changed. At post mortem examination severe lung lesions were present in a number of died animal (85%). The pathological process was expressed through purple to gray areas of consolidation of lung tissue (*Pneumonia fibrinosa in statu hepatisationis rubrae et griseae*), and macroscopically the lung lobes are very similar to the hepatic or pancreatic tissues. In chronic infections, the lesions were necrotizing, associated with fibrinous pleurisy affecting caudal parts of the lung lobes. An adhesive pleurisy was notified between the visceral and parietal pleura. The disease became most often complicated by secondary infections and spreading of pathologic process on distal lung lobes. Most frequently, we detected the picture of pleuropneumoniae, pericarditis and on the lung tissue the presence of fibrin deposits (*Inflammatio fibrinosa pleurae pulmonalis*)

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or the adhesions between the visceral and parietal pleurae (*Pleuritis adhaesiva circumscripta et diffusa*). Also, in some number of died fatteners polyserositis was observed. By bacteriological examination of samples derived from dead fatteners thefollowing bacteria was most frequently detected: *H. parasuis, P. multocida, St. aureus, M. haemolytica.*

The causative agent may be introduced into a herd in two main ways: by direct transmission following the introduction of subclinically infected pigs and by airborne transmission. Once in the herd, Mhyo is transmitted between animals in aerosolized droplets generated by coughing and sneezing or through direct contact. Horizontal transmission of infection may occur between pen mates or, in continuous flow production systems, from older to younger animals (Maes et al., 2008; Thacker and Minion, 2012). It is considered that Mhyo infection may persist in the respiratory tract of adult animals for up to 185 days (Sibila et al., 2009). Macroscopic lesions, consisting of purple to grey areas of pulmonary consolidation, are mainly found bilaterally in the apical, cardiac, intermediate and the anterior parts of the diaphragmatic lobes. In the case of a pure Mhyo infection macroscopic lesions are resolved 12–14 weeks after infection (Maes et al., 2008).

The pigs health control in the slaughterhouse revealed the existence of gross pathology changes of respiratory organs indicative to Mhyo infection (Table 1). By examination of respiratory organs of 360 fatteners at the slaughter line, no visible changes in lung tissue were observed only in 19.44% of examined pigs. In others, examination of respiratory organs revealed moderate to severe pathological changes indicative for Mhyo infection (23.55%). The various level of lung consolidation, usually involving one or two lung lobes (cranial and middle lobe) was detected. Frequently, lesions were located in the cranial parts of the lung, where consolidation, discoloration were observed. The changes in the pleura were established, manifested as local pleuritis (20.08%) and chronic diffuse pleuritis (14.11%). Pleuritis was most often located on the caudal lobes, but also the surfaces of the cranial and middle lobes were affected and adhesions between them were observed.

Table 1 Distribution of the detected lung lesions indicative for Mhyo infection

Affected lung lobes (involvement of lobes in consolidation process)	%
Apical lobes	1.26
Cardiac lobes	6.13
Diaphragmatic lobes	1.94
Apical and cardiac lobes	16.77
Apical and intermediate lobes	0.32
Apical, cardiac and intermediate lobes	0.97
Cardiac and diaphragmatic lobes	1.29
Apical, cardiac and diaphragmatic lobes	20.48
Apical, cardiac, diaphragmatic and intermediate lobes	15.46
Diffuse lobular changes	31.84
Focal changes	3.54

In a number of cases parts of the lungs were missing, most likely because of firm adhesion to the thoracic wall (chronic pleuritic). Also the pathological signs indicative for *A. pleuropneumoniae* infection (*Pleuropneumonia haemorrhagica necroticans* 6.77%)

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were discovered. In some number of fatteners, the changes in the heart muscle (*Pericarditis villosa* 7.55%) and signs of purulent bacterial infection of lungs (*Pneumonia apostematosa disseminatae* 8.5%) were observed.

Information from abattoir surveillance of slaughtered pigs may indicate the nature and level of any existing respiratory disease. More than one respiratory infection may be present on a unit at the same time (Jackson and Cockcroft, 2007). Therefore, slaughter surveys can be applied to investigate lung lesions and /or pleuritic of fattening pigs to support therapeutic or preventive veterinary decisions (Jirawattanapong et al., 2010). The inspection at slaughter might be a useful tool as data source for further epidemiological studies (Dosen et al., 2014a; Prodanov-Radulovic et al., 2015).

Bacteriological examination in total 150 tissue samples obtained from selected pigs at slaughter revealed the presence of *H. parasuis* (13.53%), *P. multocida* (8.24%), *T. pyogenes* (4.7%), *St. aureus* (0.59%) and *M. haemolytica* (0.59%) while *A. pleuropneumoniae* was detected in only 2 examined tissue samples (1.18%). The tissue samples were obtained from pigs at the slaughter line which, according to veterinary records had not been treated with antimicrobial agents in the 3 weeks prior to sample collection. The blood sampling from in total 75 pigs was done directly at the slaughter line. Serological examination (ELISA test) revealed the presence of antibodies against Mhyo in 88 % of tested sera samples. In the survey, in total 30 samples indicative for Mhyo infection were examined by RT-PCR for detection of Mhyo. The positive result was established in 83% tested samples. An etiological diagnosis of Mhyo by RT-PCR was not performed in all indicative cases in our study, but the gross findings which are characteristic for enzootic pneumonia suggest a significant role of Mhyo in respiratory problems on examined swine farms. Mhyo have emerged as significant pathogen for the pig industry, especially in high health status farms. The correct diagnosis of infection is essential to establish the appropriate control measures (Savic et al., 2015; Sibila et al., 2009). Monitoring the incidence of enzootic pneumonia includes examination of batches of lungs at slaughter and serum profiling of the herd (Jackson and Cockcroft, 2007). Currently, PCR testing is the most sensitive technique for detecting infections caused by Mhyo (Maes et al., 2008; Simionatto et al., 2013). In treating mycoplasmal diseases, macrolides and tetracyclines are most frequently used during the critical production stages. However, applying medication routinely increases the risk of antimicrobial resistance (Došen et al., 2014b). To prevent both the clinical disease and the risk of antimicrobial resistance, commercial vaccines are routinely applied worldwide (Hillen et al., 2014) and recently the vaccination is widely applied on swine farms in Serbia (Prodanov-Radulovic et al., 2015).

Conclusion

In the present study, the clinical findings and gross pathology changes in the respiratory organs indicative for Mhyo infection were evident at high rate in clinically healthy fatteners. The obtained results suggest the necessity of implementation of an updated medication and vaccination program in order to control Mhyo infection, taking into consideration the isolated causative bacterial agents and organization structure of farrow-to-finish herds in the region.

Acknowledgment

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Poster presentation

FEED QUANTITY EFFECTS ON SOME WATER QUALITY PARAMETERS AND STRESS INDICATORS IN COMMON CARP (*Cyprinus carpio* L.)

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Abstract

This paper shows the effects of different feed quantity on water quality parameters (temperature, pH, dissolved oxygen and unionized ammonia) and blood parameters in common carp juveniles (plasma cortisol, and glucose). Fish were kept for three months in tanks of the recirculation aquaculture system (RAS), and fed commercial extruded feed in following quantities: 2, 3, 4 and 5% in relation to the ichthyomass (I, II, III, and IV group, respectively). According to the results, during the study, water quality was within the recommended values for common carp, except regarding unionized ammonia levels. Feed quantity significantly influenced pH and dissolved oxygen values ($p<0.001$), and the period of examination influenced all water quality parameters ($p<0.001$). Plasma cortisol and glucose values were not affected by the feed quantity ($p>0.05$) but by the period when the sampling was done ($p<0.05$). In conclusion, feeding regime may affect water quality parameters in RAS, and long-term exposure to stressors influences cortisol and glucose levels in carp blood.

Keywords: common carp, feed quantity, cortisol, glucose, stress

Introduction

Recirculation aquaculture system (RAS) is a production system where water quality should be maintained at almost constant level and in accordance with the needs of the cultivated fish. It operates by filtering water from the tanks so it can be reused. However, high level of nutrition is often the main reason for water quality deterioration due to decomposition of uneaten feed and its retention in tanks (Isla Molleda, 2007).

Inappropriate water quality results in the activation of a stress response in fish (Conte, 2004; Marino, 2008). Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms, described by Wendelaar (1997) and Barton (2002). The influence of water quality on stress and welfare indicators in common carp (*Cyprinus carpio*) is discussed in many publications including Svobodová et al. (1991), EFSA (2008) and Relić et al. (2011). Feed quantity effect on stress and welfare in common carp juveniles was studied by Relić (2011); results from this study regarding effects on carps' blood parameters were previously published (Relić et al., 2014).

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In this paper effects of different feed quantity on some water quality parameters and stress indicators in carp blood are shown.

Material and Methods

The study was conveyed during three months in recirculating aquaculture system (RAS) of CEFAH (Centre for Fishery and Applied Hydrobiology - University of Belgrade, Faculty of Agriculture). Total 480 healthy specimens of common carp (*Cyprinus carpio*) yearlings were distributed in 4 groups. Each group consisted of three replicate 120-litre tanks, with constant water flow of 0.5 l/min. Fish were fed 2, 3, 4 and 5% of commercial extruded feed (38% proteins and 12% fat, "VZ Subotica", Serbia) in relation to the ichthyomass (I, II, III, and IV group respectively). At the beginning of the study, the average individual body mass was 6.43 ± 0.02 g and population density in the tanks was $2.14 \, kg/m^3$.

Water temperature, pH and dissolved oxygen (DO) were measured by appropriate probe (MULTI 340i/SET, WTW, Germany) three times a week, directly from the tanks. Twice per month water samples were taken for unionized ammonia (NH_3-N) content determination (method by Anon., 1985). Measurements and water sampling were conveyed in the morning, before feed distribution.

Beside mechanical filtration in RAS, accumulated organic material was removed manually from empty tanks, after all fish were harvested for their measuring and blood sampling.

Blood samples were collected from caudal vein of three fish per tank at 32, 64 and 96 day (at the end of the 1st, 2nd and 3rd month of the study i.e. period). Prior to blood sampling fish were anaesthetized by MS-222 (Sigma-Aldrich®). Blood plasma was isolated by centrifugation (1400G, 15 min), and samples were stored at -20°C before the measurements. Blood sampling and the samples preparation for analyses are described by Ardó et al. (2009). For determination of plasma cortisol and glucose levels appropriate reagents kits were used (Cortisol RIA kit, Izotop, Hungary and Glucose GOD-PAP kit, Reanal-Ker Ltd., Hungary).

Feed quantity and duration of the treatment i.e. period (time between two consecutive sampling) were considered as factors affecting water quality and blood parameters. Data were analyzed using parametric tests (two-way factorial ANOVA, LSD and Fisher LSD test) at significance level of $p < 0.05$; for data not normally distributed appropriate transformation was done. Relation between blood and water parameters was evaluated by Spearman's rank correlation coefficients. The results were analyzed by STATISTICA 8.0 Software (StatSoft, Inc. 2007), and Microsoft Office EXCEL 2010.

Results and Discussion

Average values of water quality parameters for a period of three months are shown in Table 1.

Table 1. Statistical parameters for water quality parameters

Parameter	Mean	SE	Cv (%)
T (°C)	21.56	0.20	5.56
pH	8.41	0.03	2.16
DO (mg/l)	4.43	0.14	18.50
NH$_3$-N (mg/l)	0.31	0.02	35.80

SE - standard error; Cv - coefficient of variation

According to the results, water quality during the study fit the needs of common carp, except in respect to unionized ammonia levels (NH$_3$-N) (Svobodová et al., 1993; Schreckenbach, 2002; Poli, 2009). However, feed quantity significantly influenced only pH and dissolved oxygen values (p<0.001). There were significant differences (p<0.001) in average pH and DO values between groups I and II (smaller feed quantity), and groups III and IV (Relić, 2011).

The period of examination significantly influenced all water quality parameters (p<0.001). Interaction between the treatment (feed quantity) and duration of the experiment (i.e. period) had no significant effect on the water quality (p>0.05).

Table 2. Effect of feed quantity and duration of the experiment on the water quality parameters

Parameters	Factors*	SS	df	MS	F	p - value
Temperature (°C)	Treatment	6.2	3	2.1	0.633	0.594
	Period	309.7	2	154.8	47.083	**0.000**
	Treatment*Period	7.5	6	1.3	0.382	0.890
	Error	841.8	256	3.3		
pH	Treatment	0.68	3	0.23	20	**0.000**
	Period	7.06	2	3.53	305	**0.000**
	Treatment*Period	0.07	6	0.01	1	0.413
	Error	2.96	256	0.01		
DO (mg/l)	Treatment	1.76	3	0.59	7.329	**0.000**
	Period	2.49	2	1.24	15.546	**0.000**
	Treatment*Period	0.95	6	0.16	1.981	0.069
	Error	20.49	256	0.08		
NH$_3$-N(mg/l)	Treatment	0.0	3	0.00	0.582	0.629
	Period	0.1	2	0.05	9.674	**0.000**
	Treatment*Period	0.0	6	0.00	0.209	0.973
	Error	0.3	60	0.01		

* treatment = feed quantity; period = duration of the experiment

Feed quantity had no significant effect on plasma cortisol and glucose levels in the groups (p>0.05), while period when sampling was done significantly influenced results in all groups (p<0.05). Tables 3 to 6 show data on plasma cortisol and glucose values.

Table 3. Statistical parameters for plasma cortisol (ng/ml)

Groups	Statistical parameters					
	N	Mean*	SE**	Min	Max	C$_V$ (%)**
I	27	210.12 [a]	25.38	3.96	428.42	62.76

II	27	234.01 [a]	22.89	4.69	428.42	50.82
III	27	184.62 [a]	20.23	2.48	395.08	56.93
IV	27	209.84 [a]	29.29	2.45	541.85	72.52

*a, b – significant at p<0.05; the same letter - no significant difference (p> 0.05)
**SE - standard error; Cv - coefficient of variation

In individuals of the same species, cortisol levels are very different and they can be affected by number of factors (Johansen et al. 2006; Ross and Ross, 2008). Differences between the groups in the mean cortisol values were not significant (p>0.05). However, single values varied a lot. Svobodova et al. (1999) found the higher levels of cortisol in individuals less exposed to stressors (chronic stress) prior to blood sampling (acute stressor). Lower level responses to acute stress can be caused by prolonged exposure to conditions of chronic stress, habituation or weakening (Hontela et al., 1992; Fast et al., 2008; Ross and Ross, 2008). In this study, chronic state of stress has been caused by gradual deterioration of the water quality due to accumulation of uneaten feed in groups with more intensive feeding (Table 4). Ortega et al. (2005) wrote about influence of high level of NH_3-N in the water on increased cortisol secretion in fish body.

Table 4. Mean values of plasma cortisol (ng/ml) - sampling at the end of the each period

Periods	Groups*			
	I	II	III	IV
first (1st - 32nd day)	239.66 [a]	285.35 [a]	224.09 [A]	336.95 [a]
second (33rd - 64th day)	240.53 [a]	274.58 [a]	214.38 [AB]	166.05 [a]
third (65th - 96th day)	150.18 [a]	142.09 [b]	115.40 [B]	126.53 [b]

*a, b – significant at p<0.05; A, B – significant at p<0.01; the same letter - no significant difference (p> 0.05)

According to table 4, significant difference in mean values is calculated in groups II and IV between the last and both previous samplings (p<0.05) and in group III between the first and the last sampling (p<0.01).

Table 5. Statistical parameters for glucose (mmol/l)

Groups	Statistical parameters					
	N	Mean	SE	Min	Max	Cv
I	27	4.93 [ab]	0.40	2.08	9.78	42.33
II	27	6.13 [a]	0.79	1.82	18.25	66.88
III	27	4.47 [b]	0.52	0.02	11.96	60.77
IV	27	5.34 [ab]	0.59	2.33	15.47	57.21

*a, b – significant at p<0.05; the same letter - no significant difference (p> 0.05)
**SE - standard error; Cv - coefficient of variation

Group III stands out both in terms of the lowest mean cortisol and glucose levels, and the impact of sampling is most pronounced precisely in this group (p <0.01). The highest mean value was in the II group, and it was significantly different than in group III (p <0.05). However, both values are within normal ranges for common carp (according to Svobodová and Vykusová, 1991).

Table 6. Mean values of glucose (mmol/l) - sampling at the end of the each period

Periods	Groups			
	I	II	III	IV
first (1st - 32nd day)	4.10 [a]	4.73 [A]	2.18 [A]	3.38 [A]
second (33rd - 64th day)	4.45 [ab]	5.50 [B]	5.55 [B]	6.05 [B]
third (65th - 96th day)	6.23 [b]	6.15 [B]	5.66 [B]	6.59 [B]

*a, b – significant at p<0.05; A, B – significant at p<0.01; the same letter - no significant difference (p> 0.05)

According to table 6, mean glucose values in all groups increased with each following sampling. High concentration of NH_3-N and decrease of the water temperature can affect glucose levels in the blood (Evans et al., 2006; Rahmanifarah et al., 2010). In this study, water temperature was in the optimal range for carp but slightly declined to the end, since the water was not heated.

Table 7. Correlation between water quality parameters and stress indicators (plasma cortisol and glucose)

Parameters	Groups**							
	I		II		III		IV	
	r_s *	p-level	r_s	p-level	r_s	p-level	r_s	p-level
temperature & glucose	0.47	0.205	0.44	0.232	0.52	0.154	0.60	0.088
temperature & cortisol	0.20	0.606	0.05	0.898	-0.05	0.898	0.25	0.516
pH & glucose	0.42	0.265	0.62	0.075	0.24	0.539	0.08	0.831
pH & cortisol	0.53	0.139	0.16	0.683	0.42	0.256	0.55	0.125
DO & glucose	-0.63	0.067	-0.47	0.205	-0.12	0.765	-0.15	0.700
DO & cortisol	-0.38	0.308	-0.25	0.516	-0.08	0.831	-0.58	0.099
NH_3-N & glucose	-0.52	0.154	-0.23	0.546	0.01	0.983	-0.70	**0.036**
NH_3-N & cortisol	-0.58	0.099	-0.13	0.732	-0.55	0.123	-0.20	0.606

* r_s – Spearman's rank correlation coefficient
**bolded value is significant (p < 0.05)

Data in Table 7 show that unionized ammonia was significantly related to glucose values; a strong negative correlation between NH_3-N and glucose levels ($p < 0.05$) in the group receiving the greatest amount of feed (5%, group IV) was found.

Conclusion

Feeding regime can affect water quality parameters in RAS, and long-term exposure to stressors influences cortisol and glucose levels in carp blood. The length of this study affected the capacity of maintaining water quality, especially in groups fed greater amount of feed (4 and 5%). Also, the length of the study has influenced the susceptibility of fish to stress.

Acknowledgement

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FREQUENCY AND RISK FACTORS OF COLIC EPISODES IN HORSES IN ALBANIA

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Abstract

Colic in horses is the most acute problem faced by veterinarians and some predisposing factors are associated with them. One year study estimated the frequency and the risk factors that affect the occurrence of colic in the horse population in the region of Tirana. Details of 72 colic episodes, collected prospectively, were analyzed for a period of one year. The number of spasmodic/idiopathic colic cases in the 2-10-year-old group and the number of surgical colics in older than 10-year-old group were significantly greater than <2 years-old age group. Horses aged between 2 to 10 had a higher risk of occurrence of disease *(P<0.05)*. 20.8% of the colic episodes were due to change of husbandry conditions, 11.1% by frequent changing of weather condition, 8.3% related to work or transport in agriculture, 2.8% from sweet/high carbohydrate diet (fruit). Nutritional causes and lack of anthelmintic control programs are important risk factors for development of colic. Types of grass and hay and poor-quality roughage are suspected causes. Little information is available about specific types of food or the measurement of specific nutrients in the food, such as minerals or fiber that cause colic in horses in Tirana Rural Areas. A greater understanding of the factors involved in the development of spasmodic/idiopathic colic episodes would be a great advancement in equine welfare in the region of Tirana.
Keywords: colic, horses, risk factors, incidence, equine welfare

Introduction

Digestive diseases, such as colic, diarrhea, or enterotoxaemia, represent 50% of the medical problems resulting in the death of adult horses (Andersen et al 2006). Colic, defined as abdominal pain of digestive origin, is the major part of them. Colic is one of the most difficult diseases to study with epidemiologic methods due to the large number of diseases, cause colic (abdominal pain) as a clinical sign. Epidemiological studies allow us to test the hypothesis of a relationship between exposure to a risk factor and the development of a disease. Determining the incidence of colic can help determine if the rate of colic on farms or in stables is excessive. Out of 100 horses in the general population 4-10 cases of colic is expected during one year (Tinker et al., 1997; Kaneene et al., 1997). Most of abdominal pain, 80-85% of cases, can be considered as simple colic because through the clinical control is not possible to determine a specific diagnosis and most horses respond to medical treatment or resolve spontaneously. Obstructing or strangulating colic that require surgical intervention represent only 2-4% of colic cases, although some predisposing factors in certain populations can increase this level (White 1990). In the normal horse farm population, equine mortality from all types of colic was 0.7 deaths per 100 horses a year with a rate of colic case fatality of 6.7% (Tinker et al., 1997a).

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No epidemiological study has been performed to determine the level of occurrence of colic on horse's rural population in Albania. This study can be considered prospective in nature and conducted on the general horse population in the rural areas of Tirana district. The objectives of this study are to 1) quantify the types of colic encountered in general practice in Tirana region; 2) identify risk factors associated with these colic types; 3) record the seasonal incidence of colic and establish whether this was correlated with changes in weather and 4) specifically, to identify any risk factors of simple spasmodic/idiopathic colic.

Material and Methods

During the period from March 2015 and onwards written records of every colic case seen are filled in by veterinarians practicing in the rural areas of Tirana district. Age, race and sex of animals, anamnestic data such as changes in management and food diet, recent drug administration, previous colic episodes, clinical findings and treatment methods have been all recorded. Each case was followed to its eventual outcome as far as possible, and post mortem examinations performed when necessary. We attempted to classify each colic episode according to diagnosis by relying on some characteristic symptoms of spasmodic/idiopathic, impaction or obstipation and flatulent colic, enteritis/colitis and intestinal obstruction that required surgical treatment.

The diagnostic criteria for spasmodic/idiopathic colic included mild to moderate pain, normal findings on rectal examination, normal passage of feces, response to analgesia and eventual resolution of the colic episode following medical treatment only. Colic episodes were classified as impactions if an impaction was clearly palpable *per rectum* at each site of intestinal tract, characterized by reduced fecal output, mild to moderate pain. The criteria used for a diagnosis of flatulent colic were moderate to severe abdominal pain, gas distended intestine palpable on rectal examination, passage of flatus and recovery following medical treatment. Enteritis/Colitis cases were defined as those with profuse diarrhea, abdominal pain and hematological changes but no other intestinal abnormalities. Cases placed in the surgical category were those involving intestinal obstruction that required surgical treatment, although in most instances this option was rejected by the owner.

The degree of correlation between monthly frequency of colic and weather will be calculated using Pearson's correlation coefficient (r). The test statistic was compared with standard tables to determine significance ($P < 0.05$). The proportion of each colic type within the different age and sex categories was compared by Chi-squared analysis. Again, the significance level was set at $P < 0.05$.

Results and Discussion

The current study evaluated the frequency and associated risk factors of colic during one year evaluation in horse farms of Tirana region. During the period from March 2015 and onwards, the records of 72 colic episodes, collected prospectively from first opinion cases were analyzed. A total of 68 different horses were recorded as having colic, 8 episodes occurring in horses that had previously suffered a colic episode during the recording period. A total of 4 horses had recurrent colic episodes, which represents 5.9 % of all horses suffering from colic in one year period. The classification of each colic episode and the age, sex are shown in Table 1.

Table 1. The number of different colic cases based on sex, and age recovered over one year period

Colic Cases	Age (years)			Sex		Total (%)
	<2	2-10	>10	F	M	
Spasmodic/idiopathic	3	23	13	18	21	39 (54.2)
Flatulent	-	2	2	2	2	4 (5.6)
Impactions	-	5	6	6	5	11 (15.3)
Surgical	-	-	3	1	2	3 (4.2)
Colitis	3	7	5	8	7	15 (20.8)
Total	**6**	**37**	**29**	**35**	**37**	**72 (100)**

A student test (t-Test: paired Two Sample for Means) was conducted to compare between sex groups in all colic types investigated. No difference was found between groups ($P<0.05$).

A Chi-square test was conducted to investigate the association between the age 2-10 and over 10 years old and the spasmodic/idiopathic colics (Agresti 2012). The odds ratio, χ^2 and its 95% confidence interval were calculated to measure the magnitude of the association. A 5% level of significance was used to evaluate significance of association, i.e. the p-value was considered significant if it was less than 0.05. The analyses were conducted using Statulator statistical program (Dhand and Khatkar 2014). The odds ratio indicates that the 2-10 years old age group has 2.02 (OR 2.02 and χ^2 1.97) times the odds of the outcome than the over 10 years old group. Also, we are 95% confident that the odds ratio in the population (from where the sample was obtained) would be between 0.75 and 5.43.

We investigate the association between sex and particular spasmodic/idiopathic colic. We employed a Chi-square test (Agresti 2012). The odds ratio and its 95% confidence limits were calculated to measure the magnitude of the association. A 5% level of significance was used to evaluate significance of association, i.e. the p-value was considered significant if it was less than 0.05. The analyses were conducted using Statulator statistical program (Dhand and Khatkar 2014). The odds ratio indicates that male group has 1.24 times (OR 1.24 and χ^2 0.21) the odds of spasmodic/idiopathic colic than female group. Also, we are 95% confident that the odds ratio in the population (from where the sample was obtained) would be between 0.49 and 3.14. However, association between the sex and particular spasmodic/idiopathic colic was not significant (P-value: 0.650; odds ratio and 95% CI: 1.24 [0.49, 3.14]), and the odds ratio results should be interpreted with caution.

Analysis of reported cases of colic in this population of horses revealed 54% spasmodic/idiopathic; 4.2% surgical; 5.6% flatulent; 15.3 % impactions and 20.8 % enteritis or colitis. In the total number of colic cases recorded in the study areas, a greater percentage of horses from 2-10 years old age group and older than 10 years, suffering more from almost all types of colic compared with group up to 2 years of age. Horses older than 10 years showed a higher percentage of surgical colics 3 (4.2%) compared with other groups ($P<0.05$). There was no significant difference between sexes ($P<0.05$). The effect of age and sex on each colic type showed only two significant differences between categories. The number of spasmodic/idiopathic colic cases in the 2-10-year-old group and the number of surgical colics in the over 10-year-old group were significantly greater than <2 years-old age group. Horses aged 2 to 10 years had a higher risk of occurrence of disease

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(P < 0.05).
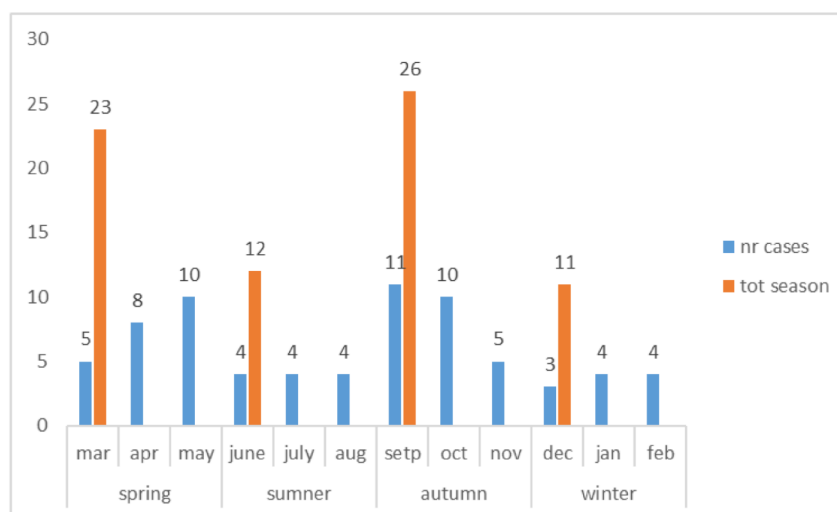
Graph 1. Seasonal frequency of colic episodes

This study showed different frequency of colic episodes based on the months of the year. Careful inspection of Graph 1 shows an increased frequency of colic during the months of spring and autumn. In the surveillance conducted so far we have not yet determined statistically whether or not there is such a correlation between weather and the incidence of colic. Of the 39 horses with spasmodic/idiopathic colic, only 31 (79.5%) had any identifiable risk factors in their histories. Table 2 lists these possible predisposing factors. 20.8% of the colic episodes were due to change of husbandry conditions, 11.1% by frequent changing of weather condition, 8.3% related to work or transport in agriculture, 2.8% from sweet/high carbohydrate diet (fruit).

Table 2. Spasmodic/idiopathic colic cases — possible risk factors

Change of husbandry conditions / related to parasites	15
Dramatic change in weather	8
Associated with exercise/ Post transportation	6
Sweet/high carbohydrate diet	2
Total	**31**

Colic is the most devastating disease in the horse population and one of the most frequent problems encountered by a large animal veterinarian. This survey was designed as a prospective study of successive colic cases. The horses showed mild to moderate signs of colic. Most of the horses in the study 50 (69.4%) showed signs of un-complicated colic and were treated by routine methods or even without medications 20 (27.8%). From all cases, 3 horses showed complicated signs of colic that required surgical treatment terminated in death. Horses who responded to medical therapy quickly, and it was not possible to place any particular diagnosis are classified as spastic colic, Table 1. Horses with acute abdominal pain are commonly seen, and most, probably greater than 90%, are un-complicated cases that respond readily to analgesic therapy.

Problems arose in the classification of most colic episodes. Horses that responded to simple medical therapy when no specific diagnosis was made had variable presenting signs and

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durations of colic symptoms (Table 1). Rollins and Clement, 1979 and Foreman and White, 1986 classified spasmodic colic as a distinct disease category. Huskamp (1982) classified spasmodic colic with other undiagnosed medical colic cases. This latter classification was adopted in the present study because the disease 'spasmodic colic' is such a poorly defined syndrome and because of the large overlap between spasmodic and 'idiopathic' colic. For the purpose of this study, this group was defined as spasmodic/idiopathic colic. Spasmodic/idiopathic colic was diagnosed most frequently. The greater proportion of colics in this category (54%) in our study, it may be interpreted by the fact that no attempt was made to separate and categorize this group of colics further and/or that a greater proportion of clinical cases may have entered in this group.

This study showed different frequency of colic episodes based on the months of the year, explained by the Mediterranean climate of Albania. Some authors have suggested, or proved, a correlation between weather and the incidence of colic (Barth 1982; Rollins and Clement 1979). However, in this study conducted so far, we have not yet defined statistically whether or not there is such a correlation. Veterinarians and owners frequently associate weather changes with increased frequency of colic, but many studies have been unable to find statistical evidence of increased risk. Early reports from Europe suggested weather changes were associated with the highest incidence of colic specifically changes to cold and damp conditions or warm and wet during advancing weather fronts (Barth 1980). A study in Texas found an increased risk of colic associated with weather changes as recalled by owners of horses with colic (Cohen *et al.* 1999b). Cold weather, which affects water intake, has been linked to increased impaction colic. When examined as a direct exposure factor in a Virginia-Maryland study, weather did not appear to be related to colic (Tinker *et al.* 1997b). Graph 1 shows an increased frequency of colic during the months of spring and autumn. This may be due to changing grass quality or changing management practices at these times of year rather than changing weather conditions. The calculation of a correlation coefficient using mean monthly temperature and mean monthly rainfall will be an attempt to give greater significance to temperature and rain during warm weather when grass is growing.

Table 2 presents some interesting potential factors related to the emergence of individual colic episodes in the spasmodic/idiopathic category. The association of these factors with the occurrence of colic episodes is circumstantial only because it is impossible to prove a direct causal relationship. It is impossible to determine the number of colic episodes in our study that were related to parasites. The association of parasitic forms may be even higher because it is not a common practice the use of anthelminthic drugs by horse owners in Tirana. Parasites (ascarids, tapeworms, strongyles) are associated with an increased colic risk of colic by in several studies (Uhlinger 1992; Proudman and Holdstock, 2000). Nutritional causes and lack of anthelmintic control programs are important risk factors for development of colic. Types of grass and hay and poor-quality roughage are suspected causes. Gradual change of diet to new types or amounts of food has long been recommended and is supported by some findings (Murray 1997). Little information is available about specific types of food or the measurement of specific nutrients in the food, such as minerals or fiber that cause colic. No regular parasitic control programs were used on all farms, and there was no documented diagnostic analysis of parasites in horses, nor in random fecal samples.

The unusual plant material reported to have been eaten most often was apples (associated with animals grazing in, or adjacent to, orchards in the autumn). The passage of readily

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available carbohydrate into the caecum and large colon would disrupt the normal microbial physiology of this section of intestine (Argenzio 1979). The change in management most commonly associated with spasmodic/idiopathic colic was turnout onto lush pasture in the spring.This study showed no significant difference between sexes for occurrence of colic (t- Test), and this is also supported by other studies (Sembert 1975; Cohen 1997; Kaneene et al., 1997). However, some special causes of colic, such as inguinal hernia in male horses, are sex-dependent. In our study, horses between 2 and 10 years of age had the highest risk of the disease (Chi-square test). Tinker et al., 1997 and Traub and Kopral 2001 reported that horses younger than 2 years have the lowest risk of colic. In the study of Kaneene et al., 1997 the risk of colic correlated with an increase in age. Change of diet, increased exposure to parasites, and decreased colon activity are factors that contribute to the occurrence of colic in adult horses (Cohen et al., 1999b; Seitzinger et al., 2000). In this study it was difficult to interpret why horses in the 2- to 10-year-old age group should suffer more from spasmodic/idiopathic colic episodes than horses in other age groups. The fact that horses in this age group face the greatest physical activity demands, and that they are subjected to more frequent changes of ownership, may be considered important reasons for the interpretation of this result. The age distribution of animals classified as surgical colics is interesting. Although the number of such colic episodes is small 3 (4.2%), 100% of them were recorded in animals older than 10 years. This is statistically different when compared with another age group (Chi-square test). We have not been able to define the type of colics in the surgical category of colics. However, the limited number of colic cases indicate to interpret the results with caution. These cases are referred by veterinarians included in the study and none of them has implemented necropsy examination to determine the exact cause of death and side where the pathological lesions happened.

Conclusion

This is the first study to estimate the frequency rate of colic in a population of horses in Albania. Colic is common in this working horse population and this study has identified factors associated with altered likelihood of colic. The study provides important information that may be used to inform future prospective studies investigating colic in working horse populations and to assist development of preventive healthcare strategies. Spasmodic/idiopathic colic was the most commonly reported cause of colic. We identified several risk factors for spasmodic/idiopathic colic. Other associated factors are the subject of further research. Most risk factors (feeding practices, parasitism, and management) require more investigations to better understand their impact on this disease. Identification of risk factors for spasmodic/idiopathic colic may highlight high risk horses and may allow intervention strategies to be introduced to reduce the incidence of the disease. The information's provided by this study confirm that colic affects the health and welfare in the aging population of horses. We conclude that age 2-10 years increase the risk of colic in horses. We have also reported the 12-month frequency of colic and possible associated risk factors in a population of working horses in Albania. This study showed a seasonal component to certain types of colic. These patterns appeared to coincide with either times of managemental change or periods when horses are more likely to be intensively managed. Further studies are required to identify the determinants of the observed seasonality. These information's together with details about management practices of horses and horse-owner perceptions about possible causes of colic can be used to inform educational and training programs for horse-owners and veterinary surgeons in this region, and in similar populations. These data provide information that may be used as a basis for performing future prospective cohort studies to examine risk factors for colic further within this and other working equid populations.

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Invited paper

THE EFFECTS OF DIET ON THE NUMBER OF PHEASANTS ON THE HUNTING GROUND

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Abstract

This paper provides an overview of local and foreign research which is related to pheasants' diet during a year, on pheasant farms or on the hunting grounds. The results of earlier research suggest that the use of new scientific findings within the diet of the parent flock and pheasant chicks at pheasant farms significantly contributes to the increase in the number of bred pheasant chicks. However, apart from diet, these results are also affected by microclimate at the facilities, population density, health hazards prevention and the effect of weather during the period of reintroduction, which is why the results obtained at pheasant farms differ considerably. After settling pheasant chicks onto hunting grounds, the possibility for control is reduced, which results in significant losses due to deficiencies in diet, diseases and predators. Previous research indicates that supplemental feeding of pheasants in the hunting grounds, during all seasons, and especially during winter, leads to positive results. However, the achieved results depend considerably on the degree of anthropogenic activity on the hunting grounds, as well as on the amount of natural food. Furthermore, supplemental feeding must be adequate, not only quantitatively, but also qualitatively. This is why, instead of single feed (most often grain, wheat, corn, etc.), complete mixtures should be used, which is an expensive solution. The worst results are obtained during the period of spring supplemental feeding on the hunting grounds, which is due to the abundance of natural food, so some authors do not recommend such measures.

Keywords: pheasant, diet, supplemental feeding, hunting ground, population

Introduction

Being the favorite game species in Serbia, pheasants are significant for their high rates of reproduction under controlled conditions (pheasant farms). Due to this, it is possible to obtain a large number of offspring per pheasant hen and then settle them into the hunting ground and thus compensate for the insufficient natural reproduction. For other game species, such opportunity practically does not exist, or it is minimal, which gives pheasants a significant advantage compared to other species such as partridge, rock partridge, rabbit, etc.

There are various factors which dictate the rate of reproduction of pheasants under controlled conditions. Among them, a special place belongs to the diet, which has been applying modern scientific discoveries in the last decades, such as precisely defined needs of animals and nutritional value of feed, modern technologies of processing feed, use of numerous additives, feeding techniques etc. However, the diet is an important factor even

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after settling pheasants into the hunting ground, even though a little or insufficient attention is dedicated to it afterward. As a result, the percentage of the survival rate of pheasants that have been brought to the hunting ground is lower and the expenses of the management are higher.

The aim of this paper is to highlight the critical points related to the diet in the process of pheasant "production" in the aviaries, settlement of pheasants into the hunting ground and their survival under natural conditions to the moment of shooting by providing a review of the results of previous researches.

Diet of Parent Flocks of Pheasants

Due to the high hunting percentage, low rate of natural reproduction, changed conditions in modern agrobiocenoses and great losses, pheasant chicks bred under completely controlled conditions on pheasant farms are brought to the hunting ground every year (Popović et al., 2011a and 2011b). Breeding the parent flock includes a series of procedures whose main purpose is the production of a larger number of eggs per hen, and a higher fertility of every egg, in order to obtain a larger number of pheasant chicks in the incubator (Đorđević et al., 2012d). These features are affected by a large number of factors such as the genetic structure of the parent flock, system of keeping, diet, the age of hens and duration of the laying season (Esen et al., 2010).

Diet affects the number and size of laid eggs either directly through necessary nutrients delete provided by intake, or indirectly through body reserves which have been provided earlier. In nature, a hen lays 12-18 eggs of 28-29 g each, while in the aviary it lays up to 60 eggs for about 3 months (Popović and Đorđević, 2009). Bojović (2012) states that the laying capacity of hens under experimental conditions can reach 100 eggs per season, and under the conditions of an extended day length even up to 140. Such productivity of hens demands adequate provision of all necessary nutrients.

The mass of eggs largely depends on the diet as well. In the experiment conducted by Kokoszynski et al. (2011) on a treatment with higher protein content, a significantly larger mass, length, width, and surface of eggs, shell mass, as well as a higher amount of egg white and yolk have been determined (table 1). Caglayan et al. (2010) have concluded that it is out of eggs of a larger mass that chicks of larger mass (p<0,001) are hatched. Ipek and Dikmen (2007) have classified eggs according to mass into three groups (27,8-29,7; 29,8-31,7 i 31,8-33,7 g) and analogically they have determined chick masses of 19,5; 21,8 i 22,6 g (p<0,01).

The diet of the parent flock is performed according to the needs of different categories by using granular food or complete concentrate mixtures, depending on the season.
Maintenance requirements for pheasants in captivity are 40-55 g of grains or some simpler mixture. It is well-known that the most frequently used grains (corn, wheat, etc.) contain only 8,5-12% of crude proteins. As opposed to this, during the laying season, the required content of protein is significantly increased and they are provided by concentrate mixtures. There are different recommendations for the protein requirements for pheasant hens and they amount to 15-25% (Đorđević et al., 2013c). According to NRC (1994) normative, the needs of pheasant laying hens are defined as the ratio between the energy

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and proteins, so that for 1 Mcal ME/kg of feed there is less than 5,6% of proteins. Also according to the mentioned normative, the needs in calcium are 2,5%.

Table 1. Characteristics of pheasant egg weight, morphometry, shell and content (Kokoszynski et al., 2011)

Parameters	Treatment 1: with 19.0% CP and 11.7 Mj ME	Treatment 2: with 15.0% CP and 12.6 Mj ME
Egg weight, g	30.8[a]	26.3[b]
Egg length, mm	43.8[a]	42.1[b]
Egg width, mm	35.6[a]	33.5[b]
Eggshell area, cm^2	46.7[a]	42.1[b]
Eggshell weight, g	3.0[a]	2.5[b]
Albumen weight, g	16.3[a]	13.9[b]
Yolk weight, g	11.5[a]	9.9[b]

Male pheasants have similar delete nutritional needs as breeding females, except those for calcium which are significantly lower (0,8%). Feed nutrients such as proteins and energy sources have a considerably greater effect on forming eggs than on hens' fertility, where vitamins and minerals have the dominant role (Đorđević et al., 2013a). Therefore, recent experiments pay more attention to micronutrients (microelements and vitamins).

Due to the influence of a large number of factors on the production indicators of parent flocks, the effect of the diet usually stays unnoticed. Thus, for instance, Popović et al. (2013) have monitored the production results of parent flocks on two pheasant farms which used mixtures with 22 and 20% of crude proteins (table 2).

Table 2. Production results of pheasant parent flock depending on the pheasant farm and year (Popović et al., 2013)

Pheasant farm	Year	Average number of laying eggs in season	Average number of egg per pheasant hen daily	Percent brooding eggs of laid eggs	Percent hatched of brooding eggs in season	Hatched by pheasant hen in season
Pheasant farm 1 (22% CP)	2008.	39.55	0.44	89.60	68.51	24.28
	2009.	34.67	0.46	84.71	67.08	19.70
	2010.	37.28	0.43	83.22	58.89	20.35
Pheasant farm 2 (20% CP)	2008.	53.16	0.51	93.19	72.08	35.71
	2009.	59.48	0.59	98.27	53.73	31.40
	2010.	53.90	0.53	97.68	55.77	29.36
Average for 2008.		46.36	0.48	91.40	70.30	30.00
Average for 2009.		47.08	0.52	91.49	60.40	25.55
Average for 2010.		45.59	0.48	90.45	57.33	24.86
Average for farm 1		37.17	0.44	85.84	64.83	21.44
Average for farm 2		55.51	0.54	96.38	60.53	32.16
Significance for pheasant farm		0.014288*	-	-	0.225205[ns]	-
Significance for year		0.259221[ns]	-	-	0.223030[ns]	-
Significance for interaction of factors		0.250971[ns]	-	-	0.121705[ns]	-

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In addition, the parent flock which was fed with the mixture with a lower level of proteins even had statistically better laying capacity. An explanation for this perhaps is in the fact that the experiment only monitored crude proteins, but not their amino acid composition, which may be an even more significant parameter. Đorđević et al. (2014) state that the needs of pheasants in the aviaries may also be met with a lower amount of proteins in a diet if an optimal ratio of the essential amino acids is achieved by accurately combining feeds. By using the synthetic amino acids, a complete substitution of feed of animal origin is enabled at the same time, as well as a lower price of the mixture (Đorđević et al., 2015b).

Preparations of the parent flock for the laying season should be started as early as January, by introducing the complete concentrate mixture into the ration. Its daily amount during the laying season is 80 g and it is recommended for it to be pelleted (4×6 mm).

Diet of Pheasant Chicks in the Aviaries

Body weight of pheasant chicks at the moment of their settlement into the hunting ground is very important for their survival during the period of adaptation (Đorđević et al., 2011a and 2011b). Therefore, great attention is dedicated to their diet, besides other important factors during breeding. There are several recommendations for pheasant chicks' diet which are significantly different in the number of single nutrients (Popović and Đorđević, 2009). The number of crude proteins is particularly important and in different recommendations and/or experiments are 20-40%. According to NRC (1994), the level of proteins in the mixtures for the first stage of pheasant breeding is 28% and for the second it is 24%. Đorđević et al. (2010) have determined that during the use of a larger amount of crude proteins in a ration, a larger weight of pheasant chicks is obtained after 42 days of breeding (457,07:373,85 g) even with a significantly higher population density (550 : 450 birds per group). In this, the energy and protein content primarily affects the conversion and body weight, but not mortality, with the exception of the extreme values of these parameters (Đorđević et al., 2013b). Thus, it is necessary to dedicate more attention to those factors which to a great extent dictate the mortality rate: microclimate inside objects, population density, health prevention and the effect of weather during the period of reintroduction (Zabranski et al., 2014).

Pheasant chicks' diet at the first stage of breeding is performed with granulated concentrate mixtures and at the second stage with pelleted ones. During feed conversion of 2:1, feed consumption per chick is from 2 g/day/chick in the first week of life to 30 g/day/chick at the end of breeding. For the whole period of feeding of 8 weeks, the total consumption amount to roughly 800g. After the fifth week of life, pheasant chicks are given grains and green mass with the purpose of adjusting them to the diet in nature. Pheasant chicks breeding is finalized at about 60 days of age when they are let into the hunting ground (Popović et al., 2011c).

Supplemental Feeding of Pheasants Following the Settlement into the Hunting Ground

The management practice in local hunting grounds includes recommendations for autumn and winter supplemental feeding of pheasants in the hunting ground, with the purpose of higher survival rate and a better condition for the following year. However, summer

supplemental feeding (after settlement) is practically not even considered. The period of settling pheasants into the hunting ground is a time of grain harvest and great droughts when food deficit in the hunting ground that are situated on large surfaces under monocultures frequently occur. According to the research in Ireland, about 70% of young birds aged up to 12 weeks die or disappear due to the lack of food, parasite infections and predators (Đorđević et al., 2012a; Popović et al., 2011d). Due to increased mortality, it is necessary to continue with the supplemental feeding of young pheasants for a certain amount of time after their release into the hunting ground (Sage et al., 2002). According to Đorđević et al. (2012b), at this critical period, 3-4 kg of grains per 100 young birds should be provided on a daily basis, and along with feeding ground water should be provided as well. Even better results are achieved while using appropriate concentrate mixtures because grains are primarily energetic feed. Additional protein diet of young pheasants, which are settled in the hunting ground, leads to an increase in body weight to a greater extent than additional energetic diet does, although its problem is the high price, primarily of the first mode. Sage et al. (2002) have examined the effect of supplemental feeding of pheasants (in the hunting ground) aged 6-16 weeks with a concentrate mixture which contained 20% of proteins, while the control group of pheasants after the tenth week of life received exclusively wheat grains which contain about 10% of crude proteins. Along with the aforementioned diets, pheasants had an unlimited supply of natural food of plant and animal origin. Despite this, the authors determined significant differences in body mass and the amount of cloacal fat in control birds aged 22-24 weeks. This means that supplemental feeding of pheasants during summer should be performed with concentrate mixtures with an increased level of proteins, in order to ensure the further growth of young birds and reduce the losses, especially during the winter period.

Winter Supplemental Feeding of Pheasants

Winter supplemental feeding is most often the only form of supplemental feeding of pheasants after settling into the hunting ground in Serbia. The purpose of winter supplemental feeding is not only to reduce losses in game species, but also to preserve their weight and condition, which may be significant for spring reproduction at the hunting ground. In order to use the additional food more effectively, it is necessary to timely begin with the adjustment of pheasants to feeding ground, best immediately after the harvest. Intensive supplemental feeding of pheasants in the plains begins in November and lasts until the end of March, in medium high terrains it begins in October and is performed until the end of March, and in the high ones from October until the end of April. The amount of food depends on the area and the length of supplemental feeding.

The additional food contains grains and even potentially pelleted food with a radius of 3-5 mm, as well as succulent food during the days with no frost. For winter supplemental feeding of pheasants, the daily amount of granular food of about 40-60 g per bird is planned. During winter, the parcels under corn are effectively used as well (Gabbert et al., 2001), which simultaneously function for feeding as well as for shelter. Feed is brought onto arranged or merely improvised spots. Improvised feeding ground is constructed if the additional diet is performed exclusively during winter. It is necessary to mask them so that other birds do not take away all the partitioned feed. Feed is given directly from the ground or out of automatic feeders.

Spring Supplemental Feeding of Pheasants

Natural production of pheasants is certainly important for the overall number of pheasants in the hunting ground. Time of reproduction of pheasants, as for the majority of other wild animals, coincides with the maximum vegetation development which is characterized by the abundance of food, both of plant and of animal origin (Đorđević et al., 2015a). Some previous research in Europe has shown that spring supplemental feeding of pheasants does not have any significant effects on reproduction. For example, in England (Clarendon Park Estate), Hoodles et al. (1999) have tested the influence of spring supplemental feeding of pheasants on reproductive results, and in it the number of pheasants in nature separately from their settling in the breeding farms. In this process, the additional diet did not cause either earlier nesting or enlargement of the nest, but the pheasant hens whose nest was damaged did quickly re-nest. As opposed to this, Draycott et al. (1998) have found that the fat reserves in females from the area with additional feeding (with wheat grains) have been on a similar level in April as they were in February (76,9±8 g), while the fat reserves in females from the area without supplemental feeding have been reduced by double (34,7±6,9 g), which means that they were in better body condition for the laying period. However, in both mentioned experiments, the additional feed for pheasants that was used was wheat (grain) which is typically carbohydrate or energetic feed, with low content of proteins (10-14% depending on the type), bad amino acidic composition of proteins, low content of calcium (which is important for the eggshell) and inadequate ratio of Ca:P. All of this indicates that in spite of the additional feeding in the specified experiment (Hoodles et al., 1999), the pheasant hens received incomplete rations, which is why there were no results. Lu and Zheng (2003) have tested the effects of the additional feeding (mostly with barley) on the endangered species of pheasants *Crossoptilon harmani* on Tibet. The authors did not find any significant differences in the number of eggs per female and they have explained these results with the fact that the birds without additional feeding have compensated for a part of the deficit in nutrients by spending more time in food searching. However, when the basic dimensions of eggs (mass, length, width, volume index) are compared, somewhat better results can be noticed in females with supplemental feeding (table 3). Draycott et al. (2005) claim that chemical contamination and mechanization have drastically reduced the natural food selection by having the mass use of insecticides that leading to a reduction of important protein food during the season of nesting and nurturing of the young. Modern mechanization reduces the dispersal of grains and seeds during harvest and sowing. These modern agrotechnical methods have negatively affected reproductive results through the pheasants' feeding in the hunting ground. Due to this, the effect of spring supplemental feeding on the reproduction of pheasants is arguable and it mostly depends on the "quality" of the very habitat or, in other words, on the level of anthropogenic activity.

Wise (1994) states that the positive results of supplemental feeding of pheasant hens during the season of reproduction may be achieved only by using a well-balanced feed and not single feed (such as grain), as well as some adequate source of calcium. In addition, this other suggests a better dispersion of feeder (mostly one per a territory of a male-rooster) which would reduce the predatory and eggs and animal losses.

Table 3. A comparison of egg size between provisioned and non-provisioned Tibetan Eared Pheasants (Lu and Zheng, 2003)

Year	Provisioned				Non-provisioned			
	n	Mean	se	Range	n	Mean	se	Range
Fresh weigh, g	55	54.81	0.45	49.7– 61.2	32	53.23	0.62	7– 59.5
Length, cm	116	5.82	0.01	5.35 – 6.18	86	5.75	0.02	5.32– 6.15
Breadth, cm	116	4.17	0.01	3.84 – 4.39	86	4.15	0.01	3.81– 4.34
Egg volume index	116	101.01	0.57	83.4–115.4	86	99.14	0.73	83.5 –111.8

Conclusion

The adequate diet of different categories of pheasants in aviaries, as well as supplemental feeding in the hunting ground during winter and spring, aims at a larger number of this game species. Yet, even beside such fact, it is not performed in an adequate way, especially after settling pheasant chicks into the hunting ground. During breeding pheasants in aviaries, more attention should be paid to the biological protein value of the rations, while supplemental feeding of pheasants in the hunting ground should be performed not only with grains, but also pelleted concentrate mixtures, and not only during winter, but also after settlement, by the end of summer and even in spring. It is recommended to coordinate the amount and quality of feed for supplemental feeding with the food potential of the hunting ground, or in other words, with the level of anthropogenic activity.

Acknowledgement

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Oral presentation

DEER FARMING IN EUROPEEN UNION AND SERBIA: VETERINARY LEGISLATION PERSPECTIVE

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"Deer farming" means husbanding of deer populations for the purpose of production of deer meat and its by-products - including hides, velvet, antlers and musk, - on a commercial basis. It is a new enterprise in Europe which during recent years has been increasingly accepted as an economically promising industry. Although, until now, we have had no registered deer farm in Serbia, we should prepare for it as part of the EU accession process. Thus, it is necessary to harmonize specific rules on breeding, health status, transport and animal health with those of the European Union.

This was a reason to conduct an analysis of the existing legislation in the European Union and compare it with Serbian laws in this field. Among others, there are following EU pieces of legislation: Notes about the movement of non domestic ungulates under the Balai Directive (Council Directive 92/65/EC) in EU countries; Regulation on the hygiene of foodstuffs (EC - No 852/2004), but also EFSA (European Food Safety Authority) scientific opinion on the public health hazards to be covered by inspection of meat from farmed game – Panel on Biological Hazards. It was also shown the importance of health control of deer animals, in order to determine the presence of health and economic significant diseases like: foot and mouth disease, tuberculosis, brucellosis, bluetongue and Chronic wasting disease (CWD).

Red deer production may be a good option for some small or part-time farming operations. But, potential producers should understand that they will need to be very active in animal health programs and be aware of the special handling requirements involved with deer (Anonymous, 2016).

Keywords: hunting, veterinary, education, European Union, health control, regulation, wild ungulates

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Introduction

The groups of animal species covered by "deer farming" are farmed deer and farmed reindeer (*Rangifer tarandus*). In the context of this opinion, farmed deer refers to all species of deer that are farmed. These are mainly red deer (*Cervus elaphus*) and fallow deer (*Dama dama*), but other species, such as roe deer (*Capreolus capreolus*), sika deer (*Cervus nippon*) and wapiti deer (*Cervus canadensis*) may also be included (EFSA, 2013).

Increased awareness by a more health-conscious public of "alternative" foods has led to significant markets for formed venison being developed. With its low fat and high iron content (compared with traditional meats), venison is now available everywhere from farm shops to major supermarket chains (BDFPA, 2016). Deer may be farmed following the conventional agricultural practices, including organic, grazing is rotated, some or all stock may be housed in the winter (not all year round) (Kotrba, 2010).

Production and consumption data for farmed game in the EU are scarce. An exception could be the report of EFSA (European Food Safety Authority), which provided useful data. According to information provided during the technical hearing on meat inspection of farmed game in the EFSA report from the year 2012., approximately 280.000 deer, predominantly red deer and fallow deer, are farmed in Europe, but less than half of these are slaughtered annually (EFSA, 2013).

Developing of deer farming globally

It was nearly 40 years ago that the first commercial farms were established in these two countries and more recently in other parts of the world. In the UK there are now approximately 35.000 farmed deer, while in New Zealand this figure is closer to 1,5 million (BDFPA, 2016).

The organization of deer farming is Austria is also interesting. About thirty years ago some farmers who were seeking a new way of utilizing grassland for meat production started farming Fallow deer. In order to support these pioneers, regional associations of deer farmers were founded. Ac. Mr Jurgen Laban, President of Austrian deer farmers association, there are 838 in the Association with 15.084 hinds (incl. fallow deer). Otherwise, there are estimated number of farms in Austria: 1623 with 28.800 hinds. But, it should be emphasis that the average farm size is relatively small: 6 Hectares'. In accordance with that the average number of animals per farm is 18 hinds (FEDFA, 2016).

Import of deer into the EU

When it comes to requirements for import of live deer in European Union countries (incl. EU movements), there are strict and detailed describe in "Balai Directive" (EU regulation, 1992). To be traded under this Directive the deer must meet the following conditions: they must be identified in accordance with the requirements of Council Directive 92/65/EEC (EU regulation, 1992) and be registered in such a way that the holding of origin can be traced; they must not be intended for slaughter due to a disease outbreak in the country of origin; they must not have been vaccinated against foot and mouth disease; they must not come into contact with ungulates other than those of a similar health status; they must come

from a holding that is not subject to any restrictions for animal health reasons; they must have been kept on the holding of origin since birth, or for at least 30 days, before dispatch;

Health certificate

The consignment should be accompanied by the certificate signed by an official veterinary surgeon of the country of origin. For all ruminants, they must come from an officially tuberculosis-free/officially brucellosis-free herd/holding or from a holding where it/they was/were subjected with negative results to the tests laid down in Council Directive 92/65/EEC (EU regulation, 1992). For bluetongue susceptible ruminants which do not originate in a country/region officially free of bluetongue, they must meet another special requirement.

Post import controls of deer animals

Blood samples may be required to be taken within a few days of the animals being imported as part of post import checks. Animals which fail any post import checks and tests may be required to be slaughtered without compensation to the importer or re-exported at the owner's expense. The animals should be isolated for 30 days (EU regulation, 1992).

Inspection of meat from farmed game

The slaughter process for deer and reindeer is similar to that for conventional livestock, such as cattle and sheep, but there can be significant differences. These arise principally when the animals are slaughtered, i.e. stunned, killed and bled, on-farm, and whether the slaughter procedure is dry or wet (EFSA, 2013).

On the EFSA Panel on Biological Hazards, there were made certain conclusions, which have practical importance. On the first place, it should be continue with inspection of meat from farmed game because of potential public health hazards. Based on EFSA Scientific Opinion, the highest priority for meat inspection in farmed deer has *Toxoplasma gondii*. It was found that *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* has not big importance as infectious agents in farmed deer. It is well known, that at the present time tuberculosis belong to the list of reemerging infectious diseases. In this content, it exist the certain risk from the farmed deer, as tuberculosis reservoirs. For this reason it is not recommended to reduce the existing plan of carcass investigation regarding post-mortem inspection procedures on tuberculosis. Because, in that way it can be endanger human health in contact with infected meat. It was also concluded, that slaughterhouse surveillance for the detection of tuberculosis in farmed deer has much more importance than when we use only the clinical examination. On the other hand, the ante-mortem inspection of farmed game animals should not be left out, because it allows the traceability in animal identification, and helps in the detection of observable abnormalities.
(EFSA, 2013).

Current legislation in Austria

For the keeping of farmed game are prescribed the minimum requirements (such as minimum size of the gate, stocking density, design of the fence, feed and water supply, documentation of input and outflow of animals, documentation of examination results and

drug use) and those who are subject to the regulations of animal welfare and under pharmaceutical law (Bogner, 1999; Deutz, 2005). Since 01.01.2005. the minimum requirements for the keeping of red deer, sika deer, fallow deer, Pere David's deer (*Elaphurus davidianus*) are established in "The first Animal keeping ordinance 2004" in Austria (Austrian regulation, 2004a; Austrian regulation, 2004b). It is shown in Table 1.

Table 1. Minimum requirement for the keeping of deer at the farms

Species	Minimum pen size	Maximum stocking density	Minimum area of weather protection
Red deer Pere David's deer	2.0 ha	10 adult animals[1]/ ha	4.0 m²/ one adult animals[1]
Fallow deer, sika deer	1.0 ha	20 adult animals[1]/ ha	2.0 m²/ one adult animals[1]

[1] Two animals up to 18 months old correspond to one adult animal
[2] Three animals up to 12 months old correspond to one adult animal
[3] Newbies up to 6 months old are not to be considered in the stocking density; Animals from 6 to 12 months old are equivalent to one adult animal

Basic requirements of deer park

Keeping of deer must be in their paddocks. A breeding group must consist of at least the one male breeding animal (stag) and three breeding hinds. Deer pen arrangement: if the pen surface not covered at least 5% with shrubs or trees, or shielded, an additional weather protection must be available. This weather protection must be at least two side walls with a roof, and provide all animals simultaneously shelter. Facilities for stock feeding (i.e. hay racks) must be also roofed. About input and outflow of animals, treatments, findings, deaths and other incidents records shall be maintained in a pen book (Austrian regulation, 2004b).
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Animal welfare requirements

Wild animals which – possibly with respect to climate, nourishment, need for free movement or social behavior – pose particular requirements for their keeping, may only be kept in compliance with the prerequisites imposed on the basis of a report on the keeping of a wild animal to be made to the authority within two weeks. Such report shall contain the name and address of the keeper, the number and maximum number of animals kept, the place where kept and further information enabling the authority to judge the matter. The fence of the pen must be such that the animals can not injure, also skipping or breaking through the fence could be not possible. The fence guide may not have corners with acute angles or form the funnel (Austrian regulation, 2004a). The fence and the gates must be checked daily (fallen on the fence trees, fence willful damage, etc.).
Alone keeping animals of one sex: under conditions of prolonged alone keeping of animals of one sex in the deer pen, it will be disturbed the function cycles of reproduction but also social and sexual behavior.

As notifiable animal diseases at Deer Farm in Austria could occur Foot-and-mouth disease, Tuberculosis and less likely Chronic Wasting Disease (CWD). On suspicion of a notifiable disease, animal holder must report the official veterinarian about this (Deutz, 2005).

Overview of legislation in Serbia relating to wildlife management

In the animal husbandry Law definition (Austrian regulation , 2016b) farm of wildlife is an enclosed or indoor facility in which there is bred by special technology one wildlife species in order to trade the products of animal origin and further reproduction". Breeder who deals with farm breeding game must meet the requirements in terms of facilities for breeding animals, the proper equipment for the breeding of certain types of wildlife and skilled staff. Breeder may enter the wild animals on the wildlife farm if he has a certificate about its state of health, in accordance with regulations governing veterinary Directorate. Breeder also must keep records on the production of individual production stages. The Minister of Agriculture shall prescribe the requirements in terms of facilities, appropriate equipment and skilled personnel to be met provided the farm or breeder.

The present program of measures for health protection for animals (Austrian regulation, 2016a) states the following: in order to monitor and control health status and determine the presence of infectious and parasitic diseases in wildlife in the intensive, or farm breeding system are carried out diagnostic tests, or immunoprophylactic measures depending on the species and the epidemiological situation, according to the program of the Ministry of agriculture, on a proposal the competent veterinary institute. It can be perform diagnostic tests of wild animals to bluetongue, foot-and-mouth disease, brucellosis, tuberculosis, Q-fever, tularemia, echinococcosis and fascioloidiasis according to the plan of the Ministry. The diagnostic test under the program of relevant scientific or specialist institute shall be make in intensive breeding of wild animals and wildlife breeding centers/farms. Funding for diagnostic tests referred in the aforementioned subsection are provided in the budget of the Republic of Serbia. Anyway it is noted, that for all infectious diseases which are not explicitly mentioned, it will apply a risk assessment on a case-by-case basis (Urosevic and Ristic, 2014).

As can be seen from the above legislation in European Union (in the case of Austria) that deer farming is regulated in detail. This refers to the aspect of health, ways of keeping (area, buildings, equipment) and the protection and welfare of animals. Until now, the precise regulations like this does not exist in our country. Because of that, we can only recommend that above mentioned list of requirements' could be a good starting point for drawing up our own legislation. Naturally, we cannot just rewrite certain provisions, but we should adapt it to the conditions of the specific situation in Serbian agriculture. Bearing especially in mind that there are no deer farms currently in our country, as opposed to the situation in the EU.

It should be make also the expert analysis and different feasibility studies for the future deer farming in Serbia. After that it is necessary to make a specific strategy for developing of these types of agribusiness. An example of this can be a United kingdom. As markets have developed, so have the skills necessary to farm deer, assisted by the work on a number of government funded research farms in United Kingdom (BDFPA, 2016). Following the example from Austria, after the start of the first deer farm, it should be establish the regional associations of deer farmers. In relation the size of the farm (area in hectares) as well as a number of heads, most similar to us may be the experience from Austria.

On the other hand, it could be a good opportunity for applying of our state institutions (i.e. scientific), farmers, breeder associations and others for EU-IPARD funds, in order to adapt

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our rules but also start-up of this business. Because, deer farming belongs to EU - accession negotiations for Serbia's membership in the EU, in the chapters of veterinary regulations as well as rural development.

Conclusion

Deer, which have grown up to expect winter housing, regular handling and rotational grazing, behave no differently than cattle and sheep, sometimes better, and as a result, with appropriate care and facilities, can be transported and even slaughtered with minimum stress. The low labor regime for deer farming means that it can easily compliment other livestock and arable enterprises (BDFPA, 2016). Dairy farms, with existing buildings, are ideally suited for conversion to deer, also in Serbia. When it comes to challenges in the future, it would be on the first place the building up specific marketing structures for bigger deer farms. It should also take into account, that nutritional and mineral supplements should be provide when necessary to maintain the deer's health, but injuries and health problems must receive prompt veterinary attention.

According the experiences in European Union countries with developed deer farming (i.e. Austria, Germany, United Kingdom), we can make some presumptions in terms of market trends in the future. Because of bigger present demand of game meat on the markets in whole Europe, deer farming production will increase in this part of world. Not only according venison, red deer farming will be more interesting because of big requests for trophy, on the first place from Chinese companies (People Republic of China). Also, the private owners - farmers from Russia show interests for import of breeding hinds and stags from European Union, on the first place from Germany, Poland, Latvia, Hungary etc. It is question of time, when will be establish first deer farm in Serbia, although we haven't all regulations about this, but this paper had the goal the help this process. Especially from the veterinary topic, but we need future publications about the other area of deer farming: breeding, housing, feeding, reproduction of animals and slaughter house incl. game meat inspection regulations.

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Oral presentation

INFLUENCE OF BODY WEIGHT ON PRODUCTIVITY OF QEEN BEES (*APIS MELLIFERA CARNICA*)

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Abstract

In order to achieve a more profitable production, it is important to raise productivity of breeding kind and work on upgrades of production. In breeding of honey bee (*Apis mellifera carnica*), we pay great attention to productivity on which genetic and environmental factors have large influence. Among the genetic factors that have influence on productivity is body weight, which is, according to many authors, in positive correlation with productivity. So far, several authors have proved the positive correlation between body weight and the numbers of ovarioles, diameter of spermatheca, number of spermatozoa in spermatheca, brood production, early start of egg-laying and age of larvae used for grafting. Every listed factor influences the quality of queen bee which, in the end, affects productivity and strength of the honey bee colony.

Keywords: productivity, *Apis mellifera carnica*, queen bee, body weight, quality

Introduction

In the honey bee colony, queen is the only one that is capable of reproduction. Production of the entire colony depends on reproductive capability of the queen bee, which is closely linked to her reproductive system. Body weight of the queen bee, along with feeding, age of the queen, number of ovarioles and size of spermatheca, is one of the factors that is proven to have a positive correlation on the productivity of the colony. The intention of this review article is to show influence of various factors on the queen bee (*Apis mellifera carnica,* Pollmann 1879) and, consequently, on the productivity of the whole colony.

Anatomy of reproductive organs and mating of queen bee

Body of the queen bee consists of a head, thorax and abdomen. Reproductive organs are placed in abdomen. In colony three castes of bees are present: drones which are reproductively capable males, facultative sterile workers and fertile queen. Both queens and workers have: ovaries, ovarioles, oviduct, vagina and seminal duct, while queen yet have spermatheca and appendicular glands, which allows her to be reproductively capable. In worker bees, oviduct was atrophied in process of evolution, seminal ducts retained just physical traces and vagina isn't sufficiently developed, therefor copulation with drones is not possible (Grout et al. 1992). Reproductive organs of the queen bee are developed enough and are capable for reproduction. Ovaries products eggs and continue to ovarioles which are connected to oviduct, and oviduct continues to vagina. After mating, spermatozoa that is now in oviduct, go to spermatheca in the next 24 hours (Woyke, 1983). Spermatheca is oval shaped organ which is placed dorsally to vagina in abdomen of queen bee. Spermatheca is wrapped with tracheal net that gives necessary conditions for survival

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of spermatozoa. Appendicular glands are attached to spermatheca and excrete secret which serves as a food for spermatozoa (Grout et al. 1992).
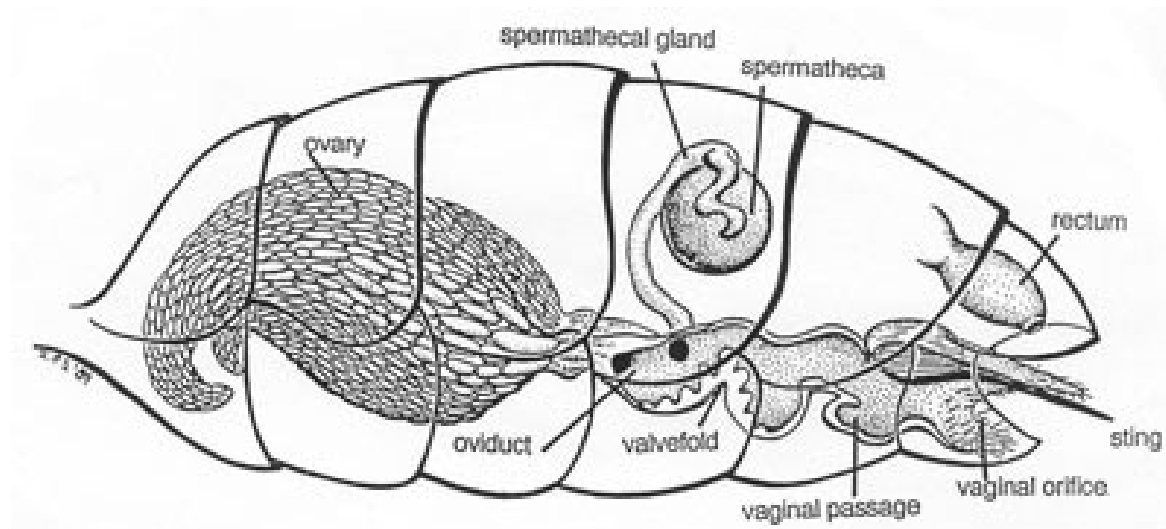
Picture 1. Reproductive system of queen bee

(source: http://www.glogster.com/whmshub/honey-bee-anatomy-by-anthony3/g-6jubs21f7v7gcp6auli51af)

Mating between the queen and drones occurs during one or more nuptial flights. Virgin queen visits so called "drone congregation areas", and mate with several drones. Recorded number of drones with which queen mates is form few to even 50 by some authors. Length of nuptial flight takes 10 to 30 minutes, and weather should be fine and non-windy (Delaney et al. 2011).

Development of the bees and feeding queen bee with royal jelly

Members of the honey bee colony are the queen, workers and drones. Colony can count up to 40 000 workers, few hundreds to few thousands of drones and one queen, which is leader of the colony (Page and Peng, 2001). Life length of workers is 3 to 6 weeks in spring and summer, but in winter they can live up to 8 months, while queen bee can live 2 to 3 years. Page and Peng (2001) report that queen bee that live in colonies for commercial use usually live in average for a year. They even report that apart from differences in organic systems and in life length, between queens and workers exist evidential difference in body size. Queens are larger than workers and they pass complete metamorphosis from egg to adult in 16 days, and workers pass it in 21 days. That divergence comes from difference in feeding from beginning of development.

The main food for the queen bee is royal jelly. Nurturer bees feed worker brood and queen with royal jelly for first three days of living. After those three days they continue to feed just queen with royal jelly while they interdict worker brood from royal jelly, and feed them with combination of honey and pollen. Authors then conclude that just three days of different feeding between queen and workers can make a lot of diversity in development and longevity (Page and Peng, 2001). Worker bees excrete royal jelly with hypopharyngeal glands. Queen bee is by first few days of life fed with honey, which enables required energy for orientation and nuptial flights. From the moment when the queen begins to lay eggs, workers feed her exclusively with royal jelly which allows her to lay up to 2000 eggs   per

day, in the peak of the season, which is equivalent to her body weight. Royal jelly is thick, homogeny matter, pale yellow color, with characteristic smell. Density is 1,1 g/cm3, and viscosity change depending on amount of water (Lercker et al. 1984). Composition is complex. In speaking of content, water have the largest level which is 57 to 70%, followed by proteins with 10 to 18%, sugars with 9 to 15 %, lipids with 3,8% and minerals with 0,7 to 1,5%. It's still not identified the whole content of royal jelly (around 3%) (Kapš, 2013). Royal jelly in her content contains every essential amino acid which have high importance for organism because they cannot be synthesized in organism, but can be taken with food, which makes Royal Jelly even more important in feeding. Enzymes that are found in royal jelly in largest amounts are: phosphatase, choline- esterase, glucose- amino oxidase and insulin- like substances. Identified vitamins in royal jelly are: B1 or Thiamin, B2 or Riboflavin, B3 or pantothenic acid, B6 or pyridoxine, PP or Niacin, H or Biotin and B9 or Folate (Lercker et al. 1984).

Quality of the queen bee and correlation between body weight and certain characteristics of queen bees

Earlier told thesis of Page and Peng (2001) how queens in hives for commercial use can live up to 1 year, was confirmed by Büchler et al. (2013) which say that for high commercial use there is need to have a production of big number of high quality queens. Hatjina el al. (2014) claims that term of quality refers to quantitative physical characteristics and characteristics of behavior and that high quality queens have: high body weight, huge number of ovarioles, large diameter of spermatheca, large number of spermatozoa in spermatheca and that they are healthy. Quality of the queen bee can be seen indirectly from characteristics of colony. In breeding work, among others, the most important evaluated traits of honey bee colonies are: fast spring development, low swarming tendency, hygienic behavior, disease resistance, honey production, low defensive behavior (aggressiveness) and calmness. Main terms that need to be fulfill for breeding a good queen are, according to Akyol et al. (2008): genotype, feeding, breeding methods, season, age of larvae used for grafting and brood production.

Table 1. Correlation between characteristics for quality of queen and other factors that effect on them (Hatjina et al., 2014).

Characteristics	Positive correlation	No correlation
Body weight – number of ovarioles	Weaver, 1957; Avetisyan, 1961; Woyke, 1971; Szabo, 1973; Wen-Cheng and Chong-Yuan, 1985; Gilley and sur., 2003	Corbella and Concalves (1982); Hatch et al., 1999; Jackson et al., 2011
Body weight – diameter of spermatheca	Akyol i sur., 2008; Kahya i sur.,2008; Bieńkowska et al., 2009; Kovačić et al, 2016	
Weight of the grafted larvae – number of ovarioles	Hoopingarner and Farrar, 1959; Woyke, 1971	
Number of ovarioles – size of spermatheca	Weaver, 1957; Woyke, 1971	Jackson et al., 2011
Number of ovarioles – brood production	Avetisyan, 1961	
Body weight – brood production	Makarov, 1969; Akyol et al., 2008	
body weight – early start of egg-laying	Taranov,1974;Siuda and Wilde, 2006	Skowronek et al., 2002
Age of larvae used for grafting – number of ovarioles	Jordan, 1960; Woyke, 1960, 1964, 1971; Szabo and Townseed, 1974	
Age of larvae used for grafting – size of spermatheca	Jordan, 1960; Woyke, 1960, 1964, 1971; Szabo and Townseed, 1974; Gilley et al., 2003; Tarpy et al., 2000	
body weight – age of larvae used for grafting	Tarpy et al., 2000	
Size of spermatheca – number of spermatozoa	Woyke, 1966; Bieńkowska et al., 2008	

In the following table, Hatjina el al. (2014) report body weight of queen bees as most commonly used characteristic for searching correlation with other characteristics. It's important to say if there not exist direct correlation between body weight of the queen bee and some other characteristic, there surely exist correlation between the body weight and some other characteristic that is correlated with the characteristic that we are searching for. As for example weight of the grafted larvae is correlated with number of ovarioles and for number of ovarioles is confirmed positive correlation with queen body weight.   Higher

number of ovarioles would allow queen to lay more eggs, where we again come to positive correlation between body weight and brood production.

Table 2. Characteristics of different groups of the queens body weight (Hatjina et al. 2014)

Group of queens by body weight	N	%	Body weight at emergence (mg)		Body weight at insemination (mg)		Size of spermatheca (mm3)	Number of spermato zoa (x 1000)
			mean	range	mean	range	mean	mean
Light	57	20,1	137,7	125-185	154	119-183	0,75	3.556
Medium	137	48,4	197,4	186-210	171,8	115-212	0,85	4.069
Heavy	89	31,4	223,9	211-250	183,1	131-218	0,92	4.210
Total	283	100	201	125-250	171,8	115-218	0,85	4.007

In table 2. Hatjina et al. (2014) divided queens in three groups according to body weight: light, medium and heavy. They researched the correlation between body weights by groups and weight at the emergence, body weight by the time of insemination, size of spermatheca and number of spermatozoa. It has been found that body weight at the emergence was bigger than body weight at insemination. Positive correlation was found between body weight at emergence, size of spermatheca, and number of spermatozoa.

As same as in the research described above, Akyol et al. (2008) separated queens in three groups by their body weight: heavy (207.63 ± 0.95 mg), medium (193.47 ± 0.96 mg) and light (175.00 ± 0.62 mg) they were observation characteristics of acceptance rates of new queens in queenless colonies, percentage of successfully mated queens, area for brood 30 days after start of oviposition, diameter of spermatheca and number of spermatozoa in spermatheca. Positive correlation was found between body weights of queens and diameter of spermatheca (r = 0.98), number of spermatozoa (r = 0,97) and area for brood (r= 0,90).

In breeding of honey bee queens, one of the most important factor for queen morphology is larvae from which is queen developed. Queens that had been obtained from newly hatched larvae have higher body weight and larger spermatheca (Gilley et al., 2003.) Woyke et al. (1971) says that breeding queens from younger larvae will gave higher body weight, number of ovarioles and number of spermatozoa. They also report that queens with higher body weight have more ovarioles, larger spermatheca and more spermatozoa so they can lay more eggs and can stay longer in colony.

Conclusion

Queen is the only reproductively capable female in colony, and the strength and productivity of colony depends on her. Body weight of queen bee is very important characteristic for which is determent correlation with many other characteristics that influence on reproductive capability and productivity for production of bee products. So

far conducted researches by many authors proves positive correlation between body weight of the queen bee and number of ovarioles, diameter of spermatheca, number of spermatozoa in spermatheca, brood production, early egg- laying capability and age of larvae used for grafting.

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Poster presentation

ECONOMIC IMPACT OF MANAGEMENT OF GAME POPULATIONS IN DIFFERENT TYPES OF HUNTING GROUNDS

Popović Z. [1]; Živković D.[1], Beuković M.[2]; Beuković D.[2]

Abstract

This analysis provides insight into financial aspect of management of *Barajevska reka* (small hilly type of hunting ground) and *Srem-Jug* (lowland type of hunting ground) hunting grounds in order to comprehend growing costs and harvest value of the most abundant game species present in the hunting grounds. The planned harvest value is EUR 22,484 and EUR 12,519 or 19.90 % and. 17.10 % of the total value of the breeding stock. Harvested big game (roe deer and wild boar) accounts for 71.12% of the planned harvest in *Barajevska reka* hunting ground while small game accounts for 28.88% of the planned harvest value. In *Srem-Jug* hunting ground planned harvest of big game accounts for 24.24% while small game accounts for 75.76%. Management result values in 2015 show that thus evaluated game species reports operating loss with cost-effectiveness ratio of 0.52 and 0.34, production profitability rate of -48.01 and -65.90 and loss of EUR 76.34 and EUR -69.38 per 100 hectares of hunting area. This clearly indicates that funding of hunting ground management requires provision of funds from other sources, which in this case means from membership of hunters. Indirect costs of management for hunting ground users account for 54.96% and 59.47%, while direct costs of game growing and maintaining hunting grounds account for 45.04% and 40.53% of total management costs.

Profit in the production may be realized through the implementation of game harvest plan whereby in the first hunting ground big game would be priced according to the price list for hunters-tourists whereas in the second hunting ground, besides big game, a share of small game (hare) would be realized as well while the remaining share of the game would be priced in accordance with the price list approved by the competent ministry. Improvement in economic performance of game population management can be achieved by improving the game management through an increase in the share of roe deer and wild boar, reduction of losses and a real growth in some game species within the acceptable biological limits.

Keywords: game population, hunting ground, value of production, expenditures

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Introduction

Game management implies multidisciplinary approach which involves both biological and social and economic elements (Tomić et al., 2005). On one hand they are reflected through the attributes of ecological balance of certain regions and on the other hand, through economic performance of economic entities engaged in management of hunting grounds. Economics prescribes that wildlife should be harvested as long as the marginal social benefits of so doing exceed the marginal social costs. In addition, (marginal) social costs include (i) the loss in situ (existence, viewing) value that wildlife provide citizens who may be located in countries other than the source country, (ii) the opportunity cost from harvesting the wildlife today rather than waiting for a more opportune time in the future when the specimen(s) may fetch a higher price, (iii) the lost future value of offspring that might result from leaving the specimen(s) in place, and (iv) the opportunity cost of the resources employed in the harvest activity (Bulte et al. 2003).

Microeconomic analysis of management of hunting game species in one hunting ground represents focusing on those populations which are used in hunting ground and which are economically significant for the user of the hunting ground. For that purpose this paper encompasses the economic analysis of the management of *Barajevska reka* hunting ground (hunting ground-1) managed by *Mića Popović* hunting association from Barajevo and *Srem-Jug* hunting ground (hunting ground- 2) managed by *Sremac* hunting association from Ruma.

To achieve optimal use of resources typically involves regulation of users. Regulation is necessary to internalize spillover effects and, when use or property rights are not secure, to account for the inter-temporal user cost associated with harvesting (Bulte et al 2003) . Same authors argue that defining property rights in physical or legal space is an important first step towards optimal resource management. Economic effects of game management in certain hunting ground depends on a number of factors beginning from habitat conditions, breeding and exploitation parameters of management of populations of certain game species, selling price of game as well as direct and indirect costs in hunting ground management. The presence of certain game in hunting grounds and its spatial distribution are primarily influenced by natural factors but the influence of anthropogenic factors is also quite pronounced, especially for some species *(*Popović et al. 2012a; Popović 2008; Popović 2006; Gajić et al.,. 1997; Popović, Bogdanović 2001).

The impact of direct and indirect costs in hunting grounds management is endowed in applicable hunting legislation. The development of hunting in Serbia is influenced by the transformation of social structure in accordance with political processes of joining the European Union, as well as international conventions (Adamič et al. 2006). The revenue is primarily influenced by price list (for tourist, members of hunting associations, price list prescribed by the Minister for the payment of fees for the exploitation of game) according to which the game animals are sold, as well as gender and trophy structure of harvested big game species. The economic results of game management depend, on one hand, on characteristics of hunting ground (size, characteristics of natural hunting grounds, game species present in the hunting ground, etc.), and on the other, on the management of economic entity which is managing operations. This analysis provides insight into the financial part of management of different types of hunting grounds (lowland and small hilly

and hilly) in order to comprehend economic effects of breeding and shooting the most abundant game species in these hunting grounds.

The aim of this paper is to carry out a microeconomic analysis of the management of small game (rabbit, quail, etc.) and large game (roe deer, wild boar) populations in different types of hunting grounds. The analysis indicated the indirect costs of management which hunting ground users are subject to in accordance with applicable legislation in Serbia as well as possibility of improving the economic effects of using different game species.

Material and Methods

The research covered game hunting bag record in *Barajevska reka* and *Srem-Jug* in the business year 2015/16 as well as expenditures related to the hunting ground management. Complete business activities of the users of this hunting grounds (*Mića Popović* hunting association Barajevo and *Sremac* hunting ground Ruma) during this hunting year. *Barajevska reka* is classified into a small hill and hilly type of hunting ground with the height above sea altitude of 100 to 408 meters, 20% forests and forest land in total hunting ground surface area. It stretches over the areas of forests, land and water of Barajevo Municipality, Belgrade County, the Republic of Serbia on a total area of 21312 ha. *Srem-Jug* hunting ground is classified as lowland type of hunting ground with height above sea altitude of 62 to 70 meters, 7.5% forests and forest lands in total hunting ground surface. It stretches over the areas of forests, land and waters of the part of the territory of Ruma Municipality, Sremski County on a total area of 21153 ha.

Game abundance and realized harvest are taken from the annual management plan of the hunting grounds and the records kept by the hunting ground users on the basis of the Law on Game and Hunting (2010). The value of the breeding stock and the value of the planned harvest in 2015/16 were determined according to the *Decision on determining the amount of fees for the exploitation of game species protected by closed season and the amount of fees for hunting ticket for the hunting year 2014/2015* (http://www.mpzzs.gov.rs/) which remained in force for 2015/2016. In the income of the hunting ground users the value realized from the sale of game in 2015/16 business year is presented. Management costs were obtained by summing up direct costs of growing the game and the part of general costs which belong to the hunting ground user according to the applicable legal regulations relevant for the hunting ground users.

As instruments of economic analysis the parameters of economic efficiency and efficacy are used including the value of production (harvest), profit, cost-effectiveness ratio, production profitability rate. Besides, the profit per 100 ha of total hunting ground surface was also analyzed. All calculations of values and costs are expressed in euros.

Results and Discussion

Total value of the stock of hunting game species in 2015/2016 business year in hunting ground-1 is EUR 112973 and in hunting ground-2 EUR 73218 (Table 1). This value does not include pheasant since it is not provided in the given price list as it is a species that is without limitation reintroduced to the hunting areas from pheasant farms. The planned harvest value in the hunting ground-1 is EUR 22484 or 19.90 % of the total value of the breeding stock while in hunting ground-2 EUR 12519 or 17.10 % of the total value of

breeding stock. Harvested big game (roe deer and wild boar) in hunting ground-1 accounts for 71.12% while small game accounts for 28.28% of the planned harvest. In this hunting ground planned roe deer harvest accounts for 67.19% of the harvest value. In hunting ground-2 planned big game harvest accounts for 24.24% while small game accounts for 75.76%, with the share of the value of hare shooting being 52.00% of the total harvest.

In overall harvest realization (Table 2) in hunting ground-1 big game accounts for 81.40% of the total harvest while small game accounts for 18.60%. In hunting ground-2 small game accounts for 78.83% of the total harvest while big game accounts for 21.17% of the total harvest.

The realization of the harvest plan value in hunting ground-1 was 78.66% where realization of big game was 90.03%, while small game was realized by 50.65%. In hunting ground-2 realization of the harvest plan was 60.66%, where big game was realized 52.98% and small game 63.12%. In *Barajevska reka* hunting ground a total value of breeding stock of roe deer's of 700 animals in 2004/05 was EUR 60,552 (*Tomić et al.* 2007), while nowadays according to this pricelist it is EUR 51,389.

Analysis of the expenditures of the users of hunting ground-1 (Table 2) shows that the direct costs of production in hunting ground-1 account for 45,04%, while in hunting ground-2 they account for 40.53%. Indirect costs of production in hunting ground-1 account for 54.96% of the total costs while in hunting ground-2 they account for 59.47%. Building and maintenance of hunting facilities, planting fields for game and feedstuffs for game in hunting ground-1 account for 29.66% of total costs and in the hunting ground-2 they account for 32.19%. Indirect costs that burden the hunting ground user of hunting ground-1 with 39.37% are costs of two employees performing the job of a gamekeeper and a professional hunting ground manager and in hunting ground-2 these costs are 47.42%. The employees are engaged according to the Law on Game and Hunting (2010) while fees for game and maintenance cost of office space account for 14.51%.

Table 1. Game stock abundance in the hunting area, planned harvest and their value in 2015/16

No.	Game species – Breeding stock		Barajevska reka hunting ground				Srem-jug hunting ground			
			Breeding stock - heads	Stock values (EUR)	Planned harvest - heads	Value of planned harvest (EUR)	Breeding stock - heads	Stock values (EUR)	Planned harvest - heads	Value of planned harvest (EUR)
1.	Roe deer	roebuck	269	34622	83	10683	77	9910	9	1158
2.	-	doe	271	11619	84	3601	96	4116	11	472
3.	*Capreolus*	fawn	200	5148	32	824	42	1081	2	51
4.	*capreolus* L.	total	740	51389	199	15108	215	15107	22	1681
5.	Wild boar-	Wild boar	3	450	2	300	7	1050	4	600
6.	*Sus*	sow	7	300	7	300	11	472	5	214
7.	*scrofa* L	gilt	10	257	11	283	13	335	21	540
8.		total	20	1007	20	883	31	1857	30	1354
9.	Hare- *Lepus europaeus* Pall.		1860	31868	250	4283	2062	35329	380	6511
10.	Mallard- *Anas platyrhynchos* L.		400	3443	40	344	500	4304	140	1205
11.	Teal -*Anas crecca* L		150	645	15	65	350	1506	25	108
12.	Other ducks - *Anas*		230	986		-	80	342	-	-
13.	Wild geese -*Anser*		100	858		-	75	644	3	26
14.	Woodcock - *Scolopax rusticola* L		500	4304	40	344	100	861	-	-
15.	Wood pigeon - *Columba palumbus* L.		1500	3898	100	260	300	780	20	52
16.	Japanese quail - *Coturnix coturnix* L		5000	8526	450	767	6000	10231	600	1023
17.	Turtle dove - *Streptopelia turtur* L		2500	4263	200	341	800	1364	300	512
18.	Collared dove - *Streptopelia decaocto* Friv.		1500	1786	100	89	1000	893	50	47
19.	**Total big game (1-6)**		-	52396	-	15991	-	16964	-	3035
20.	**Total small game (7-14)**		-	60577	-	6493	-	56254	-	9484
21.	**Total (1-14)**		-	112973	-	22484	-	73218	-	12519

Analysis of the values of management results in 2015 shows that hunting ground-1 business operation recorded losses and cost-effectiveness ratio of 0.52, and production profitability rate of -48.01, and loss of EUR 76.34 per 100 ha of total hunting ground. In hunting ground-2 business operation also recorded losses and cost-effectiveness ratio of 0.34, and production profitability rate of -65.90 and loss of EUR 69.38 per 100 ha total hunting area. This clearly indicates that funding of hunting ground management requires provision of funds from other sources, which in this case, that is, hunting ground-1 and hunting ground-2 means from membership of hunters.

By analyzing the calculated values, Feureisel (2012) found that the transport costs and accrued time spent working in the context of fulfilling the statutory duties of the district with the highest percentage of users is represented. The most important position represents the labor input in meeting the statutory duty of the wild-feeding (20.5%), followed by game

count and hunting (12.6%), regulation of wild stocks - individual hunting deer, common hunting of black-and individual and small game hunting predators (10.8%). Other significant amounts are the salaries of Gamekeeper and hunting hosts to ensure that the legal duty of protecting wildlife and the annual hunting industry (22.7%). The cost of feed for shell and small game together amounts to 5.9% of total costs.

According to the research of Popović et al. (2014) economic performance can be increased by management evaluation where the game shooting would be charged according to the price list of Serbian hunting association Hunting association of Serbia regulation (2015) and according to the price list for tourist hunters in which case profit would be made with the cost-effectiveness ratio of 1.54. However, in the current market conditions and poor financial situation of hunters that is impossible to achieve. The same authors suggest that in *Barajevska reka* hunting ground fulfilling the projected plan for roe deer and wild boar harvest and the fulfilment of the plan of realization of small game would magnify the income. Such realization of management would make profit with the cost-effectiveness ratio of 1.03, the production profitability rate of 2.50 and profit of EUR 5 per 100 ha of hunting area.

Table 2. Economic results of game management in the hunting year 2015/16 in *Barajevska reka* and *Srem-Jug* hunting grounds

	Hunting ground			
	„Barajevska reka"		„Srem-Jug"	
Elements	Amount *Val.*	%	Amount *Value*	%
A) *Value of production (euros)*	17685	**100.00**	**7594**	**100.00**
Big game	14396	81.40	1608	21.17
Small game	3289	18.60	5986	78.83
B) *Costs of production (Euros)*	**34019**	**100.00**	22271	**100.00**
Direct costs of production: Hunting facilities, animal feed	10089	29.66	7170	32.19
Maintenance of field vehicles and trailers	2617	7.69	1858	8.34
Insurance damage caused by wildlife	1817	5.34	-	
Processing trophies	800	2.35	-	
Indirect costs of production: Salaries for 2 workers	13392	39.37	10560	47.42
Bookkeeping services	-		383	1.72
Maintenance costs of office space	2689	7.90	1048	4.71
Compensation for wild game	2249	6.61	1252	5.62
Charges, taxes	366	1.08		
A-B *Profit (Euros)*	**-16334**		**-14677**	
I *Ratio of economics*	0,52		0,34	-
II *Ratio income production* (%)	-48.01	-	-65.90	-
III *Profit per 100 ha (Euros)*	-76.34	-	-69.38	-

From the results obtained, it may be inferred that the abundance and density of the analyzed game species per hunting districts are not uniform, and also that the extent of shot individuals is not harmonized with its number (Popović, 2006; Popović et al., 2008; Beuković et al., 2009; Popović et al., 2012). This is one of the main factors that causes the unsatisfied game abundance in Serbia. The most abundant game species analyzed in this paper is brown hare, whose number is decreasing in last years (Popović et al., 2012a) and it is below its number in the surrounding countries, such as Hungary or Austria (Popović et al., 2012a; Hackländer, 2012).

In order to improve the economic effects of major hunting game species Tomić et al. (2007), concluded that the activities must be directed to those factors which directly or indirectly influence the management results. First of all, losses should be reduced during game reproductive season and the exploitation rate of certain game populations should be adjusted taking into account specificities of different regions. Good planning is crucial to obtain higher benefits, because WHB is a 'no net loss' program that links area restored to wildlife habitat area removed by conversion of habitat to agriculture (Hardie et al. 2004).

Significant enhancement of the economic performance of game population management can be achieved also by improving the game management, that is, by moving relevant parameters (growth rate, losses, trophy quality) within acceptable biological limits. This is indicated by the research in roe deer breeding stock in Serbia during the hunting financial year 2003/4 (Tomić and Popović, 2005).

In order to increase the financial basis of hunting associations the hunting tourism must be increased. Hunting tourism, as a very specific branch of tourism, is a very important source of income in hunting (Beuković et al. 2004).

Provision of revenues of the association cannot be expected from the increase in number of hunters or increase in membership fee from which it is financed or from hunting ground management. On the basis of the research Popović et al. (2012b) the number of hunters in Serbia is currently in decline and the reasons for reducing the number of hunters in Serbia are reflected in difficult financial situation, pronounced rural-urban migrations, old age hunters, poor interest of young people in hunting, decreased abundance of the game and pressure of the anti-hunting organization on the hunting sector.

Conclusion

Based on the financial analysis of hunting ground management in financial year 2015, in order to comprehend the economic effects of growing and harvesting of the most abundant game species in the hunting ground the following has been concluded:

The value of the planned harvest in hunting ground-1 was EUR 22484 or 19.90 % of the total value of the breeding stock while the realization of the value of the planned harvest was 78.66%. Big game (roe deer and wild boar) harvest accounts for 71.12%, while small game accounts for 28.28% of the planned harvest. In this hunting ground planned roe deer harvest accounts for 67.19% of the harvest value.

The value of the planned harvest in hunting ground-2 was EUR 12519 or 17.10 % of the total value of the breeding stock while the realization of the value of the planned harvest

was 60.66%, big game realization being 52.98% and small game realization being 63.12%. Big game harvest accounts for 24.24% of the planned harvest while small game accounts for 75.76%, in which the value of hare harvest accounts for 52.00% of the total harvest value. Small game accounts for 78.83% of total harvest while big game accounts for 21.17% harvest value out of the total planned harvest value.

Hunting grounds operated with business losses with cost-effectiveness ratio of 0.52, production profitability rate of -48.01, and loss of EUR 76.34 per 100 ha of total hunting area in the hunting ground-1. In hunting ground-2 cost-effectiveness ratio was 0.34, production profitability rate -65.90 and loss EUR 69.38 per 100 ha of total hunting area.

These results indicate that funding of this production is provided from other sources of revenues which in this case means from membership of hunters of hunting ground-1 and hunting ground-2.

Direct costs of management in hunting ground-1 account for 45,04%, while in hunting ground-2 they account for 40.53% of total management costs. Indirect costs of management in hunting ground-1 account for 54.96% of total management costs and in hunting ground-2 they account for 59.47%.

The highest share in direct financial costs goes to the building and maintenance of hunting facilities, then planting fields for game and feedstuffs for game and it accounts for 29.66% in hunting ground-1 and 32.19% in hunting ground-2.

Indirect costs that burden the hunting ground user of hunting ground-1 with 39.37% are costs of two employees performing the job of a gamekeeper and a professional hunting ground manager according to the Law on Game and Hunting (2010) while in hunting ground-2 these costs are 47.42%.

In order to realize profit from game management harvest plan accomplishment must be pursued as well as the realization of big game harvest according to price list for tourist hunters in small hills and hilly hunting grounds while in lowland hunting ground as to the realization of the share of small game (hare) as well.

Improvement of the economic performance can be achieved also by improving the ways of game management by increasing the share of big game in medal, reducing losses and increasing real growth rate in some game species within the acceptable biological limits.

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Poster presentation

VETERINARY LEGISLATION IN HUNTING IN SERBIA: ARE WE CLOSER OR FURTHER AWAY FROM THE EUROPEAN UNION?

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Abstract

This paper analyzes the regulations concerning the health status of wildlife populations and game meat hygiene in Republic Serbia: Law on game and hunting (2010), Veterinary medicine law (2010) and subsidiary legal acts in comparison with EU legislative (in Austria and Croatia).

The legal framework in Serbia related to game meat is divided between the jurisdictions of rules that regulate hunting, veterinary, food safety and others. That implies also responsibilities in the food chain. In relation to legal regulations in the EU (i.e. Austria) evident are major differences compared to the relatively large competencies there having hunter and trained person, practically as a veterinary inspector in Serbia. Thus, the export of game meat from Serbia in an EU country would be complicated process. If we look the hunting legislation in Croatia, there are significantly used resources from the EU pre-accession funds for education and general harmonization of these regulations. This was not the case with Serbia, because according to available information there was nothing from EU projects directly related to hunting, including game meat hygiene.

According to the strategy of Serbia's accession to the European Union, there is space for correction and supplement of legislation relating wildlife health management including game meat hygiene. It is also necessary previously to do extensive scientific researches about the situation concerning the meat hygiene of game animals in Serbia, due to lack of information about it. Specifically, how many facilities we have for temporary storage of game meat in accordance with the regulations of Serbia and/or from EU. It is also important to investigate how the current regulations in Serbia are applied in practice, and how much hunters and authorized people know about this legislation.

Keywords: EU, wildlife, meat hygiene, regulations, Serbia

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Introduction

In Serbia and all West Balkan region, wild game meat has great economic (hunting), and nutritional significance - in cooking because of the high content of valuable protein and less fat, but with more valuable composition (polyunsaturated). Certainly, ensuring the safety of game meat has an important role in the entire system of food safety, particularly in terms of zoonoses (Urosevic et al., 2011). Within the hunting economy game management in European Union, a significant place takes legislation of veterinary medicine. I.e. in Austria, meat from wild game is a highly valued product. To ensure food safety "from forest/field to fork", hunters supplying meat to consumers or retailers have to undergo specific training (Winkelmayer, 2009). Serbia adopted a many different veterinary medicine and hunting regulations including by-law relating documents (Urosevic et al., 2011). In terms of hunting tourism and the export of game meat Serbia needs to harmonize the veterinary and sanitary regulations with European Union member countries. Currently, it is a big obstacle standing in the way of the arrival of a larger number of foreign tourists in our hunting grounds, the greater shooting of especially on small game, and then legal game meat transportation to their country of origin. In relation to Serbia and other countries in the region, Austria is an example of countries with similar hunting tradition and highly developed economic impact of hunting with regulations (Regulation on the inspection of meat, Anonymous, 2006) that comply with the European Union (Anonymous, 2004c). This was the reason that we make short analysis of regulations in the field of veterinary medicine in relation to game management in general.

Material and methods

This paper analyzes the regulations concerning the health status of wildlife populations and game meat hygiene in Republic Serbia: Law on game and hunting (Anonymous, 2010a), Veterinary medicine law (Anonymous, 2010b), the Law on food safety (Anonymous, 2009), "Regulation on veterinary-sanitary conditions, and general and specific requirements for food hygiene to be met by facilities for handling with shot wild game, and how to carry out official controls shot wild game (Anonymous, 2010c) and other subsidiary legal acts.

Results and discussion

Overview of legislation in Serbia relating to wildlife management: Law on Food Safety (Anonymous, 2009). In it, there is a provision about hunting and supporting activities relating to the storage, handling and transport of game carcasses from the production place to the facility. It was highlighted that in relation to the distribution of responsibilities, duties of state administration in food safety, veterinary inspections carried out as follows: in the phase of export products of animal origin, as well as the retail trade of game meat in specialized stores. In the Law on Veterinary Medicine (Anonymous, 2010b) provides the obligations to that needs to be fulfilled by a hunting grounds user during the handling of game meat. This law regulates and issues of veterinary - sanitary control of game animals meat. Legal persons engaged in hunting and Hunting associations shall be obliged to temporary store carcasses and parts of the of game for the implementation of veterinary -

sanitary controls, in the case the game is safe for human consumption. If the game meat and trophies are to be exported from Serbia it shall be subject of veterinary sanitary control at border crossings. Here are included only facilities which fulfill the veterinary-sanitary conditions, general and specific requirements for food hygiene and food for animals assigned the export control number and are entered in the Register of export facilities. In general, the export consignments of animals and animal products shall be issued by the original international veterinary certificate which confirms that the shipment fulfills the conditions of the importing country, and for food of animal origin, that these foods are safe for human consumption. Trophies of wild animals can be set out without the export facility, but with adequate preparation and provision of required documentation according to EU regulations. In the transport of hunted game and its parts, the main responsibility has a hunting ground user, which is in accordance with the Law on game and hunting (Anonymous, 2010a). Hunting grounds user belongs dead or caught animals and their parts (trophies, etc..) belongs to. hunting grounds user. He shall determine by its act the price of: hunting wild game, meat of shot game, hunting services of the wildlife, etc. Hunting grounds user can export shot game shot animals or its parts, only if it registered for such activities, as mentioned above. In the 2010th the Ministry of Agriculture of Serbia passed a subsidiary act concerning to the safety of meat from game animals: „Regulation on veterinary-sanitary conditions, and general and specific requirements for food hygiene to be met by facilities for handling with shot wild game, and how to carry out official controls shot wild game (Anonymous, 2010c). In these regulations are more closely prescribed the veterinary-sanitary conditions, general and specific requirements for food hygiene, which in terms of construction and reconstruction of facilities must fulfill the temporary storage of shot game animals, and the manner of carrying out the official control shot game animals.

Overview of legislation in European Union - example from Austria

Austrian legislation on game meat hygiene from the year 1994 (Anonymous, 1994) introduced a three-step inspection system. The hunter was responsible for ante-mortem inspection and examination of the carcass and intestines upon evisceration. Trained persons were responsible for the examination of the carcass and (edible) inner organs. The official veterinarian was responsible for the inspection of carcasses entering game handling establishments and was also always responsible involved when serious abnormalities were detected during inspection by hunters or trained persons. For small game, a simplified version was established. Under the "new" EU hygiene package from year 2004 (Anonymous, 2004b), the responsibility of trained persons increased. This means, that in all situations of direct marketing, the (documented) inspection by both hunters and trained persons is mandatory, and in case of serious abnormalities (observed deviations) from normal condition, carcass and its organs must be presented to an official veterinarian for inspection. Since 1994, ca. 20.000 hunters have been trained as „trained persons", which includes theoretical lessons on anatomy, normal appearance of organs, main diseases, hygiene, and legislation. Training books have been updated regularly to address changes in legislation (Winkelmayer et al., 2008). Their experience shows that a combination of basic courses, advanced training courses, and evaluation schemes can be effective in enabling hunters to fulfill the needs for a hygienic and safe direct supply of game meat in the sense of a longitudinal integrated "from forest/field to fork" approach. Responsibilities in the food chain are the following: for supplying the local market or the consumers directly with small quantities of meat , the examination, done by the trained person, is the end-

examination of game meat. Official Veterinarian after examination, which takes into account the findings documented by the hunter and the trained person, game meat may enter the EC/EU market (Winkelmayer, 2009).

Experiences from Croatia

Harmonization of the Croatian legislation to the one in the European Union in the field of veterinary medicine has been one of the more complex packages that Republic of Croatia had to fulfill as a candidate country. In particular, they highlighted the Veterinary Law (Anonymous, 2007a), Law on Food (Anonymous, 2007b), the Law on Hunting (Anonymous, 2005) and others, that provide a framework whereby they have made many subordinate legislation which specifically elaborates action in certain cases. So, Mikus (2010) notes that wild game meat is subject to veterinary inspection (Anonymous, 2007c, 2007d) and certain procedures prior to marketing. Game venison must be inspected as soon as possible after admission to the facility for the processing of carcasses of wild game. The official veterinarian takes into consideration the statement or information in accordance with the provisions of the Regulations on the hygiene of food of animal origin (Anonymous, 2007c) delivered by a trained person. Considering the legislative framework, if observe hunting from the perspective of sustainable development, particularly where aspire following modern European heritage, it is obvious that the framework of legislative measures and regulations of Republic of Croatia is really respectable (Florijancic et al., 2010). Anyway, the Croatian experience in adapting of legislation can be very useful for future candidate countries for accession to the European Union. Especially having in mind that the legislation in Croatia is fully harmonized with the one in EU enactment of the following laws: Veterinary Law (Anonymous, 2013a), Food Law (Anonymous, 2013b), The law on food hygiene and microbiological criteria for food (Anonymous, 2013c).

Serbian legislation perspective

As shown, the legal framework in Serbia related to game meat is divided between the jurisdictions of rules that regulate hunting, veterinary, food safety and others. Therefore dealt also responsibilities in the food chain. In relation to legal regulations in the EU (for example in Austria) evident are major differences compared to the relatively large competencies there have hunter and trained person, practically as a veterinary inspector in Serbia. Thus, the export of game meat from Serbia in an EU country would be complicated process. It should be emphasized also sharing of veterinary competence in control of game meat. In Serbia it is centralized at the federal level exclusively, and in Austria the jurisdiction transferred to Government of nine federal provinces. Least but not last, if we look the hunting legislation in Croatia, there are significantly used resources from the EU pre-accession funds for education and general harmonization of these regulations. This was not the case with Serbia, because according to available information there was nothing from EU projects directly related to hunting, including game meat hygiene. An exception is the action of oral vaccination of foxes and suppression of classical swine fever as a project funded from IPA funds, which is more or less implemented in all countries/ EU candidates the our region.

Conclusion

According to the strategy of Serbia's accession to the European Union, and the commitment to harmonization of regulations related to human and animal health and health safety of food of animal origin, there is space for correction and supplement of legislation relating wildlife management including game meat hygiene. Anyway, it should be determine the transitional period before the entry into force of provisions the Regulation - EC No. 852/2004 (Anonymous, 2004a) 853/2004 (Anonymous, 2004b) and 854/2004 (Anonymous, 2004c) to amend in the current Serbian regulations. Thereby, it should take good (and bad) experiences of countries in the region, which have this passed this for example as Croatia. It is also necessary previously to do extensive scientific researches about the situation concerning the meat hygiene of meat of game animals in Serbia, due to lack of information about it. Specifically, how many facilities we have for temporary storage of game meat in accordance with the regulations of Serbia and /or from EU, how is comply valid national regulations, and how many hunters themselves or trained person know about this. It is also important to investigate how the current regulations in Serbia (the above-mentioned) are applied in practice, and how much hunters and authorized people know about this legislation. And naturally, what are needs to be done to enable the easier procedures for the export of game meat that foreign hunters hunt in Serbia. After all mentioned, we could recommend good experience according game meat inspection system from Austria, and apply this model for future use in Serbia. It should not be ignored the continuing education of all stakeholders in hunting, which would be solve by the drafting guide for collection and testing with the instructions on the type of biological agents to be monitored in the meat of wild game, to determine the presence and frequency of hazards in wild game meat, which are important for human health. However, it should be considered that for effective implementation of regulations in regard to game meat hygiene, and the future implementation of EU regulations, it is not enough just to intensify control and penalty provisions for particular violations or crimes. All these measures requires time, persistence and of course the unity of all stakeholders (mutual interests), which currently is not the case in Serbia (Federal and Local authorities, Hunting Association, Hunting Chamber, consumers and others).

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Poster presentation

CRYOPRESERVATION IN ARTIFICIAL SPAWNING OF FISH

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Abstract

Artificial spawning is a part of the process of fish reproduction that takes place under the control of man. In terms of performance it is substantially different from natural reproduction. Artificial spawning includes several interrelated phases: the preparation of male and female gametes, insemination and fertilization of eggs. Restocking and artificial spawning represent some of the methods to improve the abundance of fish populations in marine and freshwater fish species. It is used increasingly in the intensive fishing, which points to the need for conservation of sperm and eggs.

Cryopreservation of sperm represents a storage technique in liquid nitrogen for indefinitely. In this way a constant availability of sperm for conservation programs for a variety of species is provided. It allows easy transport and use for the purpose of genetic improvement of species. Cryopreservation is widely used in animal husbandry and lately, more and more used in aquaculture. In the last decades many studies were conducted with a view to defining details, and designing protocols of cryopreservation of fish sperm.

Cryopreservation of fish spermatozoa could make available high-quality gametes for hatcheries and further breeding, with an aim to ensure the genetic quality and safety in case of possible hazards such as diseases, natural disasters and other.

Keywords: artificial spawning, cryopreservation, fish

Introduction

Artificial spawning is widely applied in freshwater fish species of the genus Claroodae, Salmonidae and Cyprinidae. Restocking and artificial spawning represent some of the methods to improve the abundance of fish populations in marine and freshwater fish species. It is used increasingly in the intensive fishing, which points to the need for conservation of royal jelly and eggs (Veselinović et al., 2004). In order to increase the performance of artificial insemination various techniques of sperm preservation are used. Application of permanent preservation of sperm cryopreservation involves liquid nitrogen, liquid short-term preservation and encapsulation with various polymers.

In 1949, Polge et al., have successfully implemented avian sperm cryopreservation using glycerol as a cryoprotectant (Tsai and Lin, 2012). After that male gamete cryopreservation has become possible. The first fish sperm cryopreservation is performed by Blaxter (1953) and achieved about 80% of cell motility after thawing. He established sperm manipulation techniques for freshwater and saltwater fish, including carp, trout, catfish, cichlids, etc.

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Cryopreservation of sperm represents a storage technique in liquid nitrogen for indefinitely period of time, which is basically used for storage of genetic material of males. In this way a constant availability of sperm for conservation programs for a variety of species is available. Cryopreservation allows easy transport and application for the purpose of genetic improvement of the species in aquaculture (Horvat et al., 2012). Cryopreservation of gametes is a reliable way to preserve genetic material for scientific purposes, for modern aquaculture and biodiversity conservation. Successful cryopreservation was achieved in more than 200 fish species and many species have been appropriate for the purposes of cryo-banks. Cryopreservation of fish embryos is not sustainable because of the sensitivity on high cooling and low permeability of the membrane (Tsai and Lin, 2012). Cryopreservation of isolated cells of the embryo is one option for preserving maternal and paternal genomes. Management of techniques and storage of sperm cryopreservation is very important. At a temperature of 0 °C the sperm can be stored for a few hours to a few days depending on the species. Cryopreserved gametes can theoretically be kept between 200 and 32,000 years without any harmful effects (Suquet et al., 2000). The advantage of this technique is a synchronized access to the gametes of both sexes or the absence of animals required for the production of gametes.

Importance of cryopreservation
Cryopreservation is important not only for fish but also for the preservation and improvement of genetic resources in general. This technique is well perfected in certain species of freshwater fish, mainly salmonids. The advantages of sperm cryopreservation include: 1) synchronization of availability of gametes of both sexes, 2) sperm economy 3) simplification of the manipulation of individuals for cultivation, 4) transport of gametes from different ponds and 5) germplasm storage for programs of genetic selection, or the preservation of species. The huge impact of biotechnology in aquaculture is particularly pronounced in recent years. Several fish species are used as research models, not only for the development of aquaculture, but for the purpose of medical research. Cryopreservation of sperm significantly contributes to the storage of all transgenic lines (Cabrita et al., 2010). Fish sperm cryopreservation procedures are necessary in order to overcome the disadvantages in terms of: the standardization of methodologies and procedures, safety testing of seed quality and development tools that prevent cryo – destructive damages. Special importance should be given to molecular and genetic research. Cryopreservation of gametes is particularly important in male fish populations. There is a wide range of possibilities for improving the resistance of cells to the cryopreservation thru their diet, the addition of amino acids (taurine and hypotaurin), vitamins (E and C), and lipids. A good balance of these compounds improves semen plasma composition and protects cells from oxidative stress (lipid peroxidation, protein oxidation, DNA fragmentation, protecting enzyme) (Cabrita et al., 2010).

Sperm cryopreservation techniques
The advantage of frozen sperm in relation to the fresh is multiple. Cryopreservation allows: long-term storage of sperm, easy transfer of the containers with liquid nitrogen and use at any time. A certain percentage of the sperm do not survive freezing or become dysfunctional. The main objective of the freezing of sperm is to prevent cell from formation of lethal crystal. It is necessary to control the changes of cell volume and to prevent damage of the membrane. Otherwise the resulting consequences of the change in temperature lead to exposure of spermatozoa to free radicals of oxygen and membrane damage.

Cryopreservation is widely used in animal husbandry. Lately, more and more is used in aquaculture. Protocols for sperm cryopreservation should be species-specific, due to the specifics in terms of physical and chemical properties of sperm (Vuthiphandchai et al., 2014).

Depending on the type of cell, tissue, available equipment in laboratories methods of cryopreservation can be different. They can be grouped into several categories based on the difference substances, containers or cooling equipment. According to the substances for cooling, methods are divided to the methods where dry ice and methods where liquid nitrogen were used. In methods with dry ice, carbon dioxide has no liquid phase and it passes directly from solid to gaseous state by a process called sublimation temperature of CO_2 -79 °C. In methods where the liquid nitrogen is used (in the liquid form of molecular nitrogen N_2), at boiling point of liquid nitrogen at -196 °C, and solidifies at a temperature of -210 °C. Methods of cryopreservation according to the container for cryopreservation are the pellet method and the method with tubes. Pellets method does not use the specific container for cryopreservation, but the very structure of dry ice at a temperature of -78 °C. It is important to harmonize the rates of cooling and thawing and to ensure easy identification of the sample. The process of sperm cryopreservation involves the use of various substances that soften the negative impact of freezing. Substances used for this purpose are cryoprotectant and extenders. Research is based on finding the optimum combination and quantity of these substances. Primary it is necessary to establish a balance between their useful and possible toxic effects (Lujić et al., 2015).

Cryoprotectants are chemicals that are used to protect the cells from damage during freezing and thawing. Their role is to prevent the formation of ice crystals in the cell. The most commonly used cryoprotectant is glycerol (to be used in blood banks and sperm), ethylene glycol - (antifreeze that is used in the car industry), propylene glycol (component of an ice cream) and dimethyl sulfoxide (DMSO, which is very important in the cryopreservation of the embryo). Dimethyl sulfoxide decreases freezing of solution, minimize osmotic shock by changing the water inside the sperm cells and reduces the formation of ice crystals within the sperm cells (Leung, 1991). Sugars and polymers stabilize the membrane during freezing with optimal concentration of cryoprotectants. Too high concentration causes osmotic imbalance and rupture of the wall of sperm. Cryoprotectants are often toxic to spermatozoa. Selection of cryoprotectants and their optimum concentration is the focus of many studies.

During cryopreservation, special attention must be paid to the handling of semen. The sperm of different types are differently sensitive to the process of freezing and thawing. Special attention is given to problems related to longer storage in liquid nitrogen and the problem of contamination of seeds with urine. The problem arose with urine contamination often meets with ejaculation in some types such as carp (Cyprinus carpio), tench (Tinca tinca), tilapia (Oreochromis mossambicus) and others (Veselinović et al., 2004).

The quality of frozen or thawed sperm is assessed by standard biological tests. A two-stage technique that examines active sperm motility and the ability to adapt to different types of fertilization is used as well as a discriminatory insemination techniques. Complementary biochemical analysis (ATP, the consumption of O_2) or morphological units (electron microscopy) precisely localize damage caused by cryopreservation process. Most extenders that are used in marine fish are saline solutions or solutions of sugar. It has been found that dimethyl sulfoxide (DMSO) is generally the most effective cryoprotectant in marine fish

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(Suquet et al., 2000).

Due to the high concentration of sperm in jelly, assessment of mobility requires a high rate of dilution. The procedure requires initiating the motility of maximum number of sperm at the same time. At first, sperm is diluted in an environment which does not initiate the motility (Suquet et al., 2000). Subsequently, the sperm can be activated directly by mixing the spermatozoa under a microscope with an activating solution. Compared to freshwater fish, it is recorded a high percentage of sperm that survived cryopreservation in marine fish.

Many studies on cryopreservation of fish sperm were carried out on the economically important species of freshwater fish. Sperm of freshwater fish are generally more difficult to cryopreserve. The rate of fertilization in sea fish after cryopreservation is similar to the results obtained in the process of cryopreservation on some species of mammals (Tsai and Lin, 2012). Gradualness and controlling the slow cooling is necessary in the cryopreservation of fish sperm. During the study, carp is recorded fertilization and hatching in 95% of cases. The quality of sperm is crucial for successful cryopreservation. Features may vary from species to species depending on the period of taking the sperm during the season. Cryopreservation can cause damage to the spermplasma membrane, mitochondria and chromatin structure (Cabrita et al., 2010). Damage may occur through mechanical forces, creating ice crystals that are formed within the sperm. Preserving the structure and function of sperm depends on the protocol of cryopreservation. In order to fertilize eggs, sperm cells must maintain the integrity of the plasma membrane and the osmotic control. The sperm must retain the ability to launch when discharged into the water in order to reach the oocyte.

Sperm motility is essential and it depends on several aspects such as the status of mitochondria, the process of production of ATP, the activities channels on the plasma membrane structure and whips. Motility represents one of its most important features that allow success of fertilization. Assessment of sperm motility was previously determined by the classical method based on the personal judgment of researchers by awarding marks from 0 to 5. The modern method that is more objective involves the use automated CASA system (Eng. Computer Assisted Sperm Analysis). This computer program captures and analyzes the movements of each individual sperm following the position of his head. The resultant data provide information on the percentage share of mobile sperm and other characteristics such as the trajectory of movement, speed etc.

Data obtained through the CASA program are expressed by the average value obtained from the analysis of any sperm in the visual field. As such, they can be used in the process of improvement of methods of artificial insemination (DeGraaf et al., 2007) or a selection of the best stem cells (Kime et al., 2001).

Preserving of genetic information is very important during the cryopreservation. There is an information on damage DNA material during cryopreservation of sperm in mammals and in fish but the mechanism of these changes has not yet been explained (Pérez- Cerezales et al., 2010).

During the process of freezing, the cooling rate should be slow enough to reduce the amount of intra-cellular ice, yet fast enough to minimize the exposure of sperm freezing temperatures. Rapid dissolution is desirable in order to minimize the damage associated

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with recrystallization, or by converting the crystals into small size, during thawing. Depending on factors that may significantly affect the quality of the results: density of sample, containers for freezing, the initial temperature, the final temperature before plunging into liquid nitrogen, diluting and removing the cryoprotectants after thawing (Leibo, 2000), freezing methods must be adapted to each type and the type of individual sperm (Mauger et al., 2006).

Conclusion

Within the last decades many studies were conducted with a goal to define detailed protocols of cryopreservation of fish sperm. Some research results have contributed to the breeding of fish by using new technologies. There are some concerns that influence on delaying the use of frozen sperm in favor of reproduction. Future research should be focused on specific aspects that would definitely ensure homogeneous and optimal results after freezing and thawing of sperm (Cabrita et al., 2010).

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Poster presentation

EFFECTS OF DIFFERENT FEED TYPES ON THE GROWTH OF COMMON CARP (*Cyprinus carpio* L., 1758) FRY

Perić A.[1], Stanković M.[2], Živić I.[1], Stojanović K.[1], Božanić M.[1], Marković Z.[2]

Abstract

The aim of this study was to evaluate the effects of four feed types on the common carp growth. All individuals, 600 juveniles (initial age - 15 days) had an approximate weight at the start of the experiment. The experiment was set by forming four groups based on different feed types: (1) live zooplankton, (2) frozen zooplankton, (3) freshly hatched brine shrimp *Artemia salina*, (4) *A. salina* grown in water rich in phytoplankton. All four treatments were tested in triplicate, with stocking density of fish: 50 pcs.aquarium^{-1}. The water temperature ranged from 21.4-23.5 °C, pH 7.8-8.7, while the oxygen concentration ranged from 5.4-7.7 mgl^{-1}.

The lowest growth was found in the group 2 with average weight of only 0.11g per individual. The group 1 showed the best results with average weight of 0.45g per individual. Intermediate results were achieved in the groups 3 and 4. The average weight of fish in these groups was 0.39g and 0.44g per individual, respectively.

The results of the present study showed that fish fed with frozen zooplankton grew slower than the fish in other groups. The reason for this may be a worse utilization of frozen zooplankton in relation to other fish. This may be due to greater attractiveness of living prey such as live zooplankton and *Artemia*. The conclusion is that frozen zooplankton cannot be an adequate substitution for live feed, at least in the first month of common carp life. Outcomes of these study showed a possibility of replacing a very expensive feed, such as *Artemia*, with live zooplankton. Also, these results indicate a possibility for enriching recently hatched brine shrimp, which would contribute to even better fish growth.

Keywords: Artemia salina, common carp, feed, growth, zooplankton

Introduction

Since the '80s of the 20th century, the market is increasingly more dependent on aquaculture, and less on capture fisheries (FAO, 2016). A large number of studies related to nutrition of breeding organisms have certainly contributed to their increase quality and production. Besides the increase of yield per unit area, the artificial feed also reduces the production costs. Common carp (*Cyprinus carpio* L., 1758) is one of the most important aquaculture fish species in the world, with the production that increases every year. Use of good quality and especially extruded pelleted feed can minimize water pollution and   the

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spread of diseases owing it to high digestibility, low conversion rate - better fish growth, less organic waste per kg of fish produced (Cho et al., 2006).

The use of concentrated feed that is adapted to age category and type of fish rearing increases the meat quality (Marković and Mitrović-Tutundžić, 2003). Balanced proportion of proteins, lipids, carbohydrates, vitamins, minerals and artificial feed, increase the condition and fish health. Essential amino acids are usually the limiting factor if the alternative sources of protein originate from plants (Stanković et al., 2009). Despite the fact that fish meal satisfies fish needs for all essential amino acids, soy proteins that have most similar composition are now in use more frequently as replacement for animal proteins. In addition to the high price of fish meal, very significant reason for the increased use of plant-based protein is a high demand for fish meal, which is the reason why it is often unavailable to producers of artificial feed. However, it has been shown in practice that the live feed is most adequate for one month old common carp fry (Wang et al., 2005), with graduate adjustment to concentrated feed. In aquaculture, hatched brine shrimp *Artemia*, which has high nutritional value, 40-60 percent protein with rich amino acid composition (Koru and Turkmen, 2010), is commonly fed to the fish fry. Because of the size of nauplius stage, *Artemia* also represents the only practical feed source for the early stages of many fish (Tamaru et al., 2001).

The aim of this study was to evaluate the effects of four live feed types on the common carp fry growth. Additionally, the opportunity of further increasing the nutritional value of *Artemia* by growing it in water rich in phytoplankton was also examined. Also, the aim was to determine whether there is a possibility of replacing a very expensive food such as *Artemia*, with live or frozen zooplankton.

Material and methods

The study was achieved at the Centre for Fishery and Applied Hydrobiology "Little Danube" (CEFAH), Experimental Station Radmilovac, Faculty of Agriculture, University of Belgrade. Research lasted 30 days and was conducted at 600 fry individuals 15 days old, that were, until then, exclusively fed with *Artemia* nauplii.

It is considered that the influence of genetic variability on phenotype of subject individuals is low, since the experimental fry originates from the same parents. The approximate weight of all fish was 0.02 g per individual at start of the experiment. 12 glass aquariums (each with a 25 liter capacity) were previously filled with dechlorinated tap water and provided with aeration regulated by air compressors (Resun LP 60). The fry were distributed at a ratio of 50 fish per aquarium to acclimate and kept unfed for one day. The aquariums were cleaned every third day to remove the feces, not ingested feed and dead fry. Later, one third of the expelled water was replaced with fresh dechlorinated tap water.

Water temperature, pH and dissolved Oxygen were recorded every two days at each aquaria using a field kit MULTI 340i/SET (WTW, Weilheim, Germany). Fish were measured with digital balance (MW 120; Casbee, Samsung, Korea, precision 0.01g).

Four groups of fish, based on different type of feed, were tested in triplicate: (1) live zooplankton, (2) frozen zooplankton, (3) freshly hatched brine shrimp *A. salina*, (4) *A. salina* grown in water with phytoplankton.

Artemia cysts used in this research were produced by Artemia Koral GmbH. Hatching and enrichment of brine shrimp were prepared following the example of part of Delbos (2009) protocol. Preparation of enriched *Artemia* requires a two day lead time. One day is required for hatching of brine shrimp and another day for the enrichment process. Preparation of regular *Artemia* requires a one day lead time for hatching of nauplii.

Zooplankton organisms were collected from the fish ponds behind the CEFAH using a plankton net and were frozen before the beginning of the experiment. For the fry fed in group 1, live zooplankton organisms were collected every day. The size of zooplankton was adjusted to our fish age category.

For better use of feed, in group 2 fish were fed three times daily. Other fish were fed once a day due to the fact that live feed stays attractive for fry longer than other. Fish were fed with amount of food as much as they could ingest. At the end of the experiment, the fry in each aquarium were netted and weighed to obtain the final body weight (g/fish).

Data were tested for normality with Shapiro-Wilk W-test, and latter comparison was performed with T-test and Mann-Whitney U-test. Statistical analyses were completed in programs STATISTICA 6.0 and Pas3.

Results and discussion

During experiment the water temperature ranged from 21.4-23.5 °C, pH 7.8-8.7, while the oxygen concentration ranged from 5.4-7.7 mgl^{-1}, with the insignificant variations between aquariums during the measurements.

Group 1 showed the best results with average weight of 0.45g per individual. The lowest growth was found in the group 2 with average weight of only 0.11g per individual. Intermediate results were achieved in the groups 3 and 4. The average weight of fish in these groups was 0.39g and 0.44g per individual, respectively.
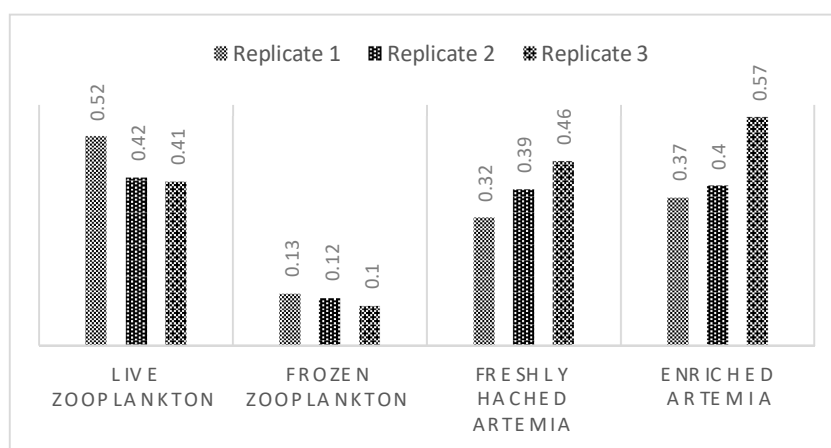
Graph 1. Average weight of *C. carpio* fry fed with tested diets for 30 days.

Table 1. Mean weights of fish at the end of experiment (ZP-live zooplankton; FZ-frozen zooplankton; FA-freshly hatched *Artemia*; EA-enriched *Artemia*).

Parameter	Group			
	ZP	**FZ**	**FA**	**EA**
Mean weight (g)	0.45 $\pm 0.06^{aa}$	0.11 $\pm 0.01^{bb}$	0.39 $\pm 0.07^{aa}$	0.44 $\pm 0.11^{aa}$

T-test p<0,05

Group 2 showed statistically significant (p<0.05) lower mean weight compared to all other groups, while other groups were not significantly different among each other.

The number of survived specimens also follows this distribution. Thus, group 1 showed the best results with average number of 44.66 individuals survived per treatment. The highest mortality was in group 2, where the average number of survived fish per treatment was 31. Intermediate mortality was in the groups 3 and 4. The average number of fish survived in these groups was 43.66 and 44.33 individuals per treatment, respectively. However, there is not statistically significant difference (p>0.05) between treatments.
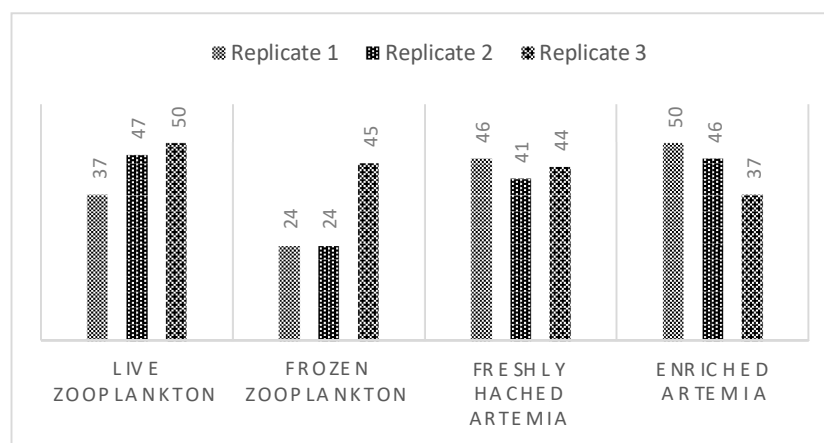
Graph 2. Survival of *C. carpio* fry fed with tested diets for 30 days.

The results of the present study showed that fish fed with frozen zooplankton grew slower than fish in other groups. The reason for this may be a worse utilization of frozen zooplankton in relation to other fish feed. This may be due to greater attractiveness of living prey such as live zooplankton and *Artemia*. Other explanation is given by Grabner et al. (1981) assumed that frozen zooplankton when introduced into water, enzymes activity is rapidly lost. Also, Sharma and Chakrabart (1999) found that the highest proteolytic enzyme activity was observed in fish fed with live feed.

Conclusion

This research shows that frozen zooplankton cannot be an adequate substitution for live feed, at least in the first month of common carp life. Outcomes of these study showed a possibility of replacing a very expensive feed such as *Artemia*, with live zooplankton. Also, these results indicate the opportunity of enriching recently hatched brine shrimp, which would contribute to even better growth and increase in fish health.

Acknowledgement

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Poster presentation

THE EFFECT OF SURVIVAL OF KOI CARP LARVAE WITH DIFFERENT WATER AERATION INTENSITY

Stanković M.[1], Radosavčev N.[1], Perić A.[1], Marković Z.[1]

Abstract

Koi carp is a famous ornamental fish species. It is commonly kept in aquariums and water gardens. Although it receives much less exposure than the other farming species from family of Cyprinidae, certain specimens of the koi carp can reach much higher prices on the market.

The experiment was realized in two stages in the fish tanks in Center for Fisheries and Applied Hydrobiology "Little Danube" Faculty of Agriculture University of Belgrade. During the first stage, koi carp larvae have been subjected to different water aeration intensities in order to test their survival rate. During the second stage, larvae had been additionally subjected to different feeding methods in order to observe the effects of different methods on their growth and color intensity.

The 3 groups of larvae, after being transferred in to the aquariums, are met with 3 different combinations of water aeration intensity and feeding method which led to different survival rate. The best result has been recorded in group A with 62% (low water aeration intensity). Group B with 54% (medium water aeration intensity) while only 21% in group C (high water aeration intensity).

Based on the results, we can conclude that besides expressing sensitivity to ambient conditions, koi carp larvae are also highly sensitive to the intensity of water aeration. Strong water aeration additionally exhausts young specimens which eventually results with lower survival rate.

Keywords: koi carp, larvae, water aeration, survival

Introduction

Koi carp is freshwater ornamental fish species from family Cyprinidae. Originate in Japan where according to the earliest records from the 18th century it has been described on paintings and drawings different color variations (KF, 2006) since it's breeding started (Ikuta and Yamaguchi, 2005). In order to obtain different varieties of koi carp, back in 1820's in the town of Ojiya on the island of Honshu (Japan) people began with the crossbreeding of fish with different colors. By the end of the 20th century it was established a production of koi carp with 13 main varieties (Blasiola, 2005). Today it is considered and recognized 22 basic varieties with constant development of new and different types of koi carp. Using different programs of selection for desired traits, it has been created more than 100 colors and designs of koi carp (Wang and Li, 2004) with over 1000 (Brewster et al., 2007) different types present in a wide range of different colors.

Koi carp basic colors are orange, yellow, red, blue, white and black as well as various combinations of these colors and shapes on the body of the fish. Depending on the   color

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and patterns on the fish body there were established different varieties and types of the fish which are particularly popular and wanted on the market. Nowadays it is believed in Japan that every variety of koi carp represents a symbol: friendship, love, masculinity, prosperity, wealth, happiness and strength.

In accordance with the availability of some varieties, koi carp on the market can reach extremely high prices. Active promotion of international trade and the maintenance of competition and auction are important events in Japan (Ikuta and Yamaguchi, 2005) and the price of these fish can be over $ 500,000 (Green, 2008).

As it belongs to the family Cyprinidae, generally it can be said that the koi carp is resistant and tolerant species of fish, with the ability to adapt to different habitats (Jones and Stuart, 2009). However like other animals, younger categories are more sensitive and less tolerant than the older category.

The purpose of this study was to evaluate the survival rate of koi carp larvae after replacing them in new environmental conditions with variable intensity of water aeration.

Material and Methods

The experiment was realized in two stages in the laboratory of the Centre for Fishery and Applied Hydrobiology "Little Danube" Faculty of Agriculture University of Belgrade. During the first stage koi carp larvae were subjected to the influence of different intensity of water aeration in order to test their survival rate. During the second phase of the research, the larvae were additionally subjected to a different diet in order to follow their growth and intensity of body colors.

Each fish aquarium, measuring 60 * 40 cm, was filled with water to a height of 10 centimeters (working volume 24 l) and placed with 50 koi carp larvae. Larvae were randomly collected from hatcheries and immediately transported into the prepared fish aquarium.

Water aeration is regulated with air compressors (Resun LP 60). Through plastic hoses air is transported to each fish tank. In the central part of fish tanks, air stones (associated with air supply) with valves, which regulate intensity of aeration, have been placed. Three treatments with nine repetitions have been set. First treatment had very low intensity of water aeration. In the second treatment water aeration intensity was optimal (medium strength) while in the third treatment the intensity of the water aeration was strong.
Duration of the experiment depended upon adaptation of larvae and their survival. Survival coefficient of koi carp larvae has been calculated based on the obtained results:

$$KS = \text{the number of surviving individuals} * 100 / 50$$

Results and Discussion

The experiment was carried out after two days when in some of the fish tanks all of koi carp larvae have died. Based on the number of fish, the survival rate is calculated both for fish tanks and treatments.

Table 1. The number of survivors and dead individuals per fish aquariums and treatments

Number	Treatments	Aquarium								
	1	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Survivors		**10**	46	27	41	35	**47**	40	20	12
Died	(poor aeration)	**40**	4	23	9	15	**3**	10	30	38
KS (%)		**20**	92	54	82	70	**94**	80	40	24
Number	2	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
Survivors		35	**48**	19	29	42	41	13	**6**	8
Died	(optimum aeration)	15	**2**	31	21	8	9	37	**44**	42
KS (%)		70	**96**	38	58	84	82	26	**12**	16
Number	3	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*
Survivors		21	**44**	**0**	12	1	17	**0**	**0**	**0**
Died	(strong aeration)	29	**6**	**50**	38	49	33	**50**	**50**	**50**
KS (%)		42	**88**	**0**	24	2	34	**0**	**0**	**0**

Larvae from the treatment 3 had the lowest survival rate from 0 to 88%. In 4 of the 9 fish tanks specimens did not survived the impact of strong water aeration. Much better survival rate of larvae was recorded in treatment 2, from 12 to 96%. Specimens which were treated with a weak aeration, survival rate ranged from 10 to 47 specimens, namely from 20 to 94%.

Graph 1. The average survival rate of koi carp larvae per treatment
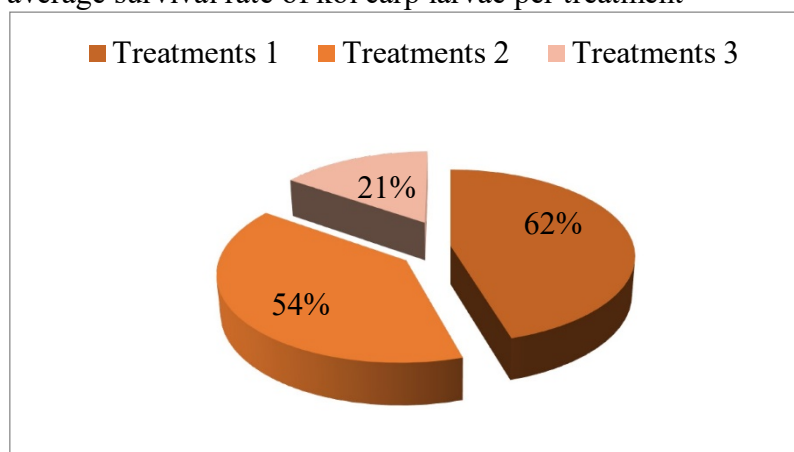
Considering the number of survived larvae from all of treated fish tanks, the worst KS had specimens from treatment 3 with 21%. The effect of the optimal water aeration for koi carp larvae resulted with value of 54% for KS. The best survival rate was observed in the first treatment where KS was 62%.

Jelkić et al. (2012) reported that survival rate of larvae carp in recirculating system (RAS) in the first 10 days of age high, from 93 to 96%, depending on the density of plantation. Since in our experiment larvae changed environmental conditions, survival was significantly lower than in the study Jelkić et al. (2012).

In a study conducted by Enache et al. (2011) in RAS on older carp offspring with average weight 65 grams, the survival rate was 98-100%. Answer for that is due to the resistance of older offspring compared to younger larvae that were used in this study as well as the effect of different aeration intensity. Adult fish are laying their eggs on

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aquatic plants in stagnant or slow-flowing parts of the water flow therefore in the first days of life newborns remain in the slow-moving water parts. With the growth of the larvae, resistance increases and more later carp off springs can easily bear the faster moving water.

Conclusion

Based on obtained results it can be concluded that in addition to the sensitivity of change of environmental conditions, koi carp larvae are also extremely sensitive to the intensity of water aeration. Strong water aeration is exhausting to young specimens which eventually results in a lower rate of survival. Poorly implemented water aeration gave the best results of KS.

Acknowledgement

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Poster presentation

APPLICATION OF GOOD MANUFACTURING PRACTICE IN BEEKEEPING

Bojanić Rašović M.[1], Saičić I.[1], Davidović V.[2], Joksimović Todorović M.[2], Relić R.[2]

Abstract

In order to investigate the application of Good Manufacturing Practice in beekeeping a total 50 beekeepers from Montenegrin municipalities of Podgorica, Kolašin, Bar and Ulcinj were surveyed. All tested apiaries are located in places with sufficient sunlight, 80% are shielded from the wind and 70% are on drained terrain, whereas 14% of apiaries have access to fresh spring water. There are 84% apiaries at the appropriate distance from neighboring houses, farms, power lines, and railroads, while 60% have access to quality pasture. Of the 30 beekeepers that carried out migration of their honey bee colonies, 17 of perform health control of bee colonies before moving (56.67%). Measures of good hygiene practices are strictly implemented by 40% of all beekeepers in this survey. They disinfect equipment and tools after each use and implement pest and rodent control as part of prevention measures once a year by commissioning an authorized service for the task at hand. Replacement of queen bee is conducted annually by 30% of beekeepers, once every two years by 58%, whereas 12% beekeepers replace the queen every three years. Full replacement of honeycomb is performed once a year by 10% of beekeepers, 30% change it once in two years, 50% - every three years, while the remaining 10% replace the honeycomb completely every four years. Feeding bees with sugar syrup is resorted to by 32% of beekeepers, 30% feed the bees solely with nectar honey, for 8% of beekeepers the bees nutrition is based on honeydew, while 30% of beekeepers implement a combined feeding approach. As part of Varroa mite control, beekeepers apply chemicals and biological method of building up frame. After extracting honey, beekeepers use preparations for bee health protection, for therapeutic or preventive purpose. Of the total number of beekeepers surveyed, only 40% analyze quality and health safety of the honey. On the basis of obtained survey results, it may be concluded that it is necessary to work consistently on beekeepers education in order to introduce measures typical of good beekeeping practices in their regular practice for the purpose of obtaining safe bee products. It is also necessary to work on compiling a manual for good beekeeping practice which would be mandatory for all honey producers.

Keywords: good beekeeping practice, Montenegro, survey, feeding bees, hygiene measures

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Introduction

Quality bee production is based on the implementation and respect for good beekeeping practices, in conjunction with HACCP as a system for food safety. For the introduction of HACCP the prerequisite programs are essential and include good manufacturing practice (GMP), good hygiene practice (GHP) and standard operating procedures (SOP). Application of GMP and GHP has an impact on reducing number of the potential physical, microbiological and chemical hazards on the health safety of products (*Anon.* 2009; *Anon.* 2011). Good beekeeping practice includes all procedures in the process of production of honey and other bee products (pollen, royal jelly, propolis, beeswax and bee venom) aimed at preserving the health of bees, the environment and safety of bee products. It is implemented of the apiary to the final product *(Anon. 2014a)*. For the safety and quality of the product is responsible beekeeper (*Anon. 2010*). Implementation of preventive measures play a major role in the improvement of beekeeping, honey production and increase bees' resistance to diseases. A key factor in the control of bee diseases represents the organization and method of technological process (*Anon. 2007).* Beekeepers are obliged to protect bees from diseases and from pesticide poisoning as well, which ensures the safety of bee products (*Stajkovac et al., 2009; Bokulić et al., 2014*). Considering the importance of the facts given above, we set for ourselves the goal of examining the application of Good Manufacturing Practice in beekeeping.

Material and Methods

In order to investigate the application of good beekeeping practices, we applied the method of interviews with 50 beekeepers in the areas of Podgorica, Danilovgrad, Kolašin, Bar and Ulcinj. The survey encompassed data related to gender and age of beekeepers, apiary locations, moving bees, testing health of bee colonies before removals, implementation of hygienic measures in the apiary, feeding bees, frequency of the queen replacement and complete replacement of the honeycomb, quality control of the honey and average production of honey per beehive.

Results and Discussion

The survey results related to gender and age of beekeepers, and apiaries location are shown in table 1.

Table 1. Structure of the beekeepers and location of their apiaries

Gender	Age (%)				Location of the apiary (%)	
	< 25	25 to 40	40 to 55	> 55	Rural	Suburban
Male	0	14	50	36	84	16
Female	0	0	0	0	0	0

Beekeeping mostly takes place in rural areas, which provide ample bees pastures (*Anon. 2014a, Anon. 2014b)*. Inability to purchase honey commercially, retirement and minor

household needs for honey are some of the reasons why people in Montenegro turn to beekeeping as a hobby, mostly in their old age (*Anon. 2014a; Saičić, 2015*).
According to the results, in beekeeping we mainly have men aged between 40 and 55 (50%), while the apiaries are mostly situated in villages (84%). In this study, there were no women beekeepers.

Conditions related to the apiary location are shown in table 2.

Table 2. Conditions related to the apiary location

Conditions at the apiary location	Apiaries (%)
Enough sunlight	100
Sheltered from wind	80
Drained terrain	70
Access to fresh spring water	14
Appropriate distance	84
Access to quality pasture	60

All apiaries have sufficient sunlight (100%), and most of them are situated in places sheltered from wind (80%) and at appropriate distance from neighboring houses, farms, power lines and railroads (84%). However, other conditions are not so satisfactory.

Location of an apiary should be such so as to provide sufficient sunlight which in turn stimulates bee colonies (*Kulinčević, 2006*). However, they should not be in direct sunlight because high temperature causes stress in bees. They must be sheltered from the north wind and rain (*Relić and Relić, 2004*). The terrain must be well drained (*Nedialkov, 1986; Savić and Ćerimagić, 1991*). Apiaries should be located at least 20m away from facilities for breeding animals, 100m away from railways and airports. The minimum distance from sugar refineries and other industrial plants is 500 meters (*Anon. 2014a, Plavša and Nedić, 2015*). Location of apiaries should be such so as to provide access to fresh and safe water in sufficient quantities (*Anon. 2007*). Table 3 shows data on health control of colonies before removals.

Table 3. Health control colonies before removals, frequency of removals and time removals

Moving apiaries (%)	Health control of the colonies before removals (%)			Season of the removals	
	Regularly	Not any more	Never	To the north of the country	From the north of the country
60	56.67	30	13.33	10-25 June	Beginning of October
Moving places	Žabljak, Piva, Šavnik, Pljevlja, Kolašin, Bijelo Polje, Podgorica				

Results from table 3 show that 60% of beekeepers move their colonies and 56.67% of them always check the colony health before removal. Moving to the north of Montenegro starts from 10-25 June, while moving from the north to the central region begins in early October.

The most common places for relocations are Žabljak, Piva, Šavnik, Pljevlja, Kolašin, Bijelo Polje and area of Podgorica. How beekeepers apply good hygiene measures is shown in table 4.

Table 4. Application of good hygiene practice in apiaries

Frequency	Implementation (% of beekeepers)		
	Disinfection of equipment and materials	Disinsection	Deratization
After each use in apiary	40	/	/
Once a year	20	40	40
If necessary	30	/	/
Not implemented	10	60	60

As shown in table 4, most beekeepers (40%) disinfect material and accessories after each use and work with the bees, whereas this measure is not applied by 10% of surveyed beekeepers. That said, we should point out that disinsection and deratization have been not been applied by the majority of the respondents (60% in both categories), while the remaining 40% apply those measures annually. Beekeepers cite lack of information about the importance of hygiene measures intended to prevent the occurrence of infectious diseases of bees and the cost of disinfectants as reasons for their failure to apply disinfectants. Beekeepers must check apiaries at least twice a year (spring and autumn), as well as close environment and space for honey extracting against the presence of rodents *(Anon. 2007; Savić and Ćerimagić, 1991; Anon. 2014b).*

Frequency of queen bee and complete honeycomb replacement over the course of the year is shown in graph 1.
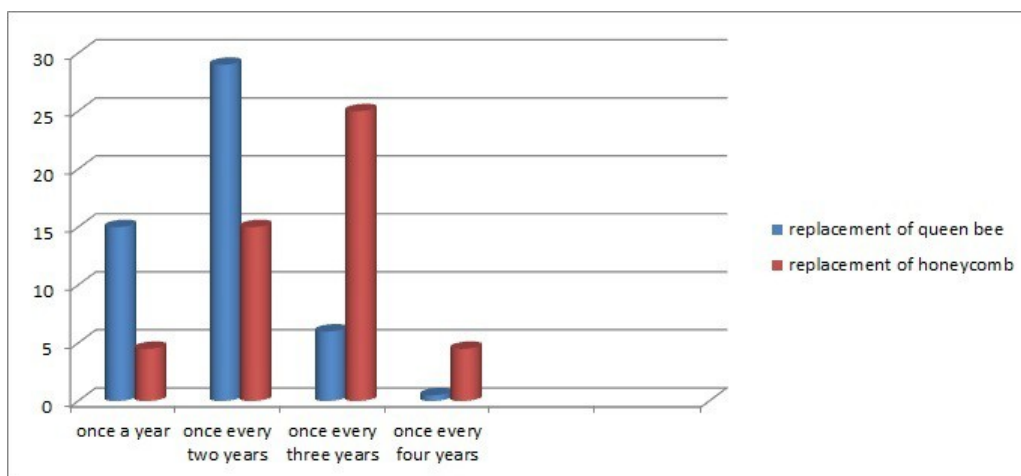
Graph 1. Frequency of queen bee and complete honeycomb replacement

According to the graph 1, replacement of the queen bee is conducted annually by 15 beekeepers (30%), once in two years by 29 beekeepers (58%), and every three years by 6

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beekeepers (12%). Complete replacement of honeycomb is conducted once a year by 5 beekeepers (10%), once in two years by 15 beekeepers (30%), once in three years by 25 beekeepers (50%), and once every four years by 5 beekeepers (10%).

The two main steps required for successful beekeeping are replacements of queens and entire honeycomb in hives *(Kantar, 2013)*. The queen has to be of extremely high quality, disease resistant and healthy, with a good reproductive characteristics *(Kulinčević, 2006; Relic, 2007)*. Young queens are more vigorous and have better reproductive performance compared to the old queen. The replacement of the nuts should be conducted at least every other year (*Anon. 2014b*). Regular renewal of honeycomb, creates the possibility of young bees to satisfy instinct construction and natural instinct for secreting wax (*Kantar, 2013*). Young honeycomb hives held in good hygienic and sanitary conditions, and this acts as prevention against various bee diseases *(Anon 2007)*.

Presence of various nutrients in bee nutrition is shown in graph 2.
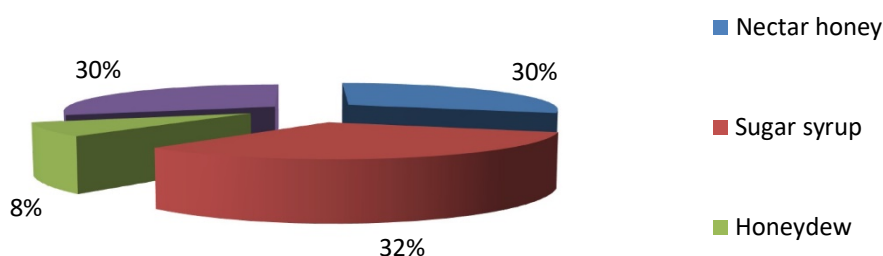
Graph 2. Percentage of nutrients in bee nutrition

According to the graph 2, sugar syrup in bees nutrition is used by 32% of beekeepers; 30% of beekeepers keep bees exclusively by nectar honey and 8% by honeydew, while the remaining 30% implement a combined nutrition.

Production of honey and other bee products is directly related to bee nutrition. Lack of honey and pollen in bee nutrition causes weakening of bee colonies due to a lack of protein in bees (*Kantar, 2013*). Honey for overwintering must be of high quality, the best is nectar honey, with no antibiotic residues, produced in their own household. Not recommended is overwintering for bees on honeydew since the bees fall ill and suffer in great numbers (*Relić and Relić, 2004*). The mistake made by some beekeepers is replacing the nectar honey by sugar products in the nutrition of bees (*Taranov*, 2006). Data on honey production and quality control are shown in table 5.

Table 5. Honey production and quality control

Average production (kg/hive)		Beekeepers who carried out the control of honey (%)
stationary type	migratory type	
8-15kg	20-30kg	40

Data in table 5 show that the average production of honey is better in migratory hives. Unfortunately, only 40% of surveyed beekeepers control the quality of honey.

Honey should be under strict laboratory control against residues of pesticides, antibiotics, industrial sugar and any other foreign matter *(Anon 2007)*.

The main goal of every beekeeper is to have strong and healthy bee colonies and to achieve high production of safe and good quality honey and other bee products. Safety of bee products means absence of any residues of chemicals, drugs and antibiotics. To reach the goal, a beekeeper must implement measures of good beekeeping and good hygiene practices.

Conclusion

Application of good beekeeping practices and HACCP in beekeeping in Montenegro is still unsatisfactory. Education of beekeepers should be focused on the application of hygiene measures in the apiary, prevention measures and methods for control of bee diseases, proper nutrition, beekeeping technology and the importance of quality and health safety of honey and other bee products. Irregular and untimely treatment of diseased bee colonies reduces the resistance of bees, there is resistance to frequently used products that leave traces in wax and honey which in turn undermines safety of the product.

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Poster presentation

FOREWING DIFFERENCES BETWEEN HONEY BEES FROM BANAT AND CENTRAL SERBIA

Nedić N.[1], Mirjanić G.[2], Jevtić G.[3], Anđelković B.[1], Plavša N.[4]

Abstract

The first written record of the varieties of honey bees in Serbia may be found in the work of Grozdanić (1926) who performed morphological comparisons of honey bee samples from Banat with *A. m. carnica*, *A. m. ligustica*, *A. m. cypria* and *A. m. syriaca* and suggested the separation of the bees from Banat into subspecies *A. m. banatica*. Due to the adaptable abilities of bees to specific habitat conditions in the Pannonian plain, it is assumed that different geographic ecotypes were created there.

Because of this the aim of our research was to analyze a part of morphological characters of honey bees from Banat and to compare them with honey bees from Central Serbia, in order to help distinguish and preserve indigenous honey bee varieties.

The samples were collected in three different locations in Banat and one location in Central Serbia. In this study, 14 morphometric characters were measured in accordance with the standard method. By means of a variance analysis very significant differences were determined between the two examined bee groups. On the basis of LSD test we have determined that some honey bee groups from Banat differed very significantly (P<0.01) from honey bees in Central Serbia regarding A4, D7 and E9 angles. Honey bees from Uljma (Southern Banat region) differed very significantly from examined honey bees as regards the size of O26 angle (42.11±3.03º). Group of bees from Central Serbia had a very significantly larger width of forewing in relation to Banat honey bees. Investigation of morphometric characters on the forewing could contribute to a clearer separation of groups of bees inside domestic population.

Keywords: honey bee, morphometry, Banat, Central Serbia

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Introduction

Apis mellifera L. is an indigenous species in Europe, Africa, and Asia (including Saudi Arabia, Iran, and the Ural Mountains in Russia). This species is widespread all over the world due to multiple migrations and introductions by humans (Meixner et al., 2013). Apis mellifera has about 29 subspecies in different geographic regions of the world and there are significant differences between them (Munoz et al., 2009).

Honey bees adapt to different environmental conditions in which they live. In order to achieve excellent production results, for which the beekeepers are most interested, breeders select lines with desirable characteristics (Nedić et al., 2011a). Selection of more productive honey bees with uniform performance can lead to uniformity of bee populations thus reducing genetic variability and adaptation to local conditions (Meixner et al., 2013).

Morphometry is a good method used in taxonomy and systematics of bees (Stevanović, 2002). Set of 36 morphometric characteristics used by Ruttner (1988) was utilized for the most famous biographical honey bee taxonomy and establishment of "classical" morphometry (Čapek and Chlebo, 2016). DuPraw (1965) introduced the measurement of 11 angles on the front wing, which proved to be very useful in research, because these characters are less influenced by environmental conditions and, thus, they represent good indicators of genetic relationships between populations (Meixner et. al. 2007; Abou-Shaara, 2013).

The first written record of the varieties of honey bees in Serbia can be found in the paper published by Živanović (1893) from the end of XIX century. He states that, in Srem, there are two varieties of honey bees, one dark and other resembles the Italian bee (*Apis mellifera ligustica*) because of two yellow rings on the abdomen.

Grozdanić (1926) suggested the separation of the bees from Banat into subspecies *A. m. banatica* based on morphological comparisons of samples of honey bee from Banat with *A. m. carnica*, *A. m. ligustica*, *A. m. cypria* and *A. m. syriaca*. Krunić (1967), studying the variability of honey bees from the area of the Pannonian Plain, concluded that they constitute a homogeneous whole, which should be distinguished as a race *A. m. panonica*. In her later studies, Stevanović (2002) researched honey bees of Sjenica-Pester, Timok and Banat ecotypes. The largest morphological differences were found between the Sjenica-Pester and Banat honey bee ecotypes. According to Mladenović et al. (2011), there is variability in the morphological characters of yellow bees in northern Serbia. Pihler (2012) notes that, in the geographical area of Vojvodina, honey bee populations are reminiscent to the races *Apis mellifera carnica*, *Apis mellifera macedonica*, *Apis mellifera ligustica*, considering their morphometric characteristics.

It is important to identify variation within Serbian honey bee population, in order to help distinguish and preserve indigenous Carniolan honeybee. The aim of this study was to analyze the size of the 14 morphometric traits of honey bee samples from Banat and from Kraljevo (central part of Serbia).

Material and Methods

Samples of 15 adult worker bees (Meixner et al., 2007 and 2013) were collected from 4 different apiaries along Serbia: Gudurica (45º17´07.22´´N, 21º44´80.25´´E) (group I), Vršac (45º13´19.17´´N, 21º35´51.06´´E) (group II), Uljma (45º05´05.81´´N, 21º16´06.52´´E) (group III), Kraljevo (43º70´00.50"N 20º60´51.44"E) (group IV). Bees were preserved in 95% ethanol, and then dissected, and the right forewing were mounted on glass slides and measured using Leica XTL-3400D binocular microscope and software package IL 1009 in accordance with the standard method.

The following characters were used for measurements: angle A4, angle B4, angle D7, angle E9, angle L13, angle J10, angle J16, angle N23, angle K19, angle G18, angle O26, cubital index (CUBI), forewing length (FWL); forewing width (FWW).

Univariate (variance) statistical analyses were conducted for 14 morphological traits of worker bee samples from different locations of the territory of Serbia. A descriptive statistical analysis was carried out and comparisons between locations were determined by LSD Test. All measurements of morphological characteristics for the bees were analyzed by multivariate discriminant analyses.

Results and Discussion

The values of descriptive statistics of morphological characteristics of honey bees from the site of Banat and Royal are shown in Table 1.

Table 1. Means and standard deviation (Sd) of selected measures for the bee samples. Sizes of angles are given in degree (°), size of other morphometrical characters are given in micrometer (μm).

Char.	Origin of Samples (locations)				Average
	Gudurica (I)	Vršac (II)	Uljma (III)	Kraljevo (IV)	
A4	29.96±1.72 ab*	28.91±1.77 a	30.53±2.42 ab	31.22±1.58 b	30.15±2.00
B4	109.77±3.99 a	108.34±5.44 a	108.94±5.41 a	104.70±4.21 a	107.94±5.08
D7	97.83±2.33 b	95.93±3.13 ab	97.13±2.93 b	93.44±2.79 a	96.08±3.22
E9	23.70±1.55 b	22.38±1.44 ab	22.99±1.33 b	21.44±1.46 a	22.63±1.64
L13	14.25±1.25 a	15.03±1.17 a	13.82±1.90 a	14.69±2.04 a	14.49±1.43
J10	56.09±3.29 a	51.22±2.36 a	52.73±2.66 a	52.77±3.88 a	53.67±3.56
J16	90.01±2.03 a	93.45±2.96 a	88.65±3.58 a	92.72±2.10 a	91.44±3.56
N23	94.87±3.19 a	93.61±2.63 a	90.26±2.40 a	91.03±2.23 a	92.57±3.62
K19	77.01±1.44 a	78.18±2.18 a	79.61±2.61 a	79.19±2.07 a	78.36±2.45
G18	91.50±2.06 a	91.26±3.94 a	90.13±4.21 a	89.16±3.51 a	90.69±3.10
O26	37.32±2.82 a	35.39±3.01 a	42.11±3.03 b	37.07±4.33 a	37.97±4.13
FWL	9061.93±109.02	8841.90±97.11	8926.35±155.56	9297.13±123.43	9031.83±211.04
FWW	3207.13±54.74 a	3143.88±91.37	3152.25±76.47 a	3320.37±69.01 b	3205.91±101.24
CUBI	2.54±0.36 a	2.52±0.42	2.45±0.44 a	2.69±0.37 a	2.55±0.40

*Means for the same characteristics followed by different letters within locations are significantly different (P<0.01) according to variance analysis followed by LSD test.

The average value of the angle A4 varied from 28.91° (group II-Vršac) to 31.22° (group IV-Kraljevo). The present results are in agreement with the results of Nedić et al. (2011b). Pihler (2012), in his research, shows slightly higher value of angle A4 from 29.74° ± 4.85° in bees from Banat. Analysis of variance showed that there is a statistically significant difference (P <0.01) between the angles A4 in group II (Vršac) and group IV (Kraljevo). The size of angle B4 in the observed samples ranged from 104.70° (group IV) to 109.77° (group I), with an average value of 107.94°. In his studies, Cermak (1999) states that the

honey bees of Carniolan race had the value for B4 angle of 107.4°. Somewhat different values are stated by Stevanović (2002) for bees of Sjenica-Pešter, Banat and Timok ecotype, that had an average size of the angle B4 109.3°, 111.2° and 109.9°, respectively.

Variation interval of the angle D7 in examined groups of bees ranged from 93.44° to 97.83° with an average value of 96.08°. These results are consistent with the results Stevanović (2002) in which the size of the angle D7 ranged from 94.9° to 96.7°. Kauhausen-Keller and Keller (1994) found a slightly higher value for the test character (97.5°), in their morphological research of bees from lines of *Apis mellifera carnica* from beekeeping institute in Kirchhain. Analysis of variance showed that there is a very significant difference (P <0.01) in the size of the angle D7 between the groups of honey bees from central Serbia and honey bees from the area of Banat (groups I and III).

In the observed groups of bees the size of the angle E9 was the lowest in the group IV (21.44°), and the largest in the group I (23.70°), with an average of 22.63°. The results are consistent with the results Ruttner (1988) who stated value of 12.23 ± 2.08° for the size of the angle E9 for *A. m. carnica*. Slightly lesser values for angle E9 in bees of Sjenica-Pešter, Banat and Timok ecotype (18.9°, 20.0° and 19.7°, respectively) were found in studies conducted by Stevanović (2002). Results of the analysis of variance showed a significant difference between the groups of bees from central Serbia and bees from all three groups from the area of Banat.

Average values for angles L13, J10, J16, N23, K19 and G18 were 14.49±1.43°, 53.67±3.56°, 91.44±3.56°, 92.57±3.62°, 78.36±2.45° and 90.69±3.10°, respectively. Determined differences of average values of these angles between the groups of bees were not statistically significant.

The angle O26 in the observed samples ranged from 35.39° (group II) to 42.11° (group III), with an average value of 37.97°. The results for this characteristic are in accordance with the values for Carniolan race. According to investigation of Maul and Hahn (1994), the size of angle O26 in the reference sample of *A. m. carnica* was 37.97 ± 3.6°. Pihler (2012), in the study of bees from Banat, found the average value of the angle E9 (39.88 ± 5.52°). Analysis of variance showed that the values of these angles differ significantly between sites (P <0.01).

Beside certain angles, the discrimination of groups of bees was very significantly influenced by the other studied morphometric characters on the wings. The data obtained by descriptive statistics (table 1) show that there are variations from 8841.90 μm (group II) to 9297.13 μm (group IV) in the length of the front wing. Bees from Central Serbia (IV) differed significantly for this character (P <0.01) from groups I, II, III and VI of bees from Banat. Also, a very significant difference (P <0.01) in the width of the front wing was found between group of bees from Central Serbia (IV) and groups of bees originated from Banat. Wing width ranged from the 3143.88 μm (II) to 3320.37 μm in the observed samples, with an average value of 3205.91 μm. A similar value of this character (3.23mm) for the bees from Banat was reported by Jevtić (2007).

Value of cubital index in our research ranged from 2.45 (III) to 2.69 (IV), with an average value of 2.55. The values were in the range for Carniolan race. Ruttner (1988) stated that

the value of the cubital index for *A. m. carnica* is 2.589±0.418. No statistically significant difference between the groups of bees was determined for cubital index.

For the characterization of subpopulations within races, multivariate statistical methods may be used to estimate similarities and/or dissimilarities (Kauhausen-Keller and Keller, 1994).
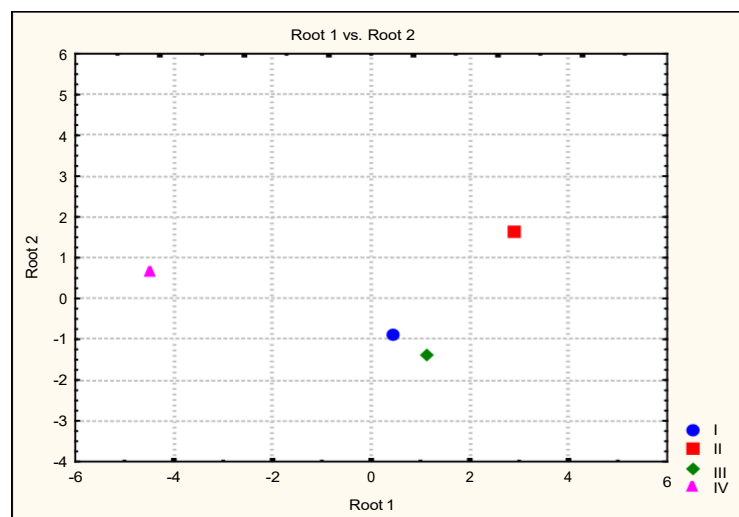
Graph 1. View of the distribution of bee groups centroids
by location (CV1 vs. CV2)

The results of multivariate discriminant analyses of the measurements of the 14 characteristics showed significant differences existed between the four groups of bees (Wilk´s λ = 0.0297, P<0.00001). The 1st axis (CV 1) explained 79.7%, the 2nd (CV 2) 15.4% and the 3rd axis (CV 3) 4.9% of the total variation. Graph 1 shows the distribution of the centroids of the analyzed bee samples according to the first discriminant axis which explains most of the total variability. According to these analyses the local honey bee samples from Kraljevo in Central Serbia (group IV) showed most difference compared to most other groups of bees originated from Banat (Graph 1).

The group of bees from Vršac was the most different from the other two groups sampled bees from Banat (Graph 1). Distinguishing between groups of honey bees is contributed mostly to morphometric characters: angle A4, angle D7, angle E9, angle O26, length and width of the front wings.

Based on the results of this analysis, we could speak about the honey bee population from Banat that are similar to each other and their common difference from the pattern of bees from central Serbia. Various geographical, climatic and botanical conditions in areas have caused a differentiation of Carniolan bee subspecies. Then there is the function of anthropogenic factors, which is reflected in the unplanned replacement of honey bee queens of unknown origin and production capabilities.

Conclusion

Based on the results obtained by morphometric analyses of 14 characteristics of the front wings of bees from the site of Banat and Central Serbia it can be concluded that there is a great phenotypic variability. The analysis of variance showed that the sampled groups of bees highly statistically significantly differed in 6 properties (A4, D7, E9, O26, FWL, FWW).

Very significant differences (P <0.01) between the bees from the area of Banat and groups of bees from central Serbia occurs in 35.7% of the observed characteristics. Very significant differences (P <0.01) between groups of bees from the area of Banat occurs only in 14.3% of the observed characteristics.

Results of the multivariate discriminant analysis show significant differences between the sampled group of bees (Wilk's $\lambda = 0.0297$, P <0.00001), where a group of bees from Central Serbia was clearly distinguished in relation to the sampled bees from Banat.

The values of certain morphometric characters of examined especially to the group of bees from Kraljevo showed a deviation from the standard values for Carniolan honey bee race. Variability in honey bee phenotype determined for groups of bees from Banat indicate the existence of a population of bees that mutually differ less in comparison with bees from central Serbia. Bees from the Banat area were sampled in the plain area, while the bees from central Serbia were sampled from the area that can be considered mountainous. Various environmental factors like relief and flora influenced the adjustment of bees to different conditions of life prevailing in these two areas, and are able to influence the inter-variability examined groups of bees. We should also not rule out the influence of man through migratory beekeeping, free replacement of honey bee queens of unknown origin and with it the possibility of an impact on the existing bee populations in Serbia.

Research of characters of the front wings has contributed to the separation of the sampled group of bees. Determined variability provides an opportunity for further promotion and selection of bees that meet the criteria for the morphometric traits established for the *Apis mellifera carnica*.

Acknowledgment

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Poster presentation

VARIABILITY OF WING SIZE AND WING INDEXES IN HONEY BEES FROM SERBIA

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Abstract

Morphological traits of honey bees have a very significant impact when determining taxonomic categories. Besides its importance for the productive activity of honey bees, wing size (length and width) has significance in determination of the honey bee race. Wing indexes are very important because, with correlation with the discoidal shift, they clearly distinguish *A. m. carnica* (carniolan honey bee) and *A. m. lingustica* from dark European honey bees. The aim of this study was to determine the variability within and between honey bee colonies for the observed traits. The research was conducted using honey bee colonies of the Institute for forage crops that originated from the areas of Kruševac, Kraljevo, Pešter, Kopaonik, Tupižnica and Banat (Bečej). The following traits were measured: the length and width of the front wing, wing indexes (cubital, precubital and dumb-bell) and discoidal shift. The study included 20 colonies with 50 individual honey bees per colony. Using descriptive statistics and analysis of variance (ANOVA) it was determined that there was variation in all of the studied traits. It was found that the average length of the wing was 8.50 mm and average width was 3.30 mm. Two homogeneous were separated considering the wing length, while for wing width there were three homogeneous groups. Cubital index and the discoidal shift showed two homogenous groups while precubital index and dumb-bell index showed three homogeneous groups. The largest coefficients of variation were found for cubital index and the discoidal shift.

Keywords: honey bee, variability, wing size, wing indexes

Introduction

Honey bees have membranous wings that are imbued with chitin nerves that serve to give rigidity and strength to the wings. Among those chitin nerves, dominant are longitudinal nerves (costa, subcosta, radius and medius) that are connected by transverse nerves (spokes - radioles) which extend in all directions. Longitudinal and transverse nerves cross each other, creating cells in wings. Morphological characteristics represent a very useful tool for identifying different populations of honey bees, and the most significant among these characters are the characteristics associated with the traits of the wing (Alpatov 1929, Ruttner 1988). Initially, the biometric measurements on the wings were significant for determining the different races and strains of honey bees. Later, these measurements become important in distinguishing the different subspecies and populations and as a

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markers of some other biological characteristics. Morphological characteristics can be the basis for determining the different races and ecotypes of honey bees (Rutner, 1988). On that basis, numerous morphometric studies were conducted, both in the wider region as well as on the territory of Serbia, whose results show the diversity of Carniolan honey bee in the former Yugoslavia (Stojanović 1992; Bubalo et al., 1994; Plužnikov 1994; Stevanović 2002 Pihler 2011; Nedić et al., 2011; Rasić 2013; Jevtić et al., 2015).

Numerous morphological parameters were used for the determination of race, but out of all of them, the most accurate are the parameters of wing nervature. They are especially significant because the wing indexes are not affected by environment (it represents relationships of individual measures), as opposed to the length of the tongue, weight, length of the tibia, width of tomentum (they represent absolute dimensions), which may vary depending on the size of the wax cell in which the bees were bred, as well as by other external factors (Rinderer, 1986).

The aim of this study was to determine the morphometric parameters (size of the wing and the wing indexes), which we can point out the racial affiliation of bee colonies, which were later used in the pollination of forage legumes (red clover). Also, intra-group variability of the observed properties of the colonies was determined, which will help us in further selection of these honey bee colonies.

Material and Methods

In this paper we analyzed honey bees from 20 colonies from the apiary of Institute for forage crops in Kruševac. These colonies were previously collected from 6 different sites in Serbia. Three sites were from mountainous regions (Pešter, Kopaonik and Tupižnica near Knjaževac), and three were from lowland regions (Banat-Bečej, Kraljevo and Kruševac). These colonies are set for many years on the same apiary, where the selection was conducted. From each colony 50 bees were sampled. After sampling, bees were kept in 96% ethyl alcohol and stored in a refrigerator at a temperature of -4°C until measurement.

Temporary isolated anatomical preparations were first scanned in resolution of 9600 dpi and then processed using computer software DrawWing (Tofilski, 2011). Length and width of the front right wing and the discoidal shift were measured and standard indexes were calculated (cubital, precubital and dumb-bell index).

Morphometric measurements of angles are performed by standard method by Ruttner et al., (1978). After measurement, for all parameters were calculated values of descriptive statistics: mean value, standard deviation, extremes and coefficient of variation. In addition, the analysis of variance was performed for the observed angles as well as the appropriate tests between the colonies.

Results and discussions

After the measurements, it was found that the average length of the front right wing of the observed colonies was 8.50 mm and width was 3.30 mm (Table 1). There was a much higher coefficient of variation for width of the wings in relation to the length, and the interval of variation was greater for length than for the width of the wings. Cubital index was 2.60 which shows that these colonies belong to the race of Carniolan honey bee (*Apis mellifera carnica* Poll). This index also has a high coefficient of variation and a large interval of variation. Pre-cubital and dumb-bell index had lower coefficients of variation and significantly lesser interval of variation (IV). Discoidal shift was 3.80 and it had the greatest variation, with a coefficient of variation of 15.87%, and the interval of variation of 2.16. Pihler (2011) found that the highest coefficient of variation for cubital index (16.67%), dumb-bell (8.57%), discoidal shift (6.54%) and the lowest variation for pre-cubital index (5.11%).

Table 1. Average value, standard deviation, coefficient of variation, extremes and interval of variation of the observed characteristics

Characteristic	Wing length	Wing width	Cubital index	Pre-cubital index	Dumb-bell index	Discoidal shift
Average	8.50	3.30	2.60	2.71	1.09	3.80
St.dev.	0.30	0.29	0.29	0.10	0.06	0.36
CV	3.54	8.72	11.00	3.68	5.51	15.87
min	7.62	2.80	2.10	2.54	0.99	2.72
max	9.31	3.80	3.10	2.87	1.19	4.88
IV	1.69	1.00	1.00	0.33	0.20	2.16

Considering individual colonies, it was found that there was variation for all observed traits between them. By testing the values for wing length it was determined that two homogeneous groups were distinguished (Table 2). Maximum wing length was found in the colonies 17, 6 and 1, and the minimum length was found in colonies 5,15,18,7,13 and 11. These colonies statistically differed between each other, but there were no differences when compared to other colonies.

Considering the wing width, there were three homogeneous groups, and the largest width was found in the colony 1 (3.36mm), and the lowest in the colony 17 (3.22mm). The colony 1, in addition to the widest wings, also had a very long wings, while the colony 17 had the longest and the narrowest front wings.

The results were slightly lower than the results by Krivcov and Lebedev (1992) who stated that Carniolan bee has wings in the range of 9.0 to 9.4 mm, as well as the results by Stojanović (1992) and Georgiev (2000). Our results are lower than results previously obtained in the same apiary (Jevtić and Mladenović 2008), which were obtained using an optical microscope to measure the morphometric characteristics. Rasić (2013) in his research found the longest front wings in colonies from Vršac in 2010 (9.09 cm), and with the shortest in colonies from Vranje in 2009 (9.00 cm). These results are however significantly higher than the results obtained in studies done by Stevanović (2002).

The results for the width of the wings are higher than the results found by Mladenović et al., (2011); Rasić (2013), and are higher than the results that have been obtained in the same apiary by Jevtic, 2007.

Table 2. Average values and t-test for length and width of front right wing

Front wing			
Length		Width	
Colony	Average	Colony	Average
5	8.42[a]	17	3.22[a]
15	8.44[a]	18	3.24[ab]
18	8.44[a]	3	3.24[ab]
7	8.45[a]	5	3.25[abc]
13	8.45[a]	8	3.26[abc]
11	8.45[a]	11	3.27[abc]
14	8.48[ab]	12	3.27[abc]
16	8.48[ab]	2	3.27[abc]
4	8.49[ab]	20	3.28[abc]
9	8.49[ab]	13	3.30[abc]
3	8.50[ab]	15	3.30[abc]
12	8.51[ab]	7	3.31[abc]
19	8.51[ab]	14	3.31[abc]
2	8.51[ab]	16	3.32[abc]
10	8.52[ab]	9	3.32[abc]
8	8.53[ab]	4	3.32[abc]
20	8.54[ab]	19	3.33[abc]
1	8.59[b]	10	3.34[bc]
6	8.59[b]	6	3.34[bc]
17	8.59[b]	1	3.36[c]

Cubital index ranged from 2.55 (colony 11) to 2.68 (colony 7) (Table 3). These two colonies have differed in comparison to other colonies. For this characteristic, two homogenous groups were distinguished. Considering that the colonies used in this study were collected from the wider area of Serbia, but that most were from area of the West Morava (Rasina valley, Morava valley, Kopaonik), it is obvious that the values of the cubital index show that the colonies belong to *A. m. carnica* and are similar to the results of Konstantinović (1965). Examining the bees from Sjenica-Pešter plateau, Stojanović (1992) stated that the cubital index varied from 2.58 to 2.69 and was significantly different from the cubital index bees from Macedonia. Examining the populations of yellow bees from Vojvodina and Slavonia, Krunić (1967) found values for cubital index of 2.96. Rašić (2013) found the highest value for cubital index in colonies line from Sutomore (2.68), and lowest value in bees from Bijelo Polje (2.57).Kozmus (2008) studied colonies of Carniolan honey bees and he found the highest cubital index in colonies from the Czech Republic (3.07), while the bees from Greece (2.34), Croatia (2.46) and Slovenia (2.53) had very similar values .

Table 3. Average values and t-test for wing indexes in studied colonies

Wing indexes							
Cubital index		Pre-cubital index		Dumb-bell index		Discoidal shift	
Colony	Average	Colony	Average	Colony	Average	Colony	Average
11	2.55^a	3	2.69^a	4	1.06^a	9	3.65^a
10	2.57^{ab}	5	2.69^a	11	1.07^{ab}	19	3.69^a
9	2.57^{ab}	2	2.69^a	19	1.08^{abc}	4	3.73^a
15	2.58^{ab}	20	2.69^a	3	1.08^{abc}	7	3.74^a
5	2.58^{ab}	1	2.69^{ab}	17	1.08^{abc}	8	3.74^a
4	2.58^{ab}	19	2.69^{ab}	14	1.08^{abc}	2	3.76^a
16	2.58^{ab}	16	2.69^{abc}	18	1.08^{abc}	14	3.76^a
1	2.59^{ab}	13	2.70^{abc}	20	1.08^{abc}	10	3.80^{ab}
13	2.59^{ab}	6	2.70^{abc}	1	1.08^{abc}	12	3.80^{ab}
18	2.59^{ab}	4	2.70^{abc}	7	1.09^{abc}	20	3.80^{ab}
3	2.59^{ab}	8	2.70^{abc}	6	1.09^{abc}	11	3.80^{ab}
14	2.59^{ab}	9	2.70^{abc}	10	1.09^{abc}	5	3.82^{ab}
6	2.59^{ab}	17	2.71^{abc}	15	1.09^{abc}	16	3.82^{ab}
8	2.62^{ab}	7	2.71^{abc}	8	1.10^{abc}	18	3.83^{ab}
2	2.62^{ab}	12	2.71^{abc}	12	1.10^{abc}	13	3.84^{ab}
20	2.63^{ab}	14	2.71^{abc}	16	1.10^{abc}	3	3.85^{ab}
12	2.64^{ab}	10	2.71^{abc}	5	1.10^{abc}	15	3.85^{ab}
17	2.65^{ab}	15	2.72^{abc}	9	1.10^{bc}	1	3.87^{ab}
19	2.65^{ab}	18	2.73^{bc}	2	1.10^{bc}	17	3.87^{ab}
7	2.68^b	11	2.74^c	13	1.11^c	6	4.00^b

The lowest value of pre-cubital index was 2.69, and the highest was 2.74. For this characteristic, colonies divided into three homogeneous groups. Dumb-bell index had three homogenous groups and discoidal shift showed two homogeneous groups of honey bee colonies.

Comparing the bees from Vojvodina, Pihler (2011) found that the value of the cubital index was 2.34, pre-cubital index was 2.74, dumb-bell index 1.05 and discoidal shift was Sift 1.53. These results are similar to results obtained in our research, in addition to the value of the discoidal shift that drastically differ.

Conclusion

Based on the results obtained by analyzing the size of the wing and the values of wing indexes of the honey bees from the Institute for forage crops, the following conclusions can be made:

It was found that the average wing length was 8.50 mm and average wing width was 3.30 mm. When it comes to the wing length there were two homogenous groups, but when it comes to wing width there were three homogenous groups.

Association number 17 had the highest length and the smallest width of the wings. The Colony's No. 1 had the longest and widest wings.

The average value of the cubital index was 2.60 which shows that the colonies belong to the race *Apis mellifera carnica*. Colonies were divided into two homogeneous groups. Colony with the lowest and highest values differed significantly.

Discoidal shift had two homogenous groups, pre-cubital and a dumb-bell index had three homogeneous groups of colonies.

The highest coefficients of variation were established for cubital index and the discoidal shift.

Acknowledgment

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Poster presentation

THE EFFECT OF WORKER BEE AGE *(APIS MELLIFERA CARNICA)* IN PRE-WINTER PERIOD ON CRUDE PROTEIN CONTENT

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Abstract

Proteins play a very important role in vital functions of worker bees. Protein content of bee body is an indicator of its vitality and resistance. This thesis deals with crude protein content of the whole honeybee body at different ages. Honey bee variety *Apis mellifera carnica* has been used in this investigation, and the trial has been carried out at the apiary of Faculty of Agriculture in Belgrade. Worker bees were divided into five different categories of age: emerging bees, foragers, nurses, bees from penultimate frame of deep super, and bees from honey super. Sample size was fifty bees from each group. Protein content was determined by the method according to Kjeldahl (automatized method). The obtained data were processed statistically, analysis of variance and tests of differences significance were carried out. Differences in protein content between investigated groups were highly significant. Few other parameters including dry matter and water content, as well as living bee mass, were investigated in addition to above mentioned ones.

Keywords: proteins, age, worker bees, *A. mellifera carnica*

Introduction

Obtaining protein profiles of an organism is the basis for assessing several aspects of biological processes. The protein content and protein composition in hemolymph, whole body or specific tissue extracts can provide valuable information on developmental stage, reproductive potential, aging processes, health status and correlated processes. Pollen is the main natural source of protein for bees; nectar collected by bees contains rather low concentrations of amino acids (*Baker and Baker, 1973*). Pollen is necessary for a colony has been known for a long time. Many papers dealt with the types of food foraged by the colonies, the influence of artificial food or the fate of foodstuffs taken up by bees. Nevertheless, many questions concerning protein metabolism remain unanswered, due to the complexity and plasticity of the social system of the bees (*Crailsheim, 1990*). One way of obtaining protein is cannibalism. In certain situations, such as lack of proteinaceous food, workers may eat eggs and brood (*Haydak, 1935*). This thesis will show differences between some age categories of bees and their function in beehive.

Material and Methods

Experiment was conducted from beginning of July and lasted till the end of August 2014. Five groups of bees of different age category were formed. Youngest category were emerging bees, then second category were working bees who feed the brood, third category were working bees from brood frame, fourth category were bees from storage frame, and fifth group were gatherer bees. During sampling fifty bees of each category were taken. Weende method was used to

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determine exact share of researched parameters of bee body. Bees were captured with tweezers, after capture bees were put in thin transparent bags. All bags were tagged, on the tag was written group to which captured bee belongs, date and number of the captured bees.

Samples were dried in the dryer at 105^0C. All samples were crushed in ceramic mortar till they became fine powder, after that, they were divided for further protein content determination. In the experiment live bee body mass was measured, amount of water in bee body was also determined along with dry matter content. Water content in bee body was determined by drying the sample at 105^0C till it achieved constant mass. With this method dry matter is directly determined and indirectly water content. Drying and measuring was repeated several times till constant mass of dry sample along with vessel was obtained. From the difference of the masses of full and empty vessel the mass of sample is calculated (Q), further percentage of water content is being determined using the formula:

% water content = (A-B) x 100/Q
A – weight of vessel with the sample before drying;
B – weight of vessel with the sample after drying;
Q = weight of the sample;
Multiplication by 100 is done in order to convert amount of water (moist) in percentage *(Đorđević et al. 2003).*

Determining of protein content using Weende method is done indirectly, using nitrogen content which is determined using Kjeldahl method. The value obtained in that way is further multiplied by factor 6,25. The aim is to determine crude proteins, because nitrogen is not compound of only proteins, but of other matters such are nitrates, nitrites, purine and pyrimidine alkalis, ammonia, urea, amino acids, some vitamins. Therefore obtained protein result is called crude proteins.

To obtain proteins Tecator system (Kjeltec Auto) was used and higher precision was achieved compared to manual titration method.

Before protein destruction it is needed to dissolve the sample with sulfuric acid. 0,2-0,5g of sample is measured and taken to kjeltec kivette, then 0,1g of selenium catalyzer is added and 15ml of concentrated sulfuric acid. Destruction of sample is speeded by adding small amount of selenium catalyzer or special tablets that contain catalyzer. Sample that is prepared in that way is cooled down and diluted using 50ml of distilled water.

Entire procedure takes approximately three minutes, for the entire amount of the prepared sample to be distilled in to titration vessel and neutralized with hydrochloric acid, titration is over when the colour of liquid in titration vessel turns red. When the level of content in the titration vessel reaches certain electrode, aperture stops working and display shows the value of titration liquid in milliliters. Amount of protein is calculated using the formula:

% protein = (14,01 x M x f x 6,25 x 100)/(mg sample) = (ml titration liquid – ml blind test result), where:
M = hydrochloric acid concentration in mol/l;
f = hydrochloric acid factor;
6,25 = standard Kjeldhal factor;
14,01 = atomic nitrogen mass. *(Đorđević et al., 2003).*

Results and Discussion

Research results regarding the water content, dry matter content and bee body weight are represented in *Table 1*.

Table 1. Additional research

Parameter	Water content	Dry matter content	One bee body weight
Bee	67,473 %	32,527 %	0,098 g

Data obtained in the research are in compliance by results obtained by *Jezef J.(2011),* whose results show that total mass that is lost when bees were dried was 68%.

In the *Table 2* data show 100% dry mater comparison is presented. Double measuring was done.

Table 2. Dry matter content *(%)*

Sampling time	Bees coming from:					
	Working bees	Brood bees	Storage bees	Open brood bees	Emerging bees	Average
I measuring	70,71	38,92	49,92	37,93	67,70	53,04
II measuring	64,30	48,82	44,95	43,14	68,59	53,96
III measuring	64,90	37,87	40,72	39,05	68,32	50,17
IV measuring	67,75	35,47	38,50	41,51	69,28	50,50
V measuring	65,07	42,05	43,43	39,44	69,64	51,93
Average	66,55	40,62	43,50	40,22	68,71	51,92
SE	1,20	2,31	1,95	0,93	0,34	0,72
SD	2,68	5,16	4,36	2,08	0,77	1,62
Variance	7,18	26,58	19,03	4,35	0,59	2,62
Minimum	64,30	35,47	38,50	37,93	67,70	50,17
Maximum	70,71	48,82	49,92	43,14	69,64	53,96
CV%	4,03	12,69	10,03	5,18	1,12	3,12
F=89,9860 (P=0,0000)						
$LSD_{0,05}$=4,48 $LSD_{0,01}$=6,11						

Based on F value and LSD test, it can be concluded that there are significant differences between groups. Between bees coming from brood, storage and open brood there are no significant statistic differences in amount of crude protein content. Between worker bees and emerging bees there is statistically very significant difference in crude protein content compared to other three categories, while between them there is no significant statistical differences.
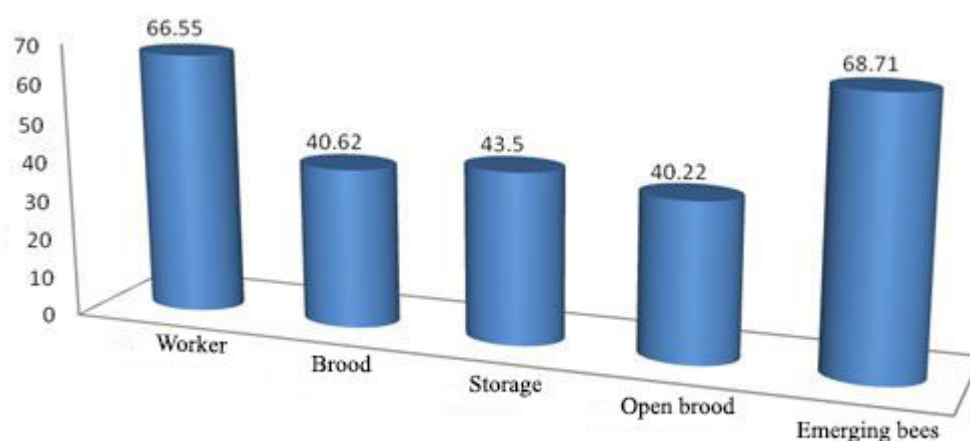
Graph 1. Amount of crude proteins in bee body (%)

Based on the experiment we can conclude that:

- It can be noted that perceiving of all parameters is almost impossible to conduct for the bees. Next to these specific factors there are many unspecific factors that influence results: queen quality, social harmony, origin of spermatozoids etc. For example first and last sample comes from same queen bee, but not from same father, which implies that worker bee genotype is different therefore expression and protein levels differ.

- Emerging bees had significantly higher protein levels because all newborn beings have higher level of proteins than fully grown developed individuals, which can be explained by high protein diet (royal jelly, bee bread and pollen)

- Adult worker bees, compared to them are the eldest individuals and it is expected that their protein content is the lowest. However, value that we obtained was high because adult worker bees were coming from cleansing brood with full digestive system, where specially in lower parts is high level of crude protein. Therefore the intestinal content influenced the protein levels, as well as autumn bees who are preparing for winter period.

- Bees coming from open brood have active milk glands and are being fed with high protein food such as pollen, bee bread and royal jelly. According to obtained results this category had least amount of crude protein, most probably because this bees use their protein reserves to feed the brood.

Bees coming from open brood according to *Haydak*-u *(1935)*, are nursing bees who use their own protein reserves to feed larvae therefore they had lowest crude protein content in abdominal level which was 11% lower than for other bees.

Usually higher protein level in bee body has positive influence on their longevity. Bees that have high level protein in the body (over 60% crude protein), are biologically stronger and can collect higher amounts of nectar, while bees that contain low amount of protein (less than 30%) are biologically weaker, have shorter life span and are more sensitive to diseases also they collect lesser amount of nectar *(Kleinschmidt 1988)*.

Proteins make 66–74% of fully adult worker bee body (*Hrassnigg and Crailsheim,* 2005). Protein content in bee body is increased in the first several days (*De Groot*, 1953) during the protein anabolism (*Crailsheim*, 1990) and it slowly lowers while bee ages. In the moment of birth, bees that are destined to survive winter had more protein in the body than summer bees (*Kunert and Crailsheim*, 1988).

Conclusion

Based on experimental work which started in July and August 2014 on the bee farm that belongs to University of Belgrade, Faculty of Agriculture in Belgrade and analysis of samples that were tested in the laboratory for animal nutrition, Zootechnics department of the same faculty it can be concluded that:

- Highest level of crude protein was obtained by the emerging bees and adult worker bees (averagely 67%), while level of crude protein coming from brood, storage and open brood bees was significantly lower (averagely 42%).

According to results it can be concluded that amount of crude protein in worker bees *(Apis mellifera carnica)* varies depending on bee age and their position (caste) in the hive, which is of very high significance for the science and for production. These experiments can be used for further researches.

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Poster presentation

THE EFFECT OF FEEDING BY FEEDBEE SUPPLEMENT ON SPRING DEVELOPMENT OF HONEYBEE COLONIES

Slobodan Dolašević[1], Nebojša Nedić[1], Nenad Đorđević[1], Mića Mladenović[1]

Abstract

If a honeybee colony lacks sufficient supplies of proper food, the beekeeper is forced to compensate that shortage by various supplements. A highly frequent supplementation method of honeybee colonies is their additional feeding by various types of patties. Two patty types have been used in this trial: one of them was sugar patty, and the other one was protein patty with the supplement Feedbee. The investigated honey bees races was Carniolan bee (*Apis mellifera carnica*). In order to evaluate influence of patty with Feedbee supplement on colony development, two groups of honeybee colonies were formed. One group received patties with Feedbee supplement, while the other received regular sugar patties. Observation and measurement of differences in honeybee productive properties was carried out by control examinations, where we established number of bees, as well as area of brood, honey and pollen in 1/10 of a frame. Development of bee colonies with Feedbee supplement patties was less affected by weather conditions which usually prevent normal working of honeybees. The observed differences between the variant with Feedbee patty and the control one in quantity of brood, bees, honey and pollen were not statistically significant.

Keywords: supplementation, patty, Feedbee, spring development, *Apis mellifera carnica*

Introduction

The development and the survival of honey bee colonies are intimately associated with the availability of environmental nutrients *(Brodschneider R, Crailsheim K, 2010).* Floral nectar, containing carbohydrates and stored as honey, is the energetic fuel of individuals, and pollen provides most of the nutrients required for their physiological development *(Brodschneider R, Crailsheim K, 2010).* Pollen is the source of proteins, lipids and minerals consumed by nurse bees, converted to nutrient rich royal jelly in hypopharyngeal glands and distributed to growing larvae and other hive members. Nutrition deficiency and starvation are likely contributing reasons for colony losses around the world *(Van Engelsdorp et al., 2009*). Evaluating pollen quality by measuring colony growth and development would provide the most pertinent information about its potential impact on honey bee fitness *(S.F. Pernal, R.W. Currie, 2000).*

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In early spring before pollen and nectar are available or at other times of the year when these materials are in short supply, supplementary feeding may help the colony survive or make it more populous and productive. If the spring weather is unusually cold and rainy, colonies may need supplemental foods for subsistence and continued brood rearing until nectar and pollen can be collected *(L. N. Standifer, 1980)*. During the shortage or complete absence of pollen, or in the presence of only poor quality pollen, beekeepers often feed colonies of honey bees with either pollen substitute (with no pollen) or supplement (with pollen) diets. Currently there are a lot of commercial pollen substitutes for honey bees in the: Bee-Pol®, Bee-Pro®, FeedBee®, MegaBee® etc. Feed-Bee® and MegaBee are non-soy-based. Commercially available pollen substitute diet for honey bees were evaluated for consumption and colony growth (brood and adult populations, amount of nectar and pollen) and compared with pure sugar cake.

Material and Methods

The experiment has been performed at the apiary of Agriculture faculty in Belgrade, in the period between March and April of 2012. with *Apis Mellifera carnica*. During the experiment two types of cakes were used, one was made out of sugar and the other was protein Feedbee cake. The ingredient used in the Feedbee cake were proteins 36,4%, fat 3,9%, carbs 41,8, sugar 10% and minerals 3,1%. Feedbee doesn't content pollen or any other bee product. Commercial pollen can be infected with various types of microorganism that may cause a lot of damage to the colony, or it may content toxic pollen. Two equally strong group of strong colonies were formed. To the one group has been added Feedbee cake and of the other group of bee colonies common sugar cake (the cake without additives so called "O" cake). Bee colonies were equally strong by number of bees, brood, honey and pollen. Trucking and control of development has been done at the period of 19.03.14. till 26.04.14. Once a week every little change in all existing parameters was written down in detail. Data collecting system is like so the measurements were done in 1\10 frames. After every data collecting what followed was there detail classification processing, analyses and compering between control and compered group. Besides parameters (number of bees, brood, honey, and pollen) what was tracked we also examine the amount of consumed cake. The final goal in this experiment was assessment and compares of effectiveness of the pollen cake and sugar cake on the development of a bee colony.

Results and Discussion

After formation of test group and planned treatment we accessed data collecting who have been statistically processed and show in the following tabs. Analysis of the quantity of bees, brood, honey and pollen in both test groups have been performed, also the analysis of differences between common sugar cake and Feedbee cake have been performed.

Analysis of the bees
In the graph number 1. is shown the number of bees expressed in 1/10 frames through different dates during the experiment.

Graph 1. Variation in the number of bees amount of bees feed by different type of food (in 1/10 frame)
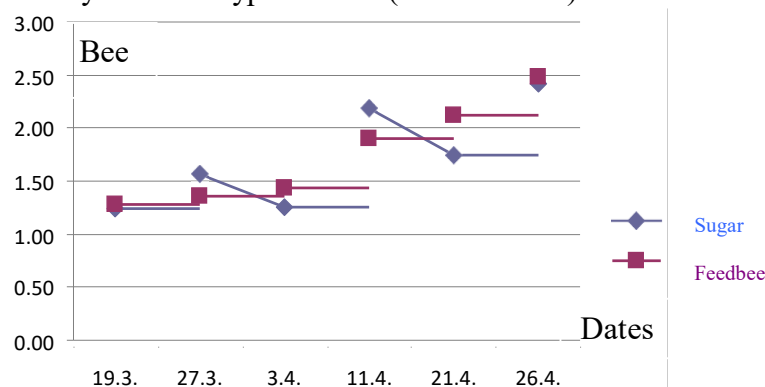
Table 1. Average (in 1/10 frame)

Date	Sugar	Feedbee
19.3.	1.24	1.28
27.3.	1.56	1.36
3.4.	1.26	1.44
11.4.	2.18	1.90
21.4.	1.74	2.13
26.4.	2.42	2.48
$\sum$	10.4	10.59
$\bar{x}$	1.73	1.76

You can see the variation (Graph 1. and Table 1.) in the number of bees are significantly more expressed in the group of colonies with common sugar cake and no oscillations with the use of Feedbee cake which with components is suitable for bee colony and the mood of the queen and her ability to lay eggs and her activity. Luck of pollen or low quality of pollen results in stagnation in the population, low weight and progress of young bees, lower development of hypopharyngeal gland and so the production of eggs by the queen bee.

Analysis of brood

In graph 2. and table 2. it's shown the variation of brood surface in both groups.

Graph 2. Variation of brood surface feed by different type of food (in 1/10 frame)
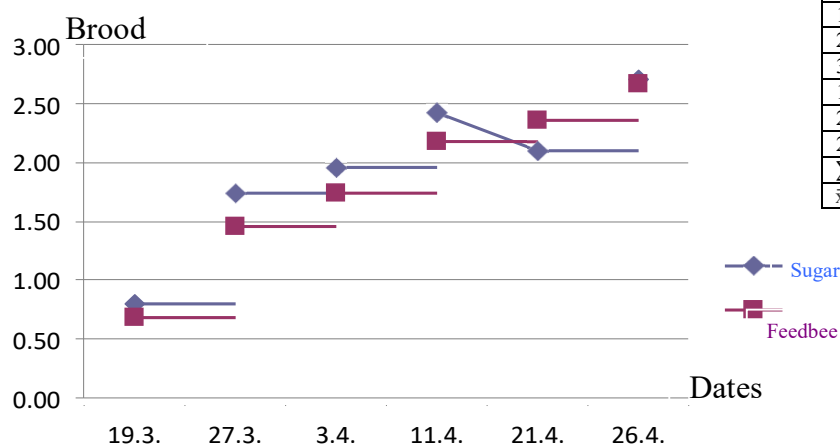
Table 2. Average amount of brood surface (1/10)

Date	Sugar	Feedbee
19.3.	0.80	0.68
27.3.	1.74	1.46
3.4.	1.96	1.74
11.4.	2.42	2.18
21.4.	2.10	2.35
26.4.	2.70	2.66
$\sum$	11.72	11.07
$\bar{x}$	1.95	1.84

In the graph number 2. we can see that the surface of brood from the beginning experiment till the and has been the same. However graph show us the variation in the group with common sugar cake are more expressed than the group with Feedbee cake. This can be interpreted with weather conditions and environments which are conditioned by higher or lower incoming nectar and pollen that are positively correlated with intensity of queen laying.
Pollen analysis

Feedbee cake is pollen substitute food that can be consumed by the bees instead of pollen. The following chart (Graph 3.) is the result of all together pollen quantity during the experiment.

Graph 3. Variation of pollen surface feed
    by different type of food (in 1/10 frame)
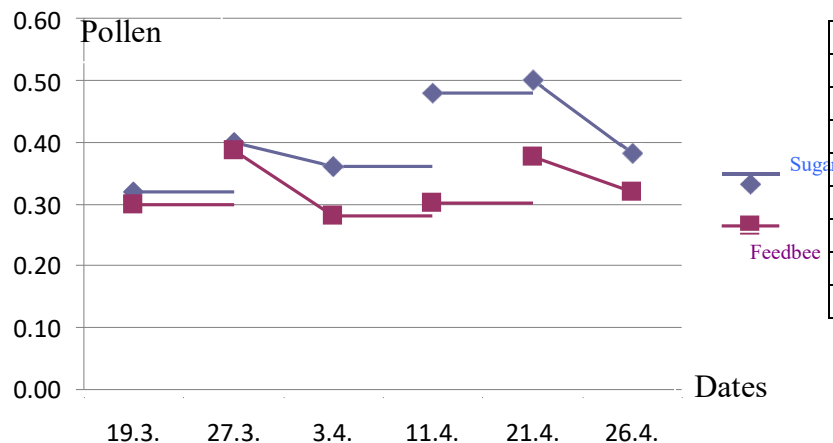
Table 3. Average surface of the pollen (in 1/10 frame)

Date	Sugar	Feedbee
19.3.	0.32	0.30
27.3.	0.40	0.44
3.4.	0.36	0.28
11.4.	0.48	0.30
21.4.	0.50	0.38
26.4.	0.38	0.32
∑	2.44	2.02
x̄	0.41	0.34

Surface of the pollen was equal in both groups (Table 3.) at the very beginning so it was at the very end. You can clearly see that the surface of the pollen in the Feedbee group had a much less need for collecting pollen because of the source of protein in Feedbee cake. In beekeeping practice, beekeepers very often adding stimulating cake with pollen substitutes. Presence of pollen in cakes trigger queen for better egg laying production and colony development.

Honey analysis

With the graph 4. and table 4. we can see that quantity of honey was always decreasing. This is probably the time when honey flow is too low, and when just little of sporadic flowering plant in parks. In this period bee colonies have huge need for proteins and energy due to maintain microclimate of the hive, and because of rearing brood. That is why the bees consume honey supply. It is common that at the end of April, there is a mild stagnation in consumption of honey, in fact daily allowance is equal to the daily consumption. However, in the group that had Feedbee cake is observed intense intake of nectar in the end of control period, which is of great importance as it will in the first honey flow these colonies make more honey, which is one of the main objectives in the successful beekeeping.

Graph 4. Variation of the honey surface feed by different type of food (in 1/10 frame)
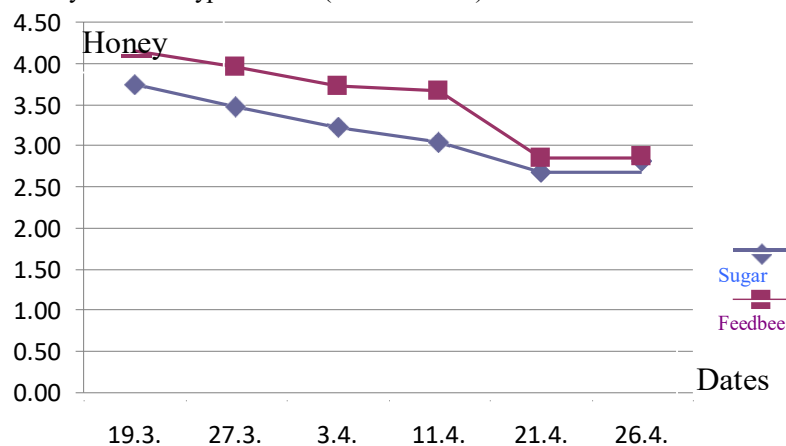
Table 4. Average amount of honey (in 1/10 frame)

Date	Sugar	Feedbee
19.3.	3.74	4.16
27.3.	3.48	3.96
3.4.	3.22	3.72
11.4.	3.04	3.66
21.4.	2.68	2.85
26.4.	2.82	2.88
∑	18.98	21.23
x̄	3.16	3.53

The dates of amount of bees, surface of brood, pollen and honey, are except tabular and graphic display, especially processed with descriptive statistical analysis and shown in Table 5.

Table 5. Descriptive statistic of all included parameters

Descriptive stat.   Bees

Group	Average	N	SD	Min	Max
1	1.73	6.00	0.48	1.24	2.42
2	1.76	6.00	0.48	1.28	2.48
All Grps	1.75	12.00	0.46	1.24	2.48

Analysis of variance

	SS	Df	MS	SS	df	MS	F	P
Bees	0.002852	1	0.002852	2.332454	10	0.233245	0.012228	0.914138

	Brood				
Group	Average	N	SD	Min	Max
1	1.95	6.00	0.66	0.80	2.70
2	1.85	6.00	0.71	0.68	2.66
All Grps	1.90	12.00	0.66	0.68	2.70

	SS	Df	MS	SS	df	MS	F	p
Brood	0.035208	1	0.035208	4.720483	10	0.472048	0.074586	0.790330

	Honey				
Group	Sr. Vr.	N	SD	Min	Max
1	3.16	6.00	0.40	2.68	3.74
2	3.54	6.00	0.55	2.85	4.16
All Grps	3.35	12.00	0.50	2.68	4.16

	SS	Df	MS	SS	df	MS	F	p
Honey	0.421875	1	0.421875	2.322017	10	0.232202	1.816847	0.207429

	Pollen				
Group	Average	N	SD	Min	Max
1	0.41	6.00	0.07	0.32	0.50
2	0.34	6.00	0.06	0.28	0.44
All Grps	0.37	12.00	0.07	0.28	0.50

	SS	Df	MS	SS	df	MS	F	p
Pollen	0.015052	1	0.015052	0.042854	10	0.004285	3.512397	0.090391

Conclusion

Based on experimental work with different sugar cakes with or without Feedbee we can conclude:

- In the unstable weather condition Feedbee cake shown very great results as nutritional supplement in the development of a bee colony.
- The consumption of Feedbee cake was 1683 grams, while common sugar cake was in the same time period 5901 grams.
- Feedbee cake has affected on continuous development of bee colonies compared to the group with common sugar cake.
- We confirmed differences between Feedbee group and sugar cake group in the amount of bees, brood, honey and pollen were not statistically significant and differences shown were probably consequences of some other uncontrolled factors.
- We can see that the Feedbee cake surly gives bee colony safe situation in the spring the formation of the brood, amount of food, and number of bees at the colony.

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ACTIVITY OF POLLEN FORAGERS IN CONDITIONS OF URBAN BEE BREEDING

Nedić N.[1], Vojt D.[1]

Abstract

Honey bee satisfies its needs for proteins by collecting pollen. The pollen is collected and brought to the hive by the foragers (pollen foragers). Pollen is mostly used for the nutrition of brood. The bees fed by a greater amount of pollen can feed a substantial part of brood and can make building elements of muscles, glands and other tissues. Pollen deficiency, especially in early spring, when development of bee brood is an intensive one, may bring to a halt the development of a bee society.

In order to study the activities of foragers in the spring period (March – April) in urban bee breeding conditions at the apiary of the Faculty of Agriculture in Belgrade, the activity of bees in two groups of societies was monitored. Both groups consisted of five beehives each. The first group included bee colonies with two-year old queen bees and the second group consisted of colonies with young one-year old queen bees. Counting of flights of foragers was carried out repeatedly during the day at 9h, 11h, 13h, 15h and 17h and in 3-minute individual intervals for each beehive. Mass of pollen balls was measured for 6 foragers of each group in diurnal time slots. Collected data have been systematized and processed by using standard mathematical statistical methods.

The activity of foragers with pollen loads from the colonies with one-year old queen bees was statistically significantly higher (P<0.05) in 11h, 13h, 15h and 17h time slots when compared to the groups of societies with older queen bees. Mass of pollen loads differed statistically significantly (P<0.05) per time slots investigated and it was the biggest (6.80 mg) at 11h time slot. The mass of individual pollen loads in the colonies with older queen bees was 6.34 mg on average while in the colonies with younger queen bees it was 6.17 mg. This goes to show that the activity of foragers in the spring period is higher in the societies which were formed in the previous year with a young one-year old queen bee and indicates a vitality of such societies for the purpose of faster spring development of bees.

Keywords: Apis mellifera, colony, pollen, forager bees, pollen load

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Invited paper

SLAUGHTER TRAITS OF CHICKENS RAISED IN ORGANIC OR CONVENTIONAL PRODUCTION SYSTEMS

Perić L.[1], Đukić Stojčić M.[1], Bjedov S.[1], Tomović V.[2]

Abstract

The aim of the study was to investigate the effect of two different rearing systems (organic and conventional production) on carcass characteristics of chickens.
Random samples of male chickens were taken from organic and conventional farm. Broilers from the conventional farm were 5 weeks old and chickens from the organic farm 8 months old. A sample of 8 broilers from each production system was used for the determination of basic slaughter traits: dressing percentage, yield and percentage of individual carcass parts, weight of internal organs and quantity of abdominal fat. The collected data were analyzed by ANOVA, using Duncan *post hoc* test, in the program Statistica 13.
The results showed that the system of meat production had a significant effect on most of the examined parameters. There was no significant difference ($P>0.05$) among groups in live body weights of chickens and the mass of the heart and the gizzard. Chickens raised in conventional system had significantly higher weight of the liver, abdominal fat pad and breast compared to the organic chickens ($P<0.01$). On the contrary, organic chickens showed significantly higher mass of drumstick, head and the neck compared to the conventional ones ($P<0.01$). The results showed that there was a significant difference in carcass quality and yield of different carcass parts between organic and conventional chickens which could have a strong influence on consumers preferences.
Keywords: chickens, carcass, conformation, organic production

Introduction

In recent years, broiler meat production is turning more to free-range or organic production systems. According to Castellini et al. (2008) the market opportunity for both organic and free range poultry products does not yet seem to be fully developed. Bogosavljević Bošković et al. (2011) pointed out that reasons for the development of extensive systems in poultry production are to improve rearing conditions, reduce environmental pollution and enhance meat quality.
In Serbia, organic production is still at the beginning, despite the fact that the demand for organic products is growing. The number of consumers who are willing and able to pay for the organic poultry products is increasing in Serbia (Rodić et al., 2006). This is caused by consumers' growing interest in the nutritional importance of human diets. Many consumers assume that the meat of free-range chickens is tastier and healthier than that of birds kept

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in a poultry house (Molee et al., 2012). However, this statement is still not scientifically confirmed. For example, Fanatico et al. (2007) reported that the consumer panel did not indicate differences in liking between conventional and specialty products.

The influence of the rearing system on the performance, carcass quality and finally on the meat quality and its sensory characteristics is the result of the interactive effects among facilities (type of floor, the space, environmental temperature, physical activity), feeding level and genotype used in the production systems (Araújo, 2011). Age at slaughtering, genetic strains (fast- and slow- growing), physical activity, and pasture intake are key factors in determining meat quality (Castellini et al. 2008).

The aim of this work was to determine and to compare the slaughter characteristics of chickens raised in two different production systems: conventional and organic system.

Material and Methods

Organic chickens were raised on the certified organic farm in northern part of Vojvodina. Two thousand day-old chicks from various autochthonous and pure breeds (Naked Neck, Leghorn, Plymouth Rock and crossbreeds) were placed into poultry house in the early spring. They were raised inside the barn for 8 weeks and fed with the concentrated feed. At the age of two months the birds had access to the pastured range. During the next 2-3 months the feeding was based on organically grown triticale (70% of the diet), corn and pasture. After that period, birds were basically fed with organic corn and the feed from the pasture. The birds were slaughtered at the age of 8 months.

The second group of birds - broiler chickens - was raised on the conventional farm for 5 weeks. ROSS 308 broilers were raised inside the barn, on a deep litter, with stocking density of 17 birds/m². They were fed with standard mixtures (starter, grower and finisher) and the feed was provided *ad libitum*.

At the end of the fattening periods, 8 male birds from each group were randomly selected, and slaughtered by cervical dislocation. Chicken carcasses were manually processed and "conventionally processed carcasses", carcasses "ready to roast" and "ready to grill" were weighed. Following the chilling procedure, the carcasses were weighed to obtain the dressing percentage. Thereafter, the dressed cold carcasses were dissected into primal cuts (head, neck, legs, breast, thigh, wing and back) following the method prescribed by the Regulation on Poultry Meat Quality (Rašeta and Dakić,1984). Edible giblets (liver, heart and gizzard) and abdominal fat pad were also removed from the carcass and measured. For all carcass parts, the relative yield was calculated.

Data were analyzed by ANOVA and means were separated by Duncan's post hoc test using StatSoft computer package (STATISTICA 15, 2016). Significance was determined at $P<0.01$.

Results and Discussion

The results of slaughter characteristics of conventional broilers and organically raised chickens are presented in the following tables (Tables 1-3). Regarding the live body weight, the chickens from organic system were 150 g heavier, but the difference was not statistically significant, mostly because of the high variability inside the groups. The differences between dressing percentages were highly significant ($P<0.01$). Organic birds

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had significantly lower dressing percentages compared to conventional ones.

Table 1. Effect of production system on dressing percentage (mean ±SD)

Trait	Conventional system	Organic production
Live body weight, g	2077 ± 120	2238 ± 204
Conventional processing, %	84.13 ± 0.81	81.24 ± 1.09**
Ready to grill, %	77.85 ± 0.88	73.87 ± 1.65**
Ready to broil, %	67.67 ± 0.96	63.65 ± 1.81**

**Means within the same raw are significantly different (P<0.01)

The assumption that the rearing system and pasture intake may affect overall carcass quality was stated by many authors. However, the results are somewhat contradictory. Skomorucha et al. (2008) reported that 42-day old Cobb broilers reared indoors were characterized by higher dressing and breast muscle percentage compared to birds grown with outdoor access. On the contrary, Bogosavljević-Bosković et al. (2006) reported that Hybro G broilers with free-range access for 49 days of age showed better slaughter characteristics compared to those reared indoors only. Likewise, Castellini et al. (2002) showed that birds that had access to free-range achieved a higher percentage of breast and thigh muscles in the carcass. There is also a significant group of researchers (Fanatico et al., 2005; Wang et al., 2009; Mikulski et al., 2011; Poltowitcz and Doctor , 2011) who didn't get any differences between carcass yield of birds raised indoor or in free range. The similar results are reported by Djukić Stojčić et al. (2016) who established that housing system didn't have significant effect on carcass eviscerated weights, dressed carcasses weights (ready to grill), percent yield breast, drumsticks, thighs and abdominal fat in Red Bro broilers. It is important to emphasize that in these trials all birds were from the same breed reared with or without outdoor access. Large discrepancies found in the literature concerning the evaluation of the effect of housing systems on carcass characteristics of chickens probably result from different age and genotype of the birds studied.

Table 2. Effect of production system on proportion of carcass cuts (mean ±SD)

Carcass cuts	Conventional system	Organic production
Head yield, %	2.66 ± 0.23	4.66 ± 0.76**
Neck yield, %	4.49 ± 0.27	6.58 ± 0.58**
Legs yield, %	4.81 ± 0.46	4.42 ± 0.65
Wings yield, %	9.32 ± 0.37	9.84 ± 0.44
Thigh yield, %	25.74 ± 0.84	29.21 ± 0.67**
Breast yield, %	28.44 ± 1.62	20.07 ± 1.92**
Back yield, %	16.93 ± 0.89	19.21 ± 0.73**

**Means within the same raw are significantly different (P<0.01)

The results of the carcass dissection (Table 2) showed a significant effect of the production system on proportion of carcass parts. Organic birds had significantly lower breast yield, but higher thigh and back yields. The head and neck yields were also significantly higher in organic birds. This is mostly because broiler chickens have a genetic predisposition for high breast meat yield compared to pure breeds. On the other hand, the organic chickens had higher thigh and leg yields because of their higher physical

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activity on the range. These findings are in accordance to the results of Funaro et al. (2014) who stated that the conventional birds had dramatically higher carcass and breast meat yield, whereas free range birds had higher wing and leg yields. Bogosavljević Bošković et al. (2011) reported that rearing system had a significant effect on the percentage yield of back, but not on the other parts. According Fanatico et al. (2005), fast growth genotype birds has greatest breast yield (%) and the lowest wing yield (%), while slow growth genotype birds exhibit lowest breast yield (%) and the greatest leg quarter yield (%) in the same production systems. Comert et al. (2016) confirmed in their research that fast growing birds had the higher breast yield, whereas slow growing had the higher thigh-drumstick yield. These results also suggests that the genetic strain might be more significant that system of rearing.

Production system significantly affected the proportion of edible giblets (Table 3). The organic chickens had lower percentage of abdominal fat and liver. Lower content of abdominal fat can be explained by the effect of genotype and increased movements of organic birds. This is in accordance to the results of Castellini et al. (2002). Higher liver weight in conventional birds was also reported by Bogosavljević Bošković et al. (2004), while Djukić Stojčić et al. (2016) did not find any effect of housing system on liver weight.

Table 3. Effect of production system on proportion of edible giblets and abdominal fat (mean ±SD)

Trait	Conventional system	Organic production
Gizzard, %	3.03 ± 0.56	2.83 ± 0.36
Liver, %	2.66 ± 0.25	2.03 ± 0.26**
Heart, %	0.69 ± 0.13	0.75 ± 0.12
Abdominal fat pad, %	1.22 ± 0.42	0.60 ± 0.21**

**Means within the same raw are significantly different (P<0.01)

It is interesting that that percentage of the gizzard was bigger in broiler chickens, although not significantly (P>0.05). This is not in line with the results of other authors. Araujo et al. (2011) stated that when birds begin to eat forage they have a greater development of the gastrointestinal tract as an adaptation to increased dietary fiber. The same effect was reported by Djukić Stojčić et al. (2016) who found highly significant differences in gizzard weight in chickens kept on free range compared to chickens raised in the barn.

Conclusion

From the results of this work it can be concluded that the production system had a significant effect on carcass characteristics of chickens. Chickens raised in conventional system had significantly higher weight of the liver, abdominal fat pad and breast compared to the organic chickens. On the contrary, organic chickens showed significantly higher mass of drumstick, head and the neck compared to the conventional ones. The results confirmed the differences in carcass quality and yield of carcass parts between organic and conventional chickens which could have a strong influence on consumers preferences.

Acknowledgement

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Invited paper

UNMANNED AERIAL VEHICLES FOR PRECISION AGRICULTURE AND LIVESTOCK PRODUCTION

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Abstract

The trend of climate change is to a great extent well-known. The consequences of such change have different intensity and manifestation in particular world regions. Climate effects are reflected in the change of the yield and product quality, in the change of food prices and market conditions, in the occurrence of new vermin and diseases, etc. Furthermore, the rapid growth of world population represents an additional burden for food producers. In order to mitigate the negative climate effects and adjust the agricultural production to the new conditions and enhance its efficiency, there are some proposed solutions in the field of modern biotechnology, methods of genetic engineering and modern management and farm management at disposal. Within the concept of "precision agriculture" (PA), a special position is assigned to the use of ultra-light unmanned aerial vehicles (UAV). Their potential is yet to be sufficiently explored and exploited within PA. Applying UAVs in precision agriculture contributes to maximizing the profit and increasing the efficiency of agricultural production. However, applying such technologies in their full capacity is still conditioned by specific limitations. Firstly, it is necessary to accurately define the expenses and benefits of the use of UAVs, especially for each type of crop and animal agricultural production. Information obtained in this manner should be timely, comprehensible and easily accessible in practice for averagely educated farmers. Thus, it is necessary for the UAV systems producers to closely cooperate with scientists and farmers during the production and development of such aircraft in order to adequately define the criteria and requirements under realistic conditions of specific agricultural production.

Keywords: Unmanned aerial vehicles, precision agriculture, climate change.

Introduction

What will represent a great global challenge for food producers in the following decades is an enormous population growth. It is estimated that, by 2050, there will be around 9.1 billion people (UN, 2009a), which means that, along with the overall population growth, the number of people that will not have a sufficient amount of food at their disposal will also increase. The production of sufficient amount of food is limited by a great number of factors. Beside the aforementioned population factor, increasing limitation of the most significant natural resources (water and arable land), the effect of adverse climate change, the occurrence of new vermin and diseases, etc. should all be highlighted.

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The effect of all these factors both individually and cumulatively negatively affects socio-economic trends (food prices growth, migration of people), crop yield and negative consequences are leaving mark on both ecosystem and ecology as well. Some particular species of cultivated crops display greater sensitivity on such fluctuations in comparison with some other species. Within the context of climate change, the most significant impact on agricultural production is realized through the change in the average air temperature, the change in atmospheric precipitation, the occurrence of drought, the change in carbon-dioxide concentration and ozone. The intensity of climate change is not equally spread in all regions of the world (Lobell and Gourdji, 2012; Parrya et al., 2004). Food and Agriculture Organization (FAO, 2002) has defined the most important aspects in terms of providing the sufficient amount of food in the world. The greatest attention is paid to the aspect of stability in the supply of food as well as to the accessibility of food and the possibility of its exploitation in all regions of the world. Food should be simultaneously available to all people, which would grant a healthy and active lifestyle within one's preferences and needs. The negative effect of climate change can be mitigated in different ways; for example, by applying new biotechnologies, implementing modern managements measures, using precision agriculture technologies, etc. In order to understand the trends of climate change and their impact on agricultural production, different climate models are employed. The dynamic models of crop production, the so-called "crop models" are used to assess the effect of climate change on the intensity of growth and development of crop from the time of sowing until that of harvest (Mihailović and Lalić, 2004). The currently used global and regional climate models are, for example, GFDL-CM2.1 2005- USA; BCC-CM1 2005, China; CCCM3.1 (T 47) 2005 Canada; SIRIUS, HadCM3,CERES-MaizeCrop Environment Resource Synthesis, CERES-Wheat, SWAP soil–water–atmosphere–plant, etc. By applying these, it is possible to define the future scenario of climate change for the state of crops and their productivity (Kang et al., 2009). The aim of this paper is to highlight the significance and role of UAVs in precision agriculture, regarding the aspect of enhancing efficiency in agriculture and current climate change.

Climate Change
Current researches indicate that the trend of global warming is to continue in the following decades as well. Regarding this, the change of maximum temperatures of about 0.3°C per decade in average and that of about 0.2°C of minimum temperatures is expected (Sacks et al., 2010). According to the results of 16 climate models, the similar trend is to be continued in the following 50 years (Lobell and Gourdji, 2012), where only minor disagreements occur among scientists in relation to the changes of maximum and minimum temperatures (Lobell et al., 2007). The results of research in AP of Vojvodina demonstrate that by 2040 an increase in mean annual air temperature from 1,1°C to 1,5°C can be expected (Lalić et al., 2011). The same authors state that the increase in the sum of active temperatures will significantly accelerate vegetation of all cultivated crops, but also the occurrence of weed and vermin. The increase of air temperature is not negatively reflected only in vegetation and crops, but also in reared animals and their productivity by jeopardizing the microclimate conditions in stalls, especially during summer months when the heat stress might occur among animals (Kučević et al., 2013).

Precipitation, or rather soil moisture, intensity and seasonality of precipitation, directly influence yield and crop conditions. In numerous parts of the world, there has been a significant increase of precipitation (Alexander et al., 2006) but droughts have occurred as

well, especially in the regions of Africa, South Asia, South and Middle Europe and Australia (Sheffield and Wood, 2008; Dai, 2011). The increase of precipitation is expected in regions of higher latitudes, while the decrease is expected primarily in the subtropical belts such as the Mediterranean area (South Europe), areas of Central America, and North Africa (Meehl et al., 2007). The results of the research in the region of Vojvodina correspond to global trends. Lalić et al., (2011) state that, in the last sixty years, a decrease of annual precipitation can be noticed and that such trend is to be retained in the following decades. Such decrease is not to be evenly distributed during the year; an increase in number of dry days is expected during summer and autumn, while its decrease is expected during spring months.

In the regions where no significant change in precipitation is expected, an increase in temperature is to cause higher evapotranspiration and, along with the occurrence of storms and surface waters runoff, soil drying or agricultural droughts are to occur more frequently (Dai, 2011). The concentration of atmospheric CO_2 has been constantly and rapidly increased from the introduction of the industrial revolution to 2000, in average for 2 µL L-1 per year (Peters et al., 2011). In 2010, the average concentration of CO_2 on the global level was 39% (390 µL L-1); higher than the one from the beginning of the industrial revolution (Global Carbon Project, 2011). The trend of the change of CO_2 by 2050 is not favorable, because it is expected to increase for about 25 µL L-1 per decade; i.e. a level of 500 µL L-1 is predicted (IPCC, 2001). A similar situation occurs with the change of ozone concentration (O_3) which spiked from 15 µL L-1 to about 35 µL L-1. In the decades to come, a further increase in the level of this gas is anticipated, especially in the developing countries. The said climate factors (the concentration of CO_2 and O_3, increase in temperature and change of precipitation and its distribution) affect the change of crop physiology by different mechanisms, which leads to the change in yield and product quality, and is indirectly reflected in the change of food prices and market conditions, etc.

Impacts on Crop Yield
Research on the influence of climate change can be performed for all species of crops that are cultivated today. Still, the significance of all cultivated cultures is not the same in terms of meeting the needs in calories and proteins (FAO, 2012). Due to this, the largest number of researchers decide to explore the impact of such changes on several basic types of grains such as wheat, corn, rice, soybeans, sorghum, etc. (Lobell and Gourdji, 2012).

During the period between 1960 and 2010, the yield of corn, wheat, soybeans, barley and rice has linearly increased, primarily by applying modern species and hybrids, irrigation measures, the use of chemicals for crop protection, etc. (Lobell and Gourdji, 2012). The same authors state that it is likely to expect that an increased concentration of CO_2 will positively affect the crop (grains) and that the average global yield will be increased for about 1.8% per decade but also that the effect of higher temperatures will affect it oppositely (negatively) and the global yield will thus be reduced for about 1.5% (0-4%) per decade. A large number of researchers agree that the growth of CO_2 emission has a positive effect on the crop conditions (yield) and that it exceeds the negative effects of climate change. However, the opposing results of the research by Lobell et al. (2011) state that, during the period between 1980 and 2008, the negative global net impact of CO_2 took place. Anwar et al. (2007) conducted researches for the area of Southern and Eastern Australia and stated that the higher concentration of CO_2 is to cause a decrease in yields of about 25%. The research by Ortiz et al. (2008) point out that the effect of CO_2 on grains (wheat) is positive in some regions, whereas in others it is negative, so it is thus necessary

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to conduct a selection of species that are heat-tolerant. The results of the research by Lalić et al., (2011) for the region of Vojvodina during the period until 2040 demonstrate that the increase of CO_2 concentration will positively affect the yield of field crop, particularly of winter crop, and that a significant decrease in the yield of spring crop (summer drought) will occur.

Adaptation to Climate Change
Numerous activities are undertaken aiming to adapt and mitigate the negative effects of climate change. Some measures are classified under the group of the new biotechnologiesand methods of genetic engineering which aim at the creation of new species and hybrids. This further ensures yield even under poor climate conditions (better water exploitation, higher tolerance to drought, high temperature, diseases, etc.). Another group of activities can be classified within the framework of management which is employed in order to enhance production. This involves adjusting and applying the modern agrotechnical solutions with the aim to repair the structure of the soil and increase the moisture retention capacity, crop rotation (decrease in spring crop and increase in winter crop), adjustment of the date of sowing, prevention of soil erosion and protection from evapotranspiration (agro- protective belts), enlargement of the area under irrigation (improving irrigation systems), etc. (Lalić et al., 2011). The use of systems of precision agriculture represents a special group of measures. In general, precision agriculture enables the farmers to increase the yield and work productivity, to rationalize the amount of their input, to adjust their business decisions to the current conditions of production more rapidly and efficiently, as well as to contribute to the protection of the environment. Within the concept of "precision agriculture", a special position is assigned to the use of ultra-light unmanned aircraft vehicles (UAVs). The existing literature is considerably scarce in terms of results of scientific research in relation to the role and benefits that the use of UAVs has within PA (Zhang and Kovacs, 2012).

Role of UAVs in Agriculture
Satellites and engine aircraft with a crew have been used for a long period of time in agriculture for monitoring crop vegetation, occurrence and development of disease, estimating the yield, etc. (Warren and Metternicht, 2005). The use of these systems is limited by economic validity and it is extremely demanding from the aspect of organization of human resources as well (Zhang et al. 2006). Moreover, their full use is limited under poor weather conditions (rain, snow, great cloud cover) and the quality of information (resolution) may be challenged (Wu et al. 2007; Stafford, 2000). The alternative platform to these systems is the introduction of unmanned aircraft (UAV) whose primary task would be gathering information from lower altitudes (Swain et al. 2007) for precisely defined production phases or their use for a direct performance of less complex technological operations on the production parcels. The basic advantages of applying UAVs are reflected in a relatively low cost of use, high spatial resolution (in centimeters) and a possibility of obtaining information at the approximately real time, which recommends UAVs as a convenient platform to use in precision agriculture (Hunt et al. 2005; Lelong et al. 2008; Nebiker et al. 2008). The market offers a large number of different types of UAVs, such as drones, quadcopters, motor balloons, light motor gliders, helicopters, etc. (Seang and Mund 2006; Eisenbeiss, 2004; Laliberte et al., 2007; Swain et al., 2007; Lelong et al. 2008; Nebiker et al., 2008). The performance of UAVs differs based on the speed and altitude of flight, flight duration (without landing), flight stability, ability to carry additional equipment, ability to wirelessly connect with other devices, ability to fly under harsh climate conditions, etc. (Laliberte et al. 2007). Their use in agriculture has proven to be

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extremely useful, especially in the case of using light commercial aircraft up to 50kg (Laliberte and Rango 2011).

Fig. 1. Various types of USA: A) powered glider, B) powered parachute, C) helicopter, D) fixed wing aircraft, E) dragonflyer X8 quadrocopter,F) aeryon Scout quadrocopter (from Zhang and Kovacs, 2012).

So far, the UAV platforms have mostly been associated with the use in crop production, or in farming and viticulture production where their use in some countries such as the USA and Australia is increasingly gaining popularity (Warren and Metternicht 2005). Commercial companies have developed a large number of systems which perform a complete monitoring of soil and crop conditions at each production phase by using UAVs. Use potential of these aircraft can be further upgraded, if used in combination with other systems (technologies) as is the case with, for example, installation of wireless sensor networks, GPS devices, equipping with different types of ADC multi-spectral camera, non-metric digital cameras, thermal infrared cameras, MCA mapping camera, near-infrared camera (NIR), etc. (Hunt et al. 2003; Hunt et al. 2008; Swain et al. 2007; Eisenbeiss 2004; Chandler et al.2005; Seang and Mund 2006; Lewis 2007; Lelong et al. 2008; Nebiker et al. 2008; Pudelko et al., 2012; Majidi and Bab-Hadiashar, 2005). However, depending on the selected platforms and sensors, the expenses of using these systems can be extremely high. Such is the case with equipping UAVs with a GPS system where expenses might reach even up to US $ 100,000 (Rango et al. 2009). By combining the aforementioned technologies, it is possible to gather different information which might facilitate and accelerate making business decisions for farmers regarding:

-the visual inspection of pedological characteristics of the soil (presence of moisture, structure);

-the analysis of health and physiological crop and animal condition (estimation of growth rate);

-monitor movement and behavior of the animals;

monitoring intensity of vegetation growth in pastures;

-the estimation of emergence of insects and vermin on crops;

-the anticipation of yield or the estimation of damage on crop/yield;

-the analysis of erosion occurrence;

-the precise analysis of the necessity of mineral fertilizer (50% of input expenses) with a possibility of a detailed identification of problematic areas (timely suggestions for a proper dose of level and type of fertilizer);

-the precise analysis of the necessity of protection products (pesticides) and determining the optimal time of application;

-the estimation of necessity for irrigation and optimization;

-deciding on the most optimal time of sowing/harvest;

-mapping wildfire;

-mapping the terrain and 3D image, etc.

(Primicerio et al. 2012; Laliberte and Rango 2009; Hardin et al. 2007; Berni et al. 2009; Hunt 2005; Swain et al. 2010; Swain et al. 2007; Schmale et al. 2008).

One of the most significant advantages of using UAVs is the fact that the farmer does not have to be physically present in the field in order to estimate soil and crop conditions, damage that poor weather conditions caused, etc. By combining UAVs with other technologies, for example, connecting them with sensor network in the field and forming a web platform which provides sales department with a customer service with the support of operators and experts, farmers can obtain necessary information about their production from their own home (Fig. 2).

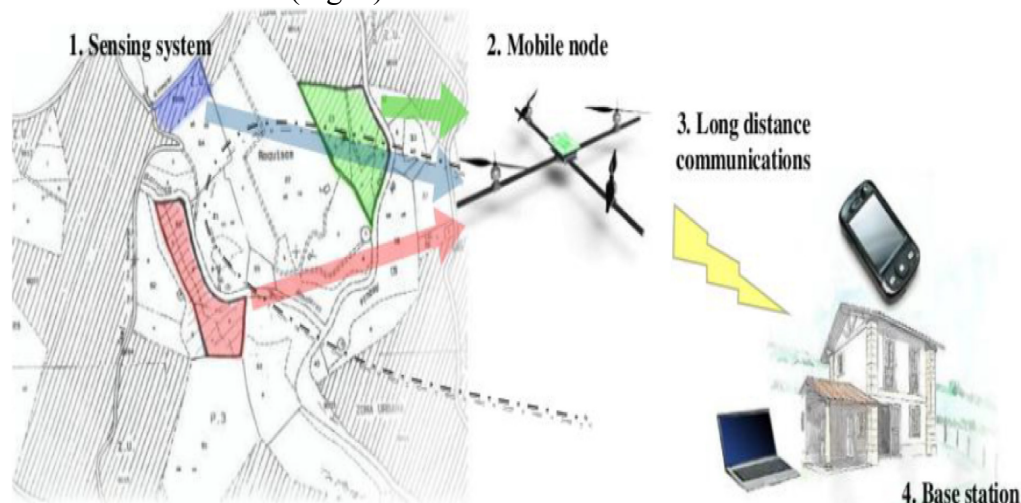Fig. 2. An illustrative example of the Wireless Sensor Networks (WSN) technology for Specific Data Acquisition (from Valente et al., 2011).
This can contribute to significant savings in time (plenty of free time) and resources (energy, transport), which further promotes life comfort for farmers. These advantages are particularly evident at less accessible fields and under poor weather conditions. Farmers

are thus enabled to timely make their business decisions and to adjust them to current (microclimate) conditions in a particular production area. UAVs are not used only as a means of gathering data, but are also used for directly performing some technological operations. Their use, for example for applying crop protection products, has several advantages because it does not damage the crops, trampling the soil is thus avoided, there is no direct exposure to chemicals, application of protection products is more precise, etc. Research by Shaw (2005) has shown that the financial savings per hectare when spraying soybeans ranged from $ 92.24 to $ 104 per hectare. Apart from in crop production, the use of UAVs is increasingly gaining significance in livestock production, as well. So far, their use has been related to livestock production organized in an open range – pastures (cattle, sheep, goats, horses). Combined with specific sensor systems, the use of UAVs enables one to monitor movement and behavior of the animals (Wark et al., 2007). Their great contribution is reflected also in monitoring the intensity of vegetation growth in pastures, in order to organize a proper arrangement for rotational livestock grazing. Apart from that, it is possible to obtain data related to the most significant patterns of natural behavior of animals (sleep, rumination, movement), and to monitor ill or injured animals. By integrating the wireless radio identification (RFID) within the sensor network, the amount of food consumed by each animal can be monitored as well (Wark et al., 2007). Agricultural production is very heterogeneous and diverse in its structure. Therefore, the requirements and criteria for the use of UAVs in precision agriculture should be thoroughly and individually defined for each type of production. Thus, for instance, in crop production, the requirements in farming, viticulture and fruit production should be separately defined, and then, within a particular type of production, criteria for cultivated crop species (e.g. wheat, corn, apples, grapes, etc.) during vegetation should also be separately defined. There is still an insufficient number of scientific studies dedicated to the specific role of UAVs in particular agricultural productions such as that of soybeans, wheat, rice, etc. (Hunt 2005; Swain et al. 2007; Lelong et al. 2008). Some trials have shown that applying UAVs for improving management of farms brings the benefit of at least $ 20/ha (Seelan et al. 2003; Robertson et al. 2007). It is essential to conduct a specific research for each type of agricultural production and for each cultivated crop species, in order to accurately quantify the significance of the use of UAVs. Tenkorang and DeBoer (2007) propose undertaking detailed analysis (expenses/benefits) with sufficient repetition in order to better define the economic significance of UAVs in precision agriculture. Those researches that include UAVs in precision agriculture are mostly performed in experimental fields and under ideal conditions. Consequently, the feedback is still meager and has no greater significance for farmers and their practice, so their adoption of UAVs is slower (Zhang and Kovacs, 2012). The rate at which farmers accept UAVs also depends on their prior knowledge and education regarding the concept of precision agriculture. Furthermore, the use of UAVs in agricultural practice is additionally burdened by aviation regulations that are considerably different across the world (Hardin and Jensen, 2011). Aviation regulations (restrictions), are generally related to the prescribed altitude of flight, the weight of the aircraft, approved locations for flight (distance from cities, airports), etc. Another problem for the mass use of UAVs is also licensing operators which is related to the requirements of insurance companies (the definition of damage for people, objects or animals) (Zhang and Kovacs, 2012).

Conclusion

The growth in world population, limited access to the most important natural resources, adverse consequences of climate change, the occurrence of diseases, etc. demands changing the approach in the current mode of agricultural and food production. It is essential to mobilize all available technologies in order to raise the efficiency of agricultural production (price cuts), increase its scope and product quality, preserve resources and mitigate the consequences of climate change. In addition to applying the new methods of biotechnology and modern management measures, what should be included as mandatory technology in order to raise efficiency and rationalization in agricultural production is the use of UAVs in precision agriculture. Their potential is currently under-explored and under-exploited so their full contribution and significance is still expected in the coming years. It is safe to say that there is no farmer who would not want to maximize their profits and increase efficiency in their production and neither is there a doubt that the application of UAVs in precision agriculture can contribute to achieving these aims. However, the adoption of this technology by farmers and its popularization is conditioned by several factors. First of all, the expenses and benefits of using UAVs in every type of agricultural production (crop or animal) should be precisely and accurately defined. Information obtained in such manner must be timely, available for practical use and comprehensible to averagely educated farmers. In order to meet these conditions, it is necessary to have a close cooperation between the producers of UAV systems, scientists and farmers, so that the criteria and requirements are better defined under realistic conditions for a specific type of production.

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Oral presentation

CORTISOL CONCENTRATION IN HAIR AND FAECES AS ANIMAL WELFARE INDICATOR IN ORGANIC DAIRY COWS

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Abstract

Cortisol is frequently used to detect changes in the activity of the hypothalamic-pituitary-adrenal axis (HPA), which in turn regulates many biological processes such as energy balance, reproduction or immune responses, but it is also activated by stress conditions. For many years, cortisol was measured primarily in blood, saliva or urine, whereas later approaches added other matrices as hair and feces for non-invasive monitoring of HPA functioning.

The aim of this study was to investigate the cortisol concentration of dairy cows reared in organic farming system obtained from two different matrices: hair and feces. The study involved 20 cows from two organic farms located near Florence (Mugello area). Samples of hair were taken from the tail switch and feces were directly picked up from the rectum ampule. Hair cortisol extraction and determination were performed according to validated methods. ANOVA test was applied to check differences between hair ad fecal cortisol. Significant differences were observed between the two biological matrices (1.6 *vs* 1.0 pg/mg respectively for hair and fecal cortisol), nevertheless the scored levels did not indicate a state of animal suffering.

Keywords: hair cortisol, feces cortisol, dairy cows, organic farm

Introduction

Dairy cattle experience stress from management and high production, than it is important to apply strategies to improve animal welfare and reduce stress.

Glucocorticoids represent a useful parameter in research on welfare of dairy cows as animal based measure. Although this parameter may be influenced by a wide variety of factors either extrinsic or intrinsic to the animal. Activity of the hypothalamic-pituitary-adrenocortical (HPA) axis is commonly assessed by measuring glucocorticoids such as cortisol (Comin et al., 2011).

Cortisol is an hormone involved in stress but it is not efficiently measured by hematic assessment because the blood drawing represents in itself an event able to alter this parameter; on the other hand either salivary and urinary samples are not reliable matrices to investigate on cortisol because are subject to circadian variation and can be confounded

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by environmental disturbances (Comin et al., 2013). Moreover, the use of non-invasive techniques to assess control of the HPA axis activity in animals is essential. Hair from the tail normally shows higher cortisol concentration in respect of other parts of the body, moreover in this area hairs grow back in very few time, suggesting that this is the most suitable location to collect hair samples for this purpose (Moya et al., 2013).

In this view, to assess stress by cortisol level, in the last years fecal and hair cortisol were increasingly used in consequence of the fact they follow the same control mechanism and are not affected by the sampling procedure, giving information on the level of this hormone before this time. Finally the sampling itself does not represent a substantial disturbance for the animals (Accorsi et al., 2008).

The aim of this study was to measure and compare cortisol concentrations in fecal and hair matrices in two organic dairy farms.

Material and Methods

The trial was conducted on 20 multiparous lactating Italian Friesian cows. The cows were selected with equal lot size from two organic farms located in the area of Mugello (Florence district) with similar environmental conditions and management. No animal enrolled experienced a change in social group or was affected by any diseases in the period before the study. Feces and hair samples were collected from the 20 cows at the same day in the spring 2014: feces were taken directly from the rectum ampule and hair was carefully cut from the tail switch using clippers.

It is important that the hair follicle itself is not included in the analysis, as a previous study found that hair follicles are capable of producing cortisol in response to corticotrophin-releasing hormone stimulation and thus may skew the results (Gow et al., 2010).

Samples were collected during the daily routinely activity to avoid disturbance for the animals and in compliance with the current legislation on animal welfare. Fecal samples were immediately frozen at the temperature of –20°C to inhibit possible metabolism by bacterial enzymes keeping fecal cortisol levels unchanged (Palme, 2012) and sent to the laboratory. Also hair samples were frozen at the same temperature to avoid the presence of lice, often presents in this body area.

Hair cortisol extraction
Extraction methodology (Accorsi et al., 2008) was modified from Koren et al. (2002). Hair was first minced into 1–3 mm length fragments and 60 mg of trimmed hair were put in a glass vial. Five milliliter methanol were added, and vials were incubated at +50°C with gentle shaking for 18 h. The vial content was then filtered to separate the liquid phase which was evaporated to dryness under an air-stream suction hood at 37°C. Dry residue was then dissolved into 0.6 ml of phosphate-buffered saline 0.05 M, pH 7.5.

Fecal cortisol extraction
Extraction methodology (Accorsi et al., 2008) was modified from Schatz and Palme (2001). Five milliliter of a methanol:water (v/v 4:1) solution were added to 500 mg (wet weight) of feces in capped glass tube vials. Vials were then vortexed for 30 min using a multitube pulsing vortexer. Following centrifugation (1500g for 15 min), 5 ml ethyl ether (BDH Italia, MI, Italy) and 0.2 ml NaHCO3 (5%) were added to 1 ml supernatant. This preparation was vortexed for 1 min on multitube pulsing vortexer and centrifuged for 5 min (1500g). The ether portion was then separated by sucking it with a pipet, and evaporated under an airstream suction hood at 37° C. Dry residue was finally redissolved into 0.5 ml PBS 0.05 M, pH 7.5.

Cortisol assay

Assay in both hair and feces were carried out according to Tamanini et al. (1983). Analysis was performed in duplicate: 100 µl of 3 H-cortisol (specific activity 100 Ci/mmol, amount 30 pg/tube vial, 12,771 dpm/100 µl) and 100 µl of an anti-cortisol antibody (dilution 1:20,000) were added to 100 µl of the solution obtained from glucocorticoid extraction. After incubation at +4°C for 18 h, free steroid was separated from bound by the addition of 1 ml of a solution of charcoal 1% + 0.025% dextran, and incubation at +4°C for 15 min followed by centrifugation (4000g) for 4 min at +4°C. The supernatant containing the hormone bound to its antibody was then decanted into scintillation vials and measured in a liquid scintillation b counter (Perkin– Elmer Life Science Inc.).

Radioactivity was determined using a liquid scintillation β counter and using a linear standard curve.

The study was carried out in accordance with EU Directive 2010/63/EU.

ANOVA test was performed to verify differences between the two biological matrices by JMP program of S.A.S. Institute (2002).

Results and Discussion

Results showed statistical differences between the two biological matrices ($P \leq 0.05$). Table 1 summarizes data regarding fecal and hair cortisol.

Table 1. Hair and feces cortisol level (pg/mg) as overall mean from both farms

Cortisol	Mean (pg/mg)	SD
In feces	1.00	0.38
In hair	1.56	1.15

Hair cortisol resulted higher than fecal cortisol, nevertheless the obtained values did not indicate a status of stress; in fact, either recorded values were into the physiological range (0.76-20.41 pg/mg) of healthy Fresian cows (Comin et al., 2013). Data regarding hair cortisol were lower the values scored in other studies: Comin et al. (2011) reported 2.57 pg/mg for dairy cows; Gonzales De la Vara et al. (2011) reported 12.15 ± 1.85 pg/mg for 2-year-old heifers.

Regarding fecal cortisol, Tallo-Parra et al. (2015) found higher cortisol concentration than ours (average value 25.27 ± 4.16 ng/g dry sample, range 10.14 to 54.83).

In relation to bibliographic data on traditional farms, the low levels of cortisol in the hair and feces observed in this study seem to indicate that the cows did not show symptoms of stress evaluated through the HPA axis response.

Conclusion

Hair sample has the benefit of an easy collection method; it is not affected by variations in water content or by circadian rhythm of cortisol or by acute environmental disturbances, and does not contain material that may bias extraction. Hair potentially offers the longest record of an animal's glucocorticoid exposure. The slow growth of hair allows to measure the animal's glucocorticoid levels in weeks and months rather than hours and days. This could make hair a useful medium for studies examining the effects of chronic stressors, but unsuitable for transient stressors.

The two methods did not result statistically superimposable, but results were similar for biological cortisol level interpretation.

The two sampling procedures resulted easily to perform without causing disturbances to the animals, however hair cortisol fits better as matrix for chronic stress evaluation.

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Oral presentation

IMMUNOCASTRATED PIGS AS AN ALTERNATIVE TO PORK PRODUCTION WITH SURGICAL CASTRATES

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Abstract

Surgical castration of piglets is routinely practiced in pig husbandry. However, this practice is presently criticized from the animal welfare point of view. Therefore, the EC strongly supports the activities that would lead to halting the surgical castration in the EU member countries. One of the possible alternatives to this is the immunocastration of pigs.

The present study investigates the effect of immunocastration on fattening, carcass and meat quality traits of entire males and compare them with surgical castrates and gilts. A total of 40 pigs were assigned to 3 groups: immunocastrates (14), barrows (13) and gilts (13) and housed by two per pen and fed standard diet according to requirements. Immunocastration of entire males was performed by double vaccination (Improvac®), the first at cca 50 kg, the second at 80 kg live weight. Beginning with 30 kg, growth rate was monitored on a monthly basis until reaching 120 kg, when they were slaughtered in an experimental abattoir. Average daily gain and feed consumption per 1 kg of daily gain were calculated and carcass traits were determined. Samples of *longissimus dorsi* muscle were taken for analyses of meat quality. Statistical analysis were done using SAS (2009). Immunocastrates had the highest daily gain and the lowest feed conversion ratio compared to barrows and gilts but differences were not significant (P>0.05). Carcass traits were not influenced by immunocastration. On the other hand, sex category affected water holding capacity with the lowest value in immunocastrated males compared to barrows and gilts.
Keywords: carcass, fattening, immunocastration, pig, pork quality

Introduction

Surgical castration of entire male pigs has been a routine practice used in pig production for decades. The main objective is to avoid the development of boar taint, an unpleasant smell produced in uncastrated male pigs at the onset of puberty. Two main compounds of boar taint – androstenone (Patterson, 1968) and skatole (Walstra and Maarse, 1970; Vold, 1970) – are accumulated in fat tissue and such meat is rejected by the consumers due to its unpleasant odor. At present, the EU legislation allows surgical castration of piglets without anesthesia and/or analgesia within the first seven days of life. However, this practice has been recently criticized from the animal welfare point of view (Prunier et al., 2006). The actions of animal welfare organizations resulted in an initiative of the European Commission (EC) to end surgical castration of pigs in all the member countries by 1 January 2018. Many representatives of European farmers, meat industry sector, retailers as

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well as scientists, veterinarians and animal welfare organizations expressed their voluntary approval with regard to the call initiated by EC (2010). If a ban on surgical castration is to happen, the impact on the whole pig sector will be immense and a lot of problems need to be addressed. Slovakia as well as other EU countries must prepare for future developments. Among several alternatives to traditional fattening of barrows only two of them seem the most feasible: production of entire males or immunocastration.

The aim of the present study was to evaluate fattening, carcass and meat quality traits of immunocastrated males and to compare them to those of surgical castrates and gilts.

Material and Methods

Forty pigs, immunocastrates (IC, n= 14), surgical castrates (SC, n = 13) and gilts (G, n = 13), progeny of Landrace sows and hybrid boars (Yorkshire x Landrase), were randomly selected for the experiment and allocated to the test station at 22-26 kg live weight. Two pigs of the same sex were housed in one pen. They were fed a commercial diet (CP 15.23 %, lysine 7.14 %, ME 12.78 MJ) according to nutrient requirements for growing-finishing pigs (Šimeček *et al.,* 1995) and had free access to water. Immunocastration of entire males was performed by double vaccination (Improvac®), the first at cca 50 kg, the second at 80 kg live weight.

Experimental observations started at 30 kg live weight. Pigs were weighed at the beginning, then once a month and from 100 kg live weight once a week for information on growth intensity – average daily gain (ADG). Feed conversion ratio (FCR) per 1 kg of body gain was calculated per pen. After reaching the average slaughter weight of 120 kg, pigs were slaughtered at the experimental slaughter house of the Research Institute for Animal Production situated approximately 200 m from the test stables. Average age at slaughter was for immunocastrates 196, for barrows 204 and for gilts 198 days. The slaughter was performed according to standard abattoir procedure e.g. electrical stunning, vertical exsanguination, vapor scalding and evisceration. Carcasses were measured for information on backfat thickness and lean meat content using TP (Two Point) method (1989). Afterwards, carcasses were chilled for 24 hours at air temperature of 2 °C to 4 °C. The second day after slaughter, the dissection of the right half of carcass was done. Weight of shoulder, neck, loin, and ham was recorded and percentage of ham, valuable meat and fatty cuts (as ratio of their weight and weight of half carcass) were calculated.

Forty-five minutes after slaughter pH in *musculus longissimus dorsi* (LD) was measured using device METTLER TOLEDO with combined electrode. This trait was measured also 24 h post mortem at the same location. Analyses of other meat quality traits were performed on samples from LD muscle in the laboratory of NAFC – RIAP Nitra. Meat color was analyzed by spectrometry (L a*, b* parameters) using MINISCAN XE Plus device and subjectively using Japanese color scale. Water holding capacity (WHC) was analyzed using method of Grau-Hamm modified by Hašek and Palanská (1976). Drip loss was determined by method of Honikel (1998). Four days after slaughter, shear force Warner-Bratzler) of meat was measured using TEXTURE ANALYSER TA-XT2i.

Statistical package SAS (2009) was employed in the analyses. Basic statistics was done using MEANS procedure. For analyses of results was used GLM model. Differences between sex groups were tested with Scheffe´s test. Results are presented as LSM and

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standard error (s_e).

Results and Discussion

Fattening characteristics of pigs according to sex category are shown in table 1. Immunocastrated pigs reached the highest average daily gain compared to surgically castrates or gilts but differences were not significant. This could be due to the fact that pigs were not fed ad libitum but according to the diet requirements. Our finding is in agreement with many other studies (e.g. Fàbrega et al., 2010; Škrlep et al., 2010b; Grela et al., 2013) whereas Pauly et al. (2009) reported slightly higher daily gain in barrows than in immunocastrates. Vaccinated pigs in our study had the best feed efficiency, however it was non-significant compared to other two groups. That is also in agreement with other published studies (Pauly et al., 2009; Fàbrega et al., 2010; Škrlep et al., 2010b; Grela et al., 2013).

Results for carcass traits of tested pigs are given in table 2. The effect of sex category was non-significant in all traits with exception of ham weight but only between surgical castrates and gilts.. Almost for all parameters of immunocastrates are positioned between the gilts and surgical castrates. Higher weights of shoulder, neck, loin and ham in vaccinated pigs compared to barrows could be explained by higher slaughter weight of immunocastrates. These pigs had lower backfat thickness than barrows. Similar results were reported in studies of Fuchs et al. (2009), Fàbrega et al. (2010), Batorek et al. (2012), Kratochvíl et al. (2011), Sattler and Schmoll (2013), and Grela et al. (2013). Lean meat content of immunocastrates in our study was slightly lower than that of surgical castrates. This is in contrast with findings of another authors (Fuchs et al., 2009; Batorek et al., 2012b; Kratochvíl et al., 2011; Sattler and Schmoll, 2013; Škrlep et al., 2010a, Pauly et al., 2009 and Grela et al., 2013).

Meat quality parameters of pigs are shown in table 3. Differences between sexes were not significant except with WHC in immunocastrated and surgically castrated pigs. Meat from IC had the lowest value followed by gilts and SC. Another traits were not affected by immunocastration. Similar results were reported in various studies (Batorek et al., 2012b; Sattler and Schmoll, 2013; Škrlep et al., 2010a; Pauly et al., 2009).

Conclusion

Immunization of entire males with GnRF vaccine had no negative effect on fattening, carcass and pork quality parameters compared to those surgically castrated and female pigs. Only water holding capacity of immunocastrated pigs was significantly lower than that of barrows. Tendency (P=0.08-0.09) to faster growth and more effective feed conversion of immunocastrates compared to barrows could be of interest for pig producers.

Table 1. Fattening traits of pigs

Item	IC (n = 14)	SC (n = 13)	G (n = 13)
Average daily gain, g	881 ± 0.02	830 ± 0.02	823 ± 0.02
Feed conversion ratio, kg	2.72 ± 0.06	2.87 ± 0.06	2.88 ± 0.05

Table 2. Carcass traits of pigs

Item	IC (n = 14)	SC (n = 13)	G (n = 13)
Slaughter weight, kg	123.12 ± 0.74	120.56 ± 0.88	121.36 ± 0.81
Backfat thickness, mm	26.37 ± 0.84	27.70 ± 1.37	24.98 ± 0.89
Lean meat, %	56.52 ± 0.60	56.88 ± 1.17	57.81 ± 0.57
Weight of shoulder, kg	5.50 ± 0.10	5.19 ± 0.16	5.54 ± 0.12
neck, kg	3.67 ± 0.05	3.58 ± 0.07	3.69 ± 0.06
loin, kg	5.45 ± 0.09	5.41 ± 0.10	5.69 ± 0.08
ham, kg	9.75 ± 0.15[ab]	9.53 ± 0.25[a]	10.38 ± 0.22[b]
Ham portion, %	20.65 ± 0.36	20.29 ± 0.59	21.67 ± 0.42
Valuable meat cuts, %	51.56 ± 0.59	50.46 ± 1.08	52.81 ± 0.66
Fatty cuts, %	13.19 ± 0.49	14.44 ± 0.97	12.56 ± 0.52

Valuable meat cuts = shoulder, neck, loin and ham
Fatty cuts = kidney fat, backfat, fat from shoulder and ham
[a,b] $P<0.05$

Table 3. Pork quality of pigs

Item	IC (n = 14)	SC (n = 13)	G (n = 13)
pH_{45}	6.42 ± 0.06	6.32 ± 0.06	6.55 ± 0.07
pH_{24}	6.08 ± 0.05	6.01 ± 0.08	6.07 ± 0.07
Color – Japanese scale	3.32 ± 0.09	3.33 ± 0.14	3.43 ± 0.10
Color – L^*	49.67 ± 0.69	48.92 ± 0.72	48.27 ± 1.11
a^*	0.99 ± 0.20	1.27 ± 0.21	1.36 ± 0.27
b^*	7.84 ± 0.21	7.91 ± 0.30	7.85 ± 0.28
Drip loss, %	3.67 ± 0.61	4.36 ± 0.71	3.68 ± 0.43
WHC, %	31.49 ± 1.26[a]	37.68 ± 2.37[b]	34.77 ± 0.98[ab]
Shear force, kg	1.13 ± 0.11	1.19 ± 0.10	1.39 ± 0.10

[a,b] $P<0.05$

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Oral presentation

THE EFFECT OF SOLAR/LUNAR RAYS AND PLANETS MOVEMENTS ON LAMBING RESULTS

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Abstract

In this study, available data acquired in 2016 were obtained from a total of 934 lambs born from 873 ewes in 12 neighboring farms located at the same coordinate (38° 34' 8.634" N, 43° 15' 53.283" E) and altitude (1656 m) in Van Province, Eastern Turkey. Routine data such as age of ewe (AE), horn size of ewe (HE), ear size of ewe (EE), sex of lamb (SL), type of birth (TB), date of birth (DB) and birth time (BT) were collected according to the 24-hour clock during lambing season between 6th February and 7th April 2016. Also, start/end dates of births were corresponded between the New Moon-3 days and the New Moon, respectively. Moreover, 63 of all ewes made twin birth. The following results of twin lambs (n=126) were found according to the analysis conducted in the Moon octal phase: Waning Gibbous (37.705%), Waxing Gibbous (21.312%), Waning Crescent (13.115%), Waxing Crescent (16.393%), First Quarter (8.197%), Full Moon (1.639%) and Last Quarter (1.639%). On the other hand, no twins were born in New Moon.

USNO (Astronomical Applications Department of The United States Naval Observatory)'s web site has been designed especially to provide information on the global and local visibility of solar and lunar rays. Both the Sun/Moon (rise/set times) and twilight (astronomical, nautical, civil) data (begin/end times) were evaluated. After, dual/quad/octal phases and apogee/perigee phases of the Moon associated with birth dates were investigated in detail. More lunar data than solar ones were collected because the Moon has non-regular movement as opposed to the Sun. Moreover, data of 7 known planets at various distances from Earth were collected via this web site. On the other hand, information gathered about the altitude and the azimuth values of celestial bodies were important in terms of the depth of the study. Then, results stemming from the data were presented after the completion of necessary processes.

Keywords: biodynamic animal husbandry, biodynamic livestock, ewe, lambing, lunar calendar

Introduction

Daily circadian rhythm is a biological process that displays an endogenous, chronobiology oscillation of about 24 hours ± 30 seconds. Both the lunar (29.53 days) and the seasonal (365.24 days) geophysical cycles like the daily circadian rhythm dominate various vital activity of plant and animal organisms on Earth. Regular vital activities and species-specific behaviors are affected by the light perceived by the eyes in mammals. So that, despite the

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imposition of social time and a world divided into time zones, especially human (Roenneberg et al., 2007) and an animal behavior are still dominated by geophysical both sunrise/sunset and moonrise/moonset times. Studies in recent years have revealed that circadian cycle plays in the important role on the habitual behavior and physiological conditions of various plants (Deep and Mittal, 2014; Jovchelevich, 2014) and animals (Palacios and Abecia, 2015). Moreover, it is known that humankind have carefully observed as highly relevant the diverse Moon phase since the Before Christ. With regard to the movement and brightness of celestial objects, results based on observations are still being followed in accordance to the Moon (Zurcher, 1999) cycles. Another known fact that, regulate of the temporal biology of living on Earth (Foster and Roenneberg, 2008) are done by the combine effects of daily, seasonal, lunar, solar, tidal cycles and the other celestial bodies. The lunar synodic cycle (FM to FM) causes a number of environmental changes that can be perceived by animals, such as the brightness of lunar light and gravitational changes, with maximum gravitational pull occurring when the Moon and Sun are aligned (at periods of NM and FM) (Grant et al., 2009; Fischer et al. 2001) .

The spectral composition names of the light are *"twilight times"*. Profound changes in irradiance at 3 begin twilights and 3 end twilights. Twilight is primarily characterized both relative enrichment of the shorter wave-lengths *(<500* nm) compared to the mid-long wavelengths (500-650 nm) and very precise spectral changes. The most obvious example for temporal specialization is the timing of activity to parts of the light, dark or twilight zones (Roenneberg et al., 1997). The time outside of day and twilights was also consider as night.

Material and Methods

Farms are located in Van/TR, with the following coordinates: latitude 38° 34' 8.634" N and longitude 15' 53.283" E, altitude 1656 m. 934 lambs data collected from 873 pregnant ewes bred in 12 neighbor farms in the Bardakci village were created live material. Infertile animals were disqualified at the beginning of the study and some of the data of pregnant ewes were gathered from normal births taken place from date of the 6th February and 7th April 2016. In the meantime, date of birth (DB) was recorded and birth time (BT) were coded as 24-hour period (Roenneberg et al., 1997). In addition, data of age of ewe (AE), horn size of ewe (HE), ear size of ewe (EE) sex of lamb (SL) and type of birth (TB) were recorded. During this study were not applied any intervention to the animals.

Thun (2005 and 2006) reference at the beginning of work on the lunar rhythm of this study was important as a guide. Starting here, DB and BT records on the basis of solar and lunar data associated with related coordinate and altitude were obtained from the USNO's official website (http://aa.usno.navy.mil/data/index.php). Meanwhile, for the timezone "2 hours and east of Greenwich" sections were marked on the web page. So that, detailed data related with the following headings were accessed.

I. Rise/Set/Transit Data
  I.I. <u>Complete Sun and Moon data for one day</u>
    I.I.I. Sun
      I.I.I.I.   Sunrise (SR)
      I.I.I.II. Sunset (SS)
    I.I.II. Moon
      I.I.II.I.   Moonrise (MR)
      I.I.II.II. Moonset (MS)
  I.II. <u>Table of twilight times for an entire year</u>
      I.II.I.   Civil twilight (CT)
      I.II.II. Nautical twilight (NT)
      I.II.III. Astronomical twilight (AT)

II. Moon phase
  II.I.   Crescent and gibbous phases
      (Waxing crescent (WXC), Waxing gibbous (WXG), Waning gibbous (WNG),
    Waning crescent (WNC))
  II.II.   Dual phase
      (New Moon (NM), Full Moon (FM))
  II.III.   Quad phase
      (NM, First Quarter (FQ), FM, Last Quarter (LQ))
  II.IV. Octal phase
      (NM, WXC, FQ, WXG, FM, WNG, LQ, WNC)

After these data, USNO data were coded for the 2nd time to ensure compliance with cases. According to this, time ranges between begin AT, begin NT, begin CT, SR, ST, SS, end CT, end NT and end AT were coded, respectively.

Data of Moon phases from the USNO for DB at midnight at Universal Time (GMT) were obtained (Grant et al., 2009). Primarily, birth data were coded to correspond to the octal phase of the Moon: NM, WXC, FQ, WXG, FM, WNG, LQ, WNC (Arliss et al., 2005; Deep and Mittal, 2014; Palacios and Albecia, 2014). This was the starting point of the study. After then, dual phase (NM, FM) and quad phase (NM, FQ, FM, LQ) data were derived there from (Benbadis et al., 2004). However, Moon dual phase (MDF), Moon quad phase (MQF) and Moon octal phase (MOF) was encoded for this study. Additionally "nearest phase of Moon" related with BT was important for this study. In this part of analysis previous (P) and next (N) phase of the Moon were used as NNM, NM, PNM, NFQ, FQ, PFQ, NFM, FM, PFM, NLQ, LQ and PLQ (Thun, 2005; 2006). So, these data were used for analyses.

During the BT, whether the combined effect of Sun, Moon and each of 7 planets (Mercury, Venus, Mars, Jupiter, Saturn, Uranus, Neptune) known of the solar system were examined.

Based on the BT, data were analyzed according to the 24-hour period (Roenneberg et al., 2007). Results of the preliminary statistical data gained from Khi-Square analysis presented in this study, which were presented in %.

Results and Discussion

In this study, begin/end dates of births were corresponded between the NM-3 days and the NM, respectively. Moreover, 126 lambs were twin. The following results of twin, birth of twin lambs compared itself, were found according to the analysis conducted in the Moon octal phase: WNG (37.705%), WXG (21.312%), WNC (13.115%), WXC (16.393%), FQ (8.197%), FM (1.639%) and LQ (1.639%).

Comparison of birth data with 6-hour periods and a variety of birth-day intervals associated with twilight times, daylight and night results were presented in the Tab.1. According to these results, parameters of EE and parameters of 6 hour periods had significant statistical result (P<0.05). Also, any statistical important was for 6 hour periods. But, percentages of results were occurred that 06-12 hours were very rush-hour for births. In fact in this case, this situation was the opposite of "sheep usually give birth at night". The highest birth rates were seen in tis interval of the day in animals.

Twilight analysis results although important, Any significant statistical results were between birth data and twilight times. Nevertheless, the highest birth rates were between SR and SS intervals of the days. The incidence of birth coincides with the period of the night was 2nd-order in the Tab.1. The lowest rates of births (6 %) were Begin AT-NT and End CT-NT intervals for all data (AE, HE, EE, SL, TB).

Comparison of the birth data related with the Sun and the Moon position in the sky were presented in the Tab.2. In this connection, percentages and statistical results of Sun R-S, Moon R-S and Sun&Moon R-S were presented. According to this, any significant results were found for analysis parameters (P>0.05). Although, the percentage of Sun R-S interval was higher than the night. Additionally, 2nd statistical analysis results of Tab. 2. were found as non- Moon R-S intervals percentage higher than the Moon R-S intervals percentage for all parameters. 3Th statistical analysis results related with Sun&Moon R-S intervals had non significant results (P>0.05) like as others in the Tab.2. But it appears that, if the Sun&Moon were not in the sky, birth rates were the highest level.

Statistical results related comparison of the birth data with dual, quad, octal phases and shine of the Moon were presented in the Tab.3. In the same table, statistical results and percentages of dual, quad, octal phases of the Moon and Moon shine are visible. According to this, Statistical significance (P<0.05) was found between EE and octal phases of the Moon.

Palacios and Albecia (2011) reported as "no evidence to support the supposition that lunar phase affected the timing of births in sheep". In an another study of the same authors related sheep, "Lunar phase was a comprising significant effect on fertility rate (p < 0.001)" were reported by Palacios and Albecia (2014). In Czechoslovakia, Horak and Potucek (1978) found in 9650 ewes that the start of cycling of seasonal breeding was associated with the occurrence of the FM (Marai and Rashwan, 2004). Births were rush at FM in dual phases, LQ in quad phases, WNG in octal phases and 51-100 % in Moon shine percentages in this study. Deep and Mittal (2014) were reported percentages that in sprouts, the potassium uptake has shot up after the FM (Super Moon) and was comparatively higher near the NM and at the FQ phases.

But the result of this study would clearly show that, results of Moon shine was not

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statistically significant (P>0.05).

Any twins were born at NM in this study. It may be due to differences in water retention according to the phases of the Moon. We know that "gravity is one major force that creates tides". The other hand, Newton's law of universal gravitation states that the greater the mass of the objects and the closer they are to each other, the greater the gravitational attraction between them (Ross, 1995). The effect of distance on tidal forces is seen in the relationship between the sun, the moon, and the Earth's waters (Anonymous, 2016).

Palacios and Abecia (2011) have previously demonstrated that the distribution of lambing dates of more than 68,000 lambing in sheep are similar and non-significantly different among the four Moon phase (Moon quad phase). But in contrast to this situation, high statistical significances (P<0.01) related TB were found between both quad and octal phases of the Moon in this study.

Comparison results of the birth data with nearest phases of the Moon were presented in the Table 4. In this table, previous and next phase of the Moon quad phases were presented. Maximum number of statistical significance was conducted at "Moon nearest phases". Results related with EE (P<0.05), SL (0.05) and TB (P<0.001) had statistical significances. In the NLQ phase of the Moon had the highest percentage in the "Nearest phases of the Moon".

Comparison of the birth data with 7 planets were calculated as statistical. These statistical results showed that Jupiter, Uranus and Neptune planets had no statistical significances (P>0.05) between them and birth data. The other hand, statistical significances were between SL and Mercury (P<0.05); Venus (P<0.05). Mars related with the significance level (P<0.01) between SL is the other important planet for this study. Finally with regard to the planets, statistical significance (P<0.05) was between SL and Saturn. When viewed from another given angle of vision, statistical significance could not be determined on the number of moons (satellites) of the planets.

Conclusion

This study supports the need to use natural light sources that the Sun and the Moon and associated with related coordinate and provides a comprehensive scientific basis for reproductive performance of small ruminants in the area of animal science. Attention was drawn to the truth power of natural light on life, the effects of celestial bodies and actually benefit from them by this study which obtained very important results.

Another important point is the effect of the gravitational pull of our World. Therefore it is very important that the locations of the planets interact with World. When the closest two planets to the Earth was thought that Venus and Mars, statistical results of the study seem to be more important. However, the association between them and lambing results, is not yet fully explained. For this reason, more extensive studies are needed.

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Appendix:

Table. 1. Comparison of birth data with 6-hour periods and a variety of birth-day intervals associated with twilight times, daylight and night

		6-hour periods						Twilight times									
		0-6	6-12	12-18	18-24	Total	Sig.	Begin AT-NT	Begin NT-CT	Begin CT-SR	SR-SS (Day)	SS-End CT	End CT-NT	End NT-AT	Night	Total	Sig.
AE	2	43 5 %	70 8.1 %	45 5.2 %	32 3.7 %	190 22 %	0.206	1 0.1 %	14 1.6 %	0 0 %	119 13.8 %	5 0.6 %	3 0.3 %	3 0.3 %	45 5.2 %	190 22 %	0.105
	3	49 5.7 %	118 13.7 %	60 7 %	59 6.8 %	286 33.1 %		3 0.3 %	9 1 %	6 0.7 %	181 21 %	8 0.9 %	1 0.1 %	1 0.1 %	77 8.9 %	286 33.1 %	
	4	56 6.5 %	90 10.4 %	60 7 %	48 5.6 %	254 29.4 %		2 0.2 %	16 1.9 %	4 0.5 %	152 17.6 %	8 0.9 %	1 0.1 %	4 0.5 %	67 7.8 %	254 29.4 %	
	5	30 3.5 %	52 6 %	33 3.8 %	11 1.3 %	126 14.6 %		0 0 %	7 0.8 %	2 0.2 %	89 10.3 %	2 0.2 %	1 0.1 %	2 0.2	23 2.7 %	126 14.6 %	
	6	1 0.1 %	3 0.3 %	3 0.3 %	0 0 %	7 0.8 %		0 0 %	0 0 %	0 0 %	4 0.5 %	2 0.2 %	0 0 %	0 0 %	1 0.1 %	7 0.8 %	
	Total	179 20.7 %	333 38.6 %	201 23.3 %	150 17.4 %	863 100 %		6 0.7 %	46 5.3 %	12 1.2 %	545 63.2 %	25 2.9 %	6 0.7 %	10 1.2 %	213 24.7 %	863 100 %	
HE	Hornless	138 16 %	264 30.7 %	152 17.7 %	121 14.1 %	675 78.4 %	0.915	5 0.6 %	39 4.5 %	9 1 %	426 49.5 %	17 2 %	4 0.5 %	7 0.8 %	168 19.5 %	675 78.4 %	0.700
	Short-horned	11 1.3 %	22 2.6 %	14 1.6 %	8 0.9 %	55 6.4 %		0 0 %	2 0.2 %	2 0.2 %	37 4.3 %	1 0.1 %	0 0 %	0 0 %	13 1.5 %	55 6.4 %	
	Long-horned	30 3.5 %	47 5.5 %	34 3.9 %	20 2.3 %	131 15.2 %		1 0.1 %	7 0.8 %	1 0.1 %	79 9.2 %	7 0.8 %	2 0.2 %	3 0.3 %	31 3.6 %	131 15.2 %	
	Total	179 20.8 %	333 38.7 %	200 23.2 %	149 17.3 %	861 100 %		6 0.7 %	48 5.6 %	12 1.4 %	542 63 %	25 2.9 %	6 0.7 %	10 1.2 %	212 24.6 %	861 100 %	
EE	Earless	5 0.6 %	13 1.5 %	19 2.2 %	7 0.8 %	44 5.1 %	0.032	0 0 %	2 0.2 %	0 0 %	31 3.6 %	1 0.1 %	1 0.1 %	0 0 %	9 1 %	44 5.1 %	0.895
	Short-eared	2 0.2 %	12 1.4 %	6 0.7 %	4 0.5 %	24 2.8 %		0 0 %	1 0.1 %	0 0 %	16 1.9 %	2 0.2 %	0 0 %	0 0 %	5 0.6 %	24 2.8 %	

	Long-eared	172 20.8 %	307 35.7 %	175 20.3 %	138 16 %	792 92.1 %		6 0.7 %	45 5.2 %	12 1.4 %	494 57.4 %	22 2.6 %	5 0.6 %	10 1.2 %	198 23 %	792 92.1 %
	Total	179 20.8 %	332 38.6 %	200 23.3 %	149 17.3 %	860 100 %		6 0.7 %	48 5.6 %	12 1.4 %	541 62.9 %	25 2.9 %	6 0.7 %	10 1.2 %	212 24.7 %	860 100 %
SL	Female	100 10.8 %	184 19.9 %	103 11.1 %	93 10.1 %	480 51.9 %	0.214	4 0.4 %	31 3.4 %	3 0.3 %	290 31.4 %	12 1.3 %	4 0.4 %	7 0.8 %	129 14.0 %	480 51.9 % · 0.131
	Male	93 10.1 %	171 18.5 %	114 12.3 %	66 7.1 %	444 48.1 %		2 0.2 %	22 2.4 %	10 1.1 %	291 31.5 %	16 1.7 %	2 0.2 %	4 0.4 %	97 10.5 %	444 48.1 %
	Total	193 20.9 %	355 38.4 %	217 23.5 %	159 17.2 %	924 100 %		6 0.7 %	53 5.7 %	13 1.4 %	581 62.9 %	28 3.0 %	6 0.7 %	11 1.2 %	226 24.5 %	924 100.0 %
TB	Single	169 18.2 %	312 33.7 %	185 20.0 %	135 14.6 %	801 86.4 %	0.610	6 0.6 %	43 4.6 %	11 1.2 %	510 55.0 %	22 2.4 %	6 0.6 %	9 1.0 %	194 20.9 %	801 86.4 % · 0.549
	Twin	24 2.6 %	44 4.7 %	32 3.5 %	26 2.8 %	126 13.6 %		0 0 %	10 1.1 %	2 0.2 %	72 7.8 %	6 0.6 %	0 0 %	2 0.2 %	34 3.7 %	126 13.6 %
	Total	181 20.8 %	336 38.4 %	202 23.4 %	149 17.4 %	927 100 %		6 0.6 %	53 5.7 %	13 1.4%	582 62.8 %	28 3.0 %	6 0.6 %	11 1.2 %	228 24.6 %	927 100 %

Table 2. Comparison of the birth data with the Sun and the Moon position (in the sky=Yes), (not in the sky=No)

		SUN R-S				MOON R-S				SUN&MOON R-S			
		Yes	No	Total	Sig.	Yes	No	Total	Sig.	Yes	No	Total	Sig.
AE	2	119 13.8 %	71 8.2 %	190 22 %	0.641	89 10.3 %	101 11.7 %	190 22 %	0.606	45 5.3 %	144 16.8 %	189 22.1 %	0.863
	3	182 21.1 %	104 12.1 %	286 33.1 %		143 16.6 %	143 16.6 %	286 33.1 %		75 8.8 %	209 24.4 %	284 33.2 %	
	4	155 18 %	99 11.5 %	254 29.4 %		112 13 %	142 16.5 %	254 29.4 %		58 6.8 %	193 22.5 %	251 29.3 %	
	5	87 10.1 %	39 4.5 %	126 14.6 %		54 6.3 %	72 8.3 %	126 14.6 %		34 4 %	91 10.6 %	125 14.6 %	
	6	4 0.5 %	3 0.3 %	7 0.8 %		3 0.3 %	4 0.5 %	7 0.8 %		2 0.2 %	5 0.6 %	7 0.8 %	
	Total	547 63.4 %	316 36.6 %	863 100 %		401 46.5 %	462 53.5 %	863 100 %		214 25 %	642 75 %	856 100 %	
HE	Hornless	246 28.6 %	429 49.8 %	675 78.4 %	0.730	323 37.5 %	352 40.9 %	675 78.4 %	0.338	175 20.4 %	497 58. 1v	672 78.7 %	0.401
	Short-horned	36 4.2 %	16 2.2 %	55 6.4 %		22 2.6 %	33 3.8 %	55 6.4 %		12 1.4 %	43 5 %	55 6.4 %	
	Long-horned	79 9.2 %	52 6 %	131 15.2 %		56 6.5 %	75 8.7 %	131 15.2 %		27 3.2 %	102 11.9 %	129 15.1 %	
	Total	544 63.2 %	317 36.8 %	861 100 %		401 46.6 %	460 53.4 %	861 100 %		214 25 %	642 75 %	856 100 %	
EE	Earless	31 3.6 %	13 1.5 %	44 5.1 %	0.264	21 2.4 %	23 2.7 %	44 5.1 %	0.984	9 1.1 %	34 4 %	43 5 %	0.739
	Short-eared	18 2.1 %	6 0.7 %	24 2.8 %		11 1.3 %	13 1.5 %	24 2.8 %		7 0.8 %	17 2 %	24 2.8 %	
	Long-eared	494 57.4 %	298 34.7 %	792 92.1 %		368 42.8 %	424 49.3 %	792 92.1 %		198 23.1 %	591 69 %	789 92.2 %	
	Total	543 63.1 %	317 36.9 %	860 100 %		400 46.5 %	460 53.5 %	860 100 %		214 25 %	642 75 %	856 100 %	
SL	Female	293 31.8 %	183 19.9 %	476 51.7 %	0.118	217 23.5 %	263 28.5 %	480 51.9 %	0.199	114 12.3 %	366 39.6 %	480 51.9 %	0.200
	Male	291 31.6 %	153 16.6 %	444 48.3 %		214 23.2 %	230 24.9 %	444 48.1 %		122 13.2 %	322 34.8 %	444 48.1 %	
	Total	584 63.5 %	336 36.5 %	920 100 %		431 46.6 %	493 53.4 %	924 100 %		236 25.5 %	688 74.5 %	924 100 %	
TB	Single	509 55.1 %	292 31.6 %	801 86.8 %	0.431	374 40.3 %	427 46.1 %	801 86.4 %	0.484	202 21.8 %	599 64.6 %	801 86.4 %	0.661
	Twin	76 8.2 %	46 5 %	122 13.2 %		58 6.3 %	68 7.3 %	126 13.6 %		34 3.7 %	92 9.9 %	126 13.6 %	
	Total	585 36.6 %	338 36.6 %	923 100 %		432 46.6 %	495 53.4 %	927 100 %		236 25.5 %	691 74.5 %	927 100 %	

Table 3. Comparison of the birth data with dual, quad, octal phases and shine of the Moon

		Dual phases				Quad phases						Octal phases										Moonshine			
		NM	FM	Total	Sig.	NM	FQ	FM	LQ	Total	Sig.	NM	WXC	FQ	WXG	FM	WNG	LQ	WNC	Total	Sig.	0-50%	51-100%	Total	Sig.
AE	2	52 6%	139 16%	191 22%	0.234	13 1.5%	58 6.7%	56 6.4%	64 7.4%	191 22%	0.098	2 0.2%	14 1.6%	11 1.35	56 6.4%	7 0.8%	73 8.4%	4 0.5%	24 2.8%	191 22%	0.115	52 6%	139 16%	191 22%	0.234
	3	101 11.6%	187 21.5%	288 33.1%		35 4%	64 7.4%	89 10.2%	100 11.5%	288 33.1%		4 0.5%	29 3.3%	8 0.9%	57 6.6%	17 2%	113 13%	10 1.1%	50 5.7%	288 33.1%		101 11.6%	187 21.5%	288 33.1%	
	4	86 9.9%	169 19.4%	255 29.3%		40 4.6%	55 6.3%	83 9.5%	77 8.9%	255 29.3%		7 0.8%	22 2.5%	9 1%	48 5.5%	14 31.8%	105 12.15%	13 1.5%	37 4.3%	255 29.3%		86 9.9%	169 19.4%	255 29.3%	
	5	50 5.7%	79 9.1%	129 14.8%		23 2.6%	27 3.1%	34 3.9%	45 5.2%	129 14.8%		6 0.7%	16 1.8%	4 0.5%	20 2.3%	5 0.6%	50 5.7%	5 0.6%	23 2.6%	129 14.8%		50 5.7%	79 9.1%	129 14.8	
	6	3 0.3%	4 0.5%	7 0.8%		2 0.2%	2 0.2%	2 0.2%	1 0.1%	7 0.8%		1 0.1%	2 0.2%	0 0%	1 0.1%	1 0.1%	2 0.2%	0 0%	0 0%	7 0.8%		3 0.3%	4 0.5%	7 0.8%	
	Total	292 33.6%	578 66.4%	870 100%		113 13%	206 23.7%	264 30.3%	287 33%	870 100%		20 2.3%	83 9.5%	32 3.7%	182 20.9%	44 5.1%	343 39.4%	32 3.7%	134 15.4%	870 100%		292 33.6%	578 66.4%	870 100%	
HE	Hornless	234 27%	444 51.2%	678 78.1%	0.656	99 11.4%	160 18.4%	197 22.7%	222 25.6%	678 78.1%	0.478	20 2.35	73 8.4%	19 2.2%	142 16.4%	33 3.8%	263 30.35	27 3.15	101 11.6%	678 78.1%	0.065	234 27%	444 51.2%	678 78.1%	0.656
	Short-horned	20 2.3%	36 4.1%	56 6.5%		4 0.5%	15 1.7%	20 2.3%	17 2%	56 6.5%		0 0%	3 0.3%	6 0.7%	11 1.3%	4 0.55	21 2.45	0 0%	11 1.3%	56 6.5%		20 2.3%	36 4.1%	56 6.5%	
	Long-horned	41 4.7%	93 10.7%	134 15.4%		13 1.5%	30 3.5%	43 5%	48 5.5%	134 15.4		0 0%	9 1%	6 0.7%	27 3.15	6 0.7%	59 6.8%	5 0.6%	22 2.55	134 15.4		41 4.7%	93 10.7%	134 15.4%	
	Total	295 34%	573 66%	868 100%		116 13.4%	205 23.6%	260 30%	287 33.1%	868 100%		20 2.3%	85 9.8%	31 3.6%	180 20.7%	43 5%	343 39.5%	32 3.7%	134 15.4%	868 100%		295 34%	573 66%	868 100%	
EE	Earless	15 1.7%	29 3.3%	44 5.1%	0.883	4 0.5%	11 1.3%	10 1.2%	19 2.2%	44 5.1%	0.037	1 0.1%	5 0.6%	3 0.3%	7 0.8%	4 0.5%	19 2.2%	0 0%	5 0.6%	44 5.1%	0.270	15 1.7%	29 3.3%	44 5.1%	0.883
	Short-eared	7 0.8%	17 2%	24 2.8%		0 0%	3 0.3%	11 1.3%	10 1.2%	24 2.8%		0 0%	2 0.2%	0 0%	6 0.7%	4 0.5%	7 0.8%	0 0%	5 0.6%	24 2.8%		7 0.8%	17 2%	24 2.8%	

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						p																	p				p
	Long-eared	272 31.4%	527 60.8%	799 92.2%		112 12.9%	191 22%	239 27.6%	257 29.6%	799 92.2%		19 2.2%	78 9%	28 3.2%	167 19.3%	35 4%	317 36.6%	32 3.7%	123 14.2%	799 92.2%		272 31.4%	527 60.8%	799 92.2%	
	Total	294 33.9%	573 66.1%	867 100%		116 13.4%	205 23.6%	260 30%	286 33%	867 100%		20 2.3%	85 9.8%	31 3.6%	180 20.8%	43 5%	343 39.6%	32 3.7%	133 15.3%	867 100%s		294 33.9%	573 66.1%	867 100%	
SL	Female	167 17.9%	315 33.8%	482 51.8%	0.782	72 7.75	115 12.4%	133 14.3%	162 17.4%	482 51.85	0.215	8 0.9%	50 5.4%	20 2.1%	90 9.7%	27 2.9%	201 21.6%	14 1.5%	72 7.7%	482 51.8%	0.444	167 17.9%	315 33.8%	482 51.8%	0.398
	Male	151 16.2%	298 32%	449 48.2%		51 5.55	114 12.2%	144 15.5%	140 15%	449 48.2%		12 1.3%	45 4.8%	17 1.8%	104 11.2%	18 1.9%	166 17.8%	18 1.9%	69 7.4%	449 48.2%		151 16.2%	298 32%	449 48.2%	
	Total	318 34.2%	613 65.8%	931 100%		123 13.2%	229 24.6%	277 29.8%	302 32.4%	931 100%		20 2.1%	95 10.2%	37 4.0%	194 20.8%	45 4.8%	367 39.4%	32 3.4%	141 15.1%	931 100%		318 34.2%	613 65.8%	931 100%	
TB	Single	271 29%	537 57.5%	808 86.5%	0.315	109 11.7%	183 19.6%	248 26.6%	268 28.7%	808 86.5%	0.010	20 2.1%	73 7.8%	27 2.9%	169 18.1%	43 4.6%	319 34.2%	31 3.3%	73 7.8%	808 86.5%	0.0003	271 29%	537 57.5%	808 86.5%	0.183
	Twin	48 5.1%	78 8.4%	126 13.5%		14 1.55	46 4.9%	30 3.2%	36 3.9%	126 13.5%		0 0%	22 2.4%	10 1.1%	26 2.8%	2 0.2%	48 5.1%	2 0.2%	22 2.4%	126 13.5%		48 5.1%	78 8.4%	126 13.5%	
	Total	319 34.2%	615 65.8%	934 100%		123 13.2%	229 24.5%	278 29.8%	304 32.5%	934 100%		20 2.1%	95 10.2%	37 4.0%	195 20.0%	45 4.8%	367 39.3%	33 3.6%	95 10.2%	934 100%		319 34.2%	615 65.8%	934 100%	

Table 4. Comparison of the birth data with nearest phases of the Moon

		Nearest phases												Total	Sig.
		NNM	NM	PNM	NFQ	FQ	PFQ	NFM	FM	PFM	NLQ	LQ	PLQ	Total	Sig.
AE	2	8 0.9 %	2 0.2 %	3 0.3 %	11 1.3 %	11 1.3 %	36 4.1 %	20 2.3 %	7 0.8 %	29 3.3 %	43 4.9 %	4 0.5 %	17 2 %	191 22 %	0.205
	3	23 2.6 %	4 0.5 %	8 0.9 %	21 2.4 %	8 0.9 %	35 4 %	22 2.5 %	17 2 %	50 5.7 %	53 6.1 %	10 1.1 %	37 4.3 %	288 33.1 %	
	4	27 3.1 %	7 0.8 %	6 0.7 %	16 1.8 %	9 1 %	30 3.4 %	18 2.1 %	14 1.6 %	51 5.9 %	43 4.9 %	13 1.5 %	21 2.4 %	255 29.3 %	
	5	10 1.1 %	6 0.7 %	7 0.8 %	9 1 %	4 0.5 %	14 1.6 %	6 0.7 %	5 0.6 %	23 2.6 %	26 3 %	5 0.6 %	14 1.6 **%**	129 14.8 %	
	6	0 0 %	1 0.1 %	1 0.1 %	1 0.1 %	0 0 %	1 0.1 %	0 0 %	1 0.1 %	1 0.1 %	1 0.1 %	0 0%	0 0 %	7 0.8 %	
	Total	68 7.8 %	20 2.3 %	25 2.9 %	58 6.7 %	32 3.7 %	116 13.3 %	66 7.6 %	44 5.1 %	154 17.7 %	166 19.1 %	32 3.7 %	89 10.2 %	870 100 %	
HE	Hornless	57 6.6 %	20 2.3 %	22 2.5 %	51 5.9 %	19 2.2 %	90 10.4 %	52 6 %	33 3.8 %	112 12.9 %	130 15 %	27 3.1 %	65 7.5 %	678 78.1 %	0.275
	Short-horned	3 0.3 %	0 0 %	1 0.1 %	2 0.2 %	6 0.7 %	7 0.8 %	4 0.5 %	4 0.5 %	12 1.4 %	9 1 %	0 0 %	8 0.9 %	56 6.5 %	
	Long-horned	10 1.2 %	0 0 %	3 0.3 %	6 0.7 %	6 0.7 %	18 2.1 %	9 1 %	6 0.7 %	28 3.2 %	27 3.1 %	5 0.6 %	16 1.8 %	134 15.4 %	
	Total	70 8.1 %	20 2.3 %	26 3 %	59 6.8 %	31 3.6	115 13.2 %	65 7.5 %	43 5 %	152 17.5 %	166 19.1	32 3.7 %	89 10.3 %	868 100 %	
EE	Earless	1 0.1 %	1 0.1 %	2 0.2 %	3 0.3 %	3 0.3 %	5 0.6 %	2 0.2 %	4 0.5 %	4 0.5 %	14 1.6 %	0 0 %	5 0.6 %	44 5.1 %	0.021
	Short-eared	0 0%	0 0 %	0 0 %	2 0.2 %	0 0 %	1 0.1 %	5 0.6 %	4 0.5 %	2 0.2 %	5 0.6 %	0 0 %	5 0.6 %	24 2.8%	
	Long-eared	69 8 %	19 2.2 %	24 2.8 %	54 6.2 %	28 3.2 %	109 12.6 %	58 6.7 %	35 4 %	146 16.8 %	147 17 %	32 3.7 %	78 9 %	799 92.2 %	
	Total	70 8.1 %	20 2.3 %	26 3 %	59 6.8 %	31 3.6 %	115 13.3 %	65 7.5 %	43 5 %	152 17.5 %	166 19.1 %	32 3.7 %	88 10.1 %	867 100 %	
SL	Female	42 4.5 %	8 0.9 %	22 2.4 %	28 3 %	20 2.1%	67 7.2 %	23 2.5 %	27 2.9 %	83 8.9 %	101 10.8 %	14 1.5 %	47 5 %	482 51.8 %	0.021
	Male	31 3.3 %	12 1.3 %	8 0.9 %	37 4 %	17 1.8 %	60 6.4 %	44 4.7 %	18 1.9 %	82 8.8 %	76 8.2 %	18 1.9 %	46 4.9 %	449 48.2 %	
	Total	73 7.8 %	20 2.1 %	30 3.2 %	65 7 %	37 4 %	127 13.6 %	67 7.2 %	45 4.8 %	165 17.7 %	177 19 %	32 3.4 %	93 10 %	931 100 %	
TB	Single	67 7.2 %	20 2.1 %	22 2.4 %	51 5.5 %	27 2.9 %	105 11.3 %	64 6.9 %	43 4.6 %	141 15.1 %	153 16.4 %	31 3.3 %	84 9 %	808 86.6 %	0.001
	Twin	5 0.5 %	0 0 %	8 0.9 %	14 1.5 %	10 1.1 %	22 2.4 %	4 0.4 %	2 0.2 %	24 2.6 %	24 2.6 %	2 0.2 %	10 1.1 %	125 13.4 %	
	Total	72 7.7 %	20 2.1 %	30 3.2 %	65 7 %	37 4 %	127 13.6 %	68 7.3 %	45 4.8 %	165 17.7 %	177 19 %	33 3.5 %	94 10.1 %	933 100 %	

Poster presentation

THE POTENTIAL APPLICATIONS OF PLANT EXTRACTS – ESSENTIAL OILS IN LIVESTOCK ORGANIC PRODUCTION SYSTEMS

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Abstract

Livestock production is under political and social pressure, especially in the European Union (EU), to decrease pollution and environmental damage arising due to animal agriculture. The EU has banned the use of antibiotics and other chemicals, which have been shown to be effective in promoting growth and reducing environment pollutants because of the risk caused to humans by chemical residues in food and by antibiotic resistance being passed on to human pathogens. As a result of this, scientists have intensified efforts in exploiting plants, plant extracts or natural plant compounds as potential natural alternatives for enhancing the livestock productivity.

The purpose of the present work was to examine the antibacterial activity of essential oils (EOs) derived from some aromatic medicinal plants (cultivated in Serbia), against pathogens from cows udder, in order to use EOs as an active substance in intramammary preparations for veterinary use, alternative to conventional drugs.

The EOs from savory (*Satureja montana* L.), thyme (*Thymus vulgaris* L.), peppermint (*Mentha* x *piperita* L.), were extracted by hydrodistillation and analyzed by gas chromatography and showed a high content of carvacrol, thymol and menthol, representing 42.1%, 34.4%, and 36.7% of the total oil, respectively. Antibacterial sensitivity of isolates (field strain originating from cattle milk) was tested *in vitro* using an Agar Dilution Method (ADM) to determine the minimal inhibitory concentration (MIC) of selected EO's.

The results obtained have shown that selected EOs performed antimicrobial activity against pathogens *in vitro* assays. EOs from savory, thyme, and peppermint were effective against all tested strains (MICs ranged from 0.39 to 3.12 µL mL^{-1}).

In addition to their well-known traditional use in food and cosmetics, the great potential of tested EOs for application in veterinary medicine encourage further research *in vitro* and *in vivo*.

Keywords: animals pathogens, antibacterial activity, essential oils

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Introduction

Plant oils and extracts have been used for a wide variety of purposes for many thousands of years. The antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Baser and Buchbauer, 2010).

The abundant use of anti-infective agents resulted in developing resistance of bacteria strains to certain antibiotics, presence of antibiotic residues in the food of animal origin etc. To overcome these problems, a variety of medicinal plants have been screened worldwide for their antimicrobial properties. Essential oils (EOs) derived from aromatic medicinal plants have been reported to exhibit exceptionally good antimicrobial effects against bacteria, yeasts, filamentous fungi, and viruses. For veterinary medicine, the antibacterial effect is of great importance, because of that EOs may have potentials in the control of infective animal diseases. The control of bovine mastitis is interesting for us because mastitis remains a major challenge to the worldwide dairy industry despite the widespread implementation of mastitis control strategies. Bovine mastitis, defined as `inflammation of the mammary gland', can have an infectious or noninfectious etiology. Organisms as diverse as bacteria, mycoplasma, yeasts and algae have been implicated as causes of the disease. There has identified 137 different organisms as a cause of mastitis. Fortunately the vast majority of mastitis is of bacterial origin and just five species of bacteria (*Escherichia coli, Streptococcus uberis, Staphylococcus aureus, Streptococcus dysgalactiae* and *Streptococcus agalactiae*) account for almost 80% of all diagnoses. Mastitis continues to be the most economically important disease of dairy cattle, accounting for 38% of the total direct costs of the common production diseases (Bradley A. J., 2002).

While some of the oils used on the basis of their reputed antimicrobial properties have well been documented *in vitro* activity, there are few published data for many others. While these data are useful, the reports are not directly comparable due to methodological differences such as choice of plant extract(s), test micro-organism(s) and antimicrobial test method (Hammer et al., 1999; Lambert et al., 2001; Ratajac R. et al., 2014). The aim of this study was to evaluated a antimicrobial activity of the EOs of peppermint (*Mentha* x *piperita* L.), savory (*Satureja montana* L.) and thyme (*Thymus vulgaris*) plants cultivated in Serbia, *in vitro* conditions against pathogens from animal mammary gland, isolates (field strains originating from cattle's milk) and bacterial strains obtained from American type culture collection (ATCC). Therefore, it is assumed that all of the tested oils can demonstrate high activity against bacteria and it might be an active substance for pharmaceutical use in intramammary preparations for veterinary use.

Material and Methods

Plant Material and Essential Oils

The plant material (herb in flower - *Menthae herba, Saturejae herba, Thymii herba*) used for the tests were collected from plants cultivated in the experimental field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia. Air-drying of the plants were performed in a shady place at room temperature for 10 days (the moisture content was about 10.00%

(m/m)). A weighed mass of plants material were submitted for 2 h to water-distillation, according to the method described in Ph. Jug. V (2.8.12.). The oils were separated from water by decantation and were separated from the solvent by distillation in a rotary vacuum evaporator at a temperature not higher than 50°C. The obtained EOs were dried over anhydrous sodium sulphate and were used for GC measurements and antibacterial tests.

Gas Chromatography

GC analyses were performed using a United Technologies Packard model 439 gas chromatograph equipped with FID, a column CP-SIL 5 CB was installed (10 m x 0.25 mm i.d., 100% dimethylpolysiloxane). Oven temperature was programmed to subsequently at 600C/min up to 2000C, at 3.50C/min, and then held isothermal for 10 min; injector and detector temperatures, 2000C and 2900C, respectively; nitrogen was used as gas carrier, adjusted to a linear flow of 86 mL/min. Oil samples were dissolved in n-hexane 1:100 (1μL of test oil in 100μL of n-hexane) and were injected in quantity of 1μL sample solution in n-hexane. The components were identified by comparing the retention time of the sample signal with retention times of reference standards (Sigma, Chemical CO.; Merck-Schuchardt). The percentage composition of the oils was computed, by the normalization method, from the GC peak areas without using correction factors. The percentage data shown are mean values of two injections.

Organisms and growth conditions

Milk samples collected aseptically from cows suffering from mastitis were examined for bacterial growth. Isolation and identification were performed by routine methods that are applied in the Scientific Veterinary Institute Novi Sad. Isolations of bacteria were done by using blood agar (BA) medium (supplemented with 5% defibrinated sheep blood) and MacConkey agar. The seeded plates were incubated at a temperature of 37°C under aerobic, microaerophilic and anaerobic conditions 24-72 hours. After seeding samples on nutrient media, colonies were macroscopic reviewed. Selected suspect colonies from solid substrates were seeded to isolate pure cultures, and then (or at the same time: directly from the sample or material-prime culture) were performed microscopic, biochemical and serological identification. The bacterial strains obtained from ATCC were: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Streptococcus agalactiae* ATCC 13813. Organisms were maintained on BA. Overnight cultures were prepared by inoculating approximately 2mL Mueller Hinton broth (MHB) with 2–3 colonies of each organism taken from BA. Broths were incubated overnight at 35°C. Inocula were prepared by diluting overnight cultures in saline to approximately 10^6 cfu mL^{-1} for bacteria.

Agar dilution method

The agar dilution method followed approved by the NCCLS with the following modification: a final concentration of 0.5% (v/v) Tween-20 (Sigma) was incorporated into the agar after autoclaving to enhance oil solubility. Briefly, a series of twofold dilutions of each oil, ranging from 2.5% (v/v) to 0.0012% (v/v) (at concentrations ranging from 25 to 0.0122 μL mL^{-1}), was prepared in Mueller Hinton (MH) agar (or MH with 5% sheep blood). Plates were dried at 35°C for 30 min prior to inoculation with 10 μL spots containing

approximately 10^4 cfu of each organism. Mueller Hinton agar, with 0.5% (v/v) Tween-20 but no oil, was used as a positive growth control. Inoculated plates were incubated at 35 to 37°C for a period of 18 hours. Minimum inhibitory concentrations (MICs) were determined after 18 h. The MICs were determined as the lowest concentration of oil inhibiting the visible growth of each organism on the agar plate. The presence of one or two colonies was disregarded.

Results and Discussion

In EO obtained from peppermint in flower, we identified 40 ingredients by GC analysis. The chemical composition of oil was dominated by menthol (36.74%), menthone (18.81%), menthyl acetate (9.42%) and limonene (5.99%) (Table 1). The main component menthol in an amount up to 50% of oil and 15 - 25% of menthone, is in accordance with the results of other researchers. In studies of Soković et al. (2009) the main components of the EO of *M. piperita* were identified: menthol (37.4%), methyl acetate (17.4%) and menthone (12.7%).

Chemical analysis of essential oils winter savory in bloom, indicated the following results: identified 27 ingredients and chemical composition of oil was dominated by carvacrol (42.12%) limonene (24.57%) and *p*-cymene (19.85%) (Table 1). The results of our tests are in accordance with studies of the chemical composition of the EO of selected and cultivated savory, while the significant difference in relation to the publication, which studied the chemical composition of the essential oils of wild savory. Namely, the selected and cultivated savory contained carvacrol in an amount from 42 to 67% as the main component in the oil, and thymol (29-43%) was the major ingredient in the wild forms, *S. hortensis*. Among the other components of the oil, these were identified: *p*-cymene (4.5 to 30.0%), γ-terpinene (15.3 to 45.0%), α-pinene (8.0%), metilthymol (5.4%), spathulenol (5.2%), 1,8-cineole (3.8%), α-terpinene (1.29%), β-caryophyllene (1.90%) and β-bisabolene (1.01%) (Adıgüzel A. et al., 2007; Mihajilov-Krstev et al., 2009a). In the studies of Milosavljevic et al. (2000), the chemical composition of the EO of savory (*Satureja kitaibelii* Wierzba. ex Heuff.) from six different localities in Eastern Serbia trans-geraniol was the dominated compound, and only in one locality *p*-cymene was the major component (34.16%). Qualitative and quantitative compositions of the most abundant compounds (in some samples from different localities, all listed components were not detected) were for trans-geraniol 12.98% - 29.66%, linalool 6.17% - 11.97%, *p*-cymene 4.37% - 34.16%, limonene 5.68% - 10.45%, linalool 4.96% - 10.66%, caryophyllene oxide 3.02% and 4.31%, borneol 5.36% - 10.41%, ß-caryophyllene 4.47% and terpinen-4-ol from 4.13% to 10.84%. The explanation for these differences in the chemical composition is in the genetic variability of cultivated and wild varieties of plants, as well as in environmental factors.

Table 1. Essential oil composition (% of major components) of *Mentha, Satureja* and *Thymus*

Compound	Essential oils / (%)		
	Thyme	Peppermint	Winter savory
Carvacrol	4.92	-	42.12
Limonene	-	5.99	24.57
Menthyl-acetate	-	9.42	-
Menthol	-	36.74	-
Menthone	-	18.81	-
Menthophuran	-	2.40	-
p-Cymene	34.04	-	-
Thymol	34.41	-	-

The results of the chemical composition of the EO of thyme herbs - thyme in bloom, showing that the dominant ingredients were presented in the following concentrations: thymol (34.41%), p-cymene (34.04%) and carvacrol (4.92%) and 34 compounds were identified (Table 1). Composition of EO in our studies is consistent with the results of the examination of the EO derived from the dried plant material: *Thymus vulgaris* L. and *Thymus tosevii* L.. Thymol (48.9%) and p-cymene (19.0%) were the main components *T. vulgaris*, while carvacrol (12.8%), a-terpinyl acetate (12.3%), cis-mirtanol (11.2%) and thymol (10.4%) dominated in *T. tosevii* (Soković et al., 2009).

A total of 45 bacterial isolates comprising *Eschericia coli, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus disgalactiae*, and *Streptococcus uberis* were isolated from the milk samples.

The MICs of the tested oils against pathogens field (isolates) and ATCC strains obtained by the agar dilution method are shown in Table 2. Peppermint (*Aetheroleum Menthae piperitae*) inhibited all organisms at the concentration of 3.12 µL mL^{-1}. *E. coli* and *Staph. aureus* were the most susceptible organisms, average MIC values were 1.34 and 1.17 µL mL^{-1}, respectively. Savory and thyme (*Aetheroleum Saturejae, Aetheroleum Thymii*) inhibited all organisms at the concentration of 0.78 µL mL^{-1}. Thyme had the lowest average MIC of 0.50 µL mL^{-1} against *E. coli*, and the lowest MIC of savory EO was against the *Streptococcus spp.* (0.43 µL mL^{-1}). Isolates and ATCC strain of *Staph. aureus* were less susceptible than other bacteria to EOs of savory and thyme (average MIC values were 0.65 µL mL^{-1}). The results obtained have shown that EOs from thyme and winter savory performed the strongest antimicrobial activity against all tested bacteria *in vitro* assays, average MIC values for thyme and savory were from 0.46 to 0.65 µL mL^{-1}. It can be observed in the correlation between the content of the major ingredients carvacrol and thymol in oils and their good antibacterial activity.

Table 2. MICs of the essential oils of *Satureja montana* L., *Mentha x piperita* L. and *Thymus vulgaris* against pathogens from cows mammary gland

Test substances (EOs)	Bacterial field and ATCC strains / MIC µL mL^{-1}		
	Escherichia coli (13 field strains + ATCC 25922)	*Staphylococcus aureus* (17 field strains + ATCC 25923)	*Streptococcus spp.* (15 field strains + *S. agalactiae* - ATCC 13813)
Thyme	0.39 - 0.78 (Ẍ =0.50)	0.39 - 0.78 (Ẍ =0.65)	0.39 - 0.78 (Ẍ =0.51)
Peppermint	0.78 - 1.56 (Ẍ =1.34)	0.78 - 1.56 (Ẍ =1.17)	1.56 - 3.12 (Ẍ =1.85)
Winter savory	0.39 - 0.78 (Ẍ =0.53)	0.39 - 0.78 (Ẍ =0.65)	0.39 - 0.78 (Ẍ =0.46)

Using the agar dilution method, tested thyme EO showed good antibacterial activity. Very similar results were published by Hammer et al. (1999) who performed testing the antimicrobial activity of EO of thyme herb against *E. coli* and *Staphylococcus aureus*. Using the agar dilution method, they came to the following results: the value of MIC (% v/v) for EO were as follows: *E. coli* 0.12 and *S. aureus* 0.25, and MIC and minimum cidal concentration (MCC) (% v/v) obtained by broth microdilution method were: for *Staph. aureus* MIC - 0.03, MCC - 0.06 and for *E. coli*, MIC - 0.03, MCC - 0.03.

EO of mint in the agar dilution test inhibited the growth of microorganisms at the concentration of 2.0 % (v/v) (Hammer et al., 1999), the value of MIC (%, v/v) was as follows: *E. coli* 0.5 and *S. aureus* 1.0, and using the broth microdilution method for determination of MIC and MCC (% v/v) the results of the EO of mint were: for *Staph. aureus* MIC - 0.12, MCC - 0.25 and for *E. coli* MIC - 0.12, MCC - 0.12. MIC values in these studies are very close to our results for the tested microorganisms *E. coli* and *S. aureus*.

In the studies conducted by Milosavljevic et al. (2000), the EOs from savory were studied for antimicrobial activity by dilution method (agar and broth). *E. coli* (ATSS 25922) and *S. aureus* showed sensitivity. MIC and minimum lethal concentration (MLC) were for *E. coli* from 2 to 8 µL mL^{-1}, and *S. aureus* from 1 to 2 µL mL^{-1}. These values are more than doubled in comparison to the results that we got in our experiments, which can be explained by the chemical composition of the oils. The dominant ingredient was carvacrol in the oil that we used, and oils used in this research had a trans-geraniol in the highest concentration. Other studies have also confirmed the antibacterial potency of EO of *S. hortensis* L., against various bacterial strains *Staphylococcus aureus* (ATCC 25923, ATCC 6538), and *Escherichia coli* (ATCC 8739, ATCC 25922). The MIC against all tested Gram-negative bacteria (microdilution method), were from 0.025 to 0.78 µL mL^{-1}. MIC values for the tested Gram-positive bacteria were in the range of from 0.20 to 0.39 µL mL^{-1} (Mihajilov-Krstev et al., 2009b). EOs of savory herb from our region, used in this study showed more activity against staphylococci compared with essential oil originating from Turkey (MIC 15.62 to 62.50 µL mL^{-1}), and also the value of MIC for *Streptococcus* (*S. pyogenes* KUKEM-676) were much higher MIC 62.50 µL mL^{-1} (Adiguzel A. et al., 2007).

Conclusion

The results of chemical composition of EOs showed the presence of carvacrol, menthol and thymol in high concentration. According to the phenolic and terpenoid characterization of the examined EOs, they could be regarded as a possible new potent source of natural products. Considering antimicrobial potential, these results undoubtedly validate the common use of these plant extracts in traditional and official medicine, as well as a potent source of natural medical substances. However, further research is necessary to confirm these results and assess the toxicity and the therapeutic effect of selected substances *in vivo*.

Acknowledgement

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Invited paper

CASE STUDIES FROM MACEDONIA ABOUT THE APPLICATION OF MOLECULAR METHODS IN DETERMINATION AND PREDICTION OF QUALITY IN AINMAL PRODUCTION

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Abstract

Molecular methods became powerful tools in the analysis of animal products quality using protein and nucleic acid based techniques. The Department for Biochemistry and Genetic Engineering as a part of the Animal Biotechnology Institute as part of the Faculty of Agriculture and Food Sciences in Skopje - Macedonia is a state stakeholder in the development of modern approaches to food quality control. In this respect, special focus is placed on a marker assisted selection related to genes that are affecting the quality and processing traits of animal products, determination of animal products origin, GMO detection in processed animal products and gene expression in different branches of livestock production. The analyses related to those applications are performed at protein, RNA and DNA levels. Marker assisted selection is systematically applied in cattle breeding using ĸ-casein as a crucial factor for milk processing traits and in swine breeding using the RYR-1 gene whose mutation is the cause for appearance of pale, soft and exudative meat. The identification of meat and dairy products origins was done using protein profiling. Gene expression in muscle growth development was done by analyzing different growth factors and inhibitors at RNA level. Most of the RNA/DNA analyses are PCR based, while protein analyses were done using different electrophoretic techniques. Marker genes were mostly characterized with Restriction Fragment Length Polymorphisms (RFLP). Muscle growth development was investigated by determination of RNA/DNA ratio and Reverse transcription PCR analysis of different genes involved in production of muscle mass. The detection of GM ingredients in animal products was performed using duplex PCR. More than 2.000 samples with animal origin were analyzed as part of this study.

Keywords: animal products, quality, nucleic acids, proteins, Macedonia

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Introduction

Food safety and food quality became top imperative for the civilization. Permanent increasing of food production, development of new products and application of new approaches in food technology raised the need for discovery of new, modern and sensitive tools in food control. Over the last few decades the efficiency of food control has been radically increased by the application of molecular methods. In that sense the animal products are specific target having in mind their irreplaceability in human nutrition (1).

Molecular analyses of animal production could be done on protein and nucleic acid level. They can be used for different purposes such as: determination of gene alleles that are closely related to quality and processing traits of animal products; identification of animal product's origin; detection of undeclared GM ingredients in animal products; predicting the growing potential of different tissues and organs as a source of animal products etc. (2).

At certain sites along the genomes the sequences vary between individuals. These sites, where differences in DNA sequence occur, are known as **molecular markers**. When differences in DNA occur within genes, these differences have the potential to affect the function of the gene and hence the phenotype of the individual. Based on the knowledge about molecular markers, specific approaches called **marker assisted selection (MAS)** have been developed. There are many markers associated with quality and processing traits of animal products (3).

The other field in food control where molecular tools took place was identification of animal product origin. This issue is especially important in control of dairy products due to their possible falsification by usage of different type of milk as a raw material (4). In terms of meat products molecular tools are crucial for control of so called halal food (5).

In last few decades the application of genetic modification in food production was widely used. The targeted crops for modification are soybean, corn, cotton, canola, sugar beet, rice whose are used as a feedstuff and as ingredients in some processed animal products. The molecular methods in this case are useful for detection of undeclared GMO presence in such product (6).

All those challenges where basis for systematic application of molecular methods in animal production in Macedonia driven by the Department of biochemistry and genetic engineering which is part of the Institute of Animal Biotechnology as part of the Faculty of Agricultural Sciences and Food in Skopje. In last 15 years more than 2000 different samples from animal origin were analyzed in the Laboratory for biochemistry and molecular biology and GMO laboratory as units of the mentioned department.

This study is compilation of our findings in: frequency of k-casein polymorphisms in cattle population and mutation in RYR-1 in swine population in Macedonia, and their influence on milk processing traits and meat quality; development of new approaches in identification of raw material origin in cheese making process and identification of meat origin in meat products; detection of GM soybean in processed meat; and determination of the growing potential of muscle tissue in fish. The achieved results are clustered on a protein and nucleic acid level.

PROTEIN LEVEL

Determination of milk protein polymorphisms in cow milk using different SDS PAGE electrophoresis

-casein is a fraction of milk proteins which is very important for the milk quality and processing. The goal of this task was to develop appropriate technique for the discrimination of different types of -casein in cow milk. As a starting material for such analysis we used scream and lyophilized milk. In accordance to the molecular weight and isoelectric point of the -casein we developed different approaches in separation of polymorphic types of those proteins. For the separation of different types of -casein we used vertical electrophoresis in 15% SDS polyacrylamide gel (7). Using this method we found six different haplotypes of milk proteins including k-casein shown in Fig.1.
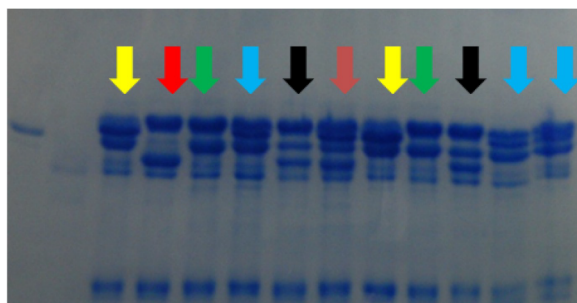

Fig. 1. Determination of polymorphisms of milk protein using 12.5% SDS Page.

SDS - PAGE electrophoresis for origin identification of the different diary products

The major milk proteins are caseins, lacto-albumin, and lacto-globulin. These globular proteins are significant indicator of the milk and dairy products quality. Knowing that caseins, lacto-albumins and lacto-globulins vary in molecular weight and concentration in different types of milk, this fluctuation can be used for determining the milk origin. The aim of this task was to develop an appropriate method for distinction of milk protein from different origin. Twelve samples of milk, white cheese, yellow cheese and curd from cow, sheep and goat were obtained and studied. The protein separation was made with SDS-PAGE. For determination of the proteins were used protein standards. The results have shown differences, as well as other fractions that can be used for identification of the origin (8). The differences between different types of dairy products are shown in Fig 2.
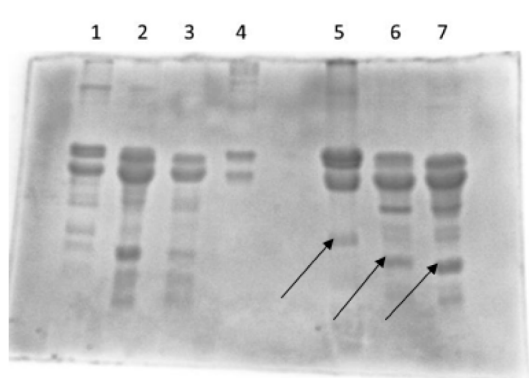

Fig. 2. 15% SDS-PAGE:1. Cow white cheese, 2. Goat white cheese, 3. Sheep white cheese 4. Mix (10 µg), 5. Cow yellow cheese, 6. Goat yellow cheese (approx. 33µg of proteins), 7. Sheep yellow cheese.

Different approaches in identification of the meat origin based on the protein profiling

The meat as one of the most popular ingredient and its origin is a fundamental factor that has an impact on the quality and the usage of the meat products. In this study the meat origin was determined by protein characterization using the gel electrophoresis techniques. The SDS-PAGE method optimized by changing the running conditions, amount of loaded sample materials and the concentration of the gel itself, has shown that different types of meat could be distinguished. The differences in protein profile of poultry meat were observed compared to the other samples where two specific fractions between 116 and 200 kD are identified in the zone of myosin heavy chains and one bellow 45 kD in the zone of actin. In the beef samples there is a specific fraction in the zone of tropomyosin, while in pork and beef samples a fraction appears in the zone of myosin light chain (9). This technique is suitable and can only be used for internal control in the production and during the processing. (Fig.3)
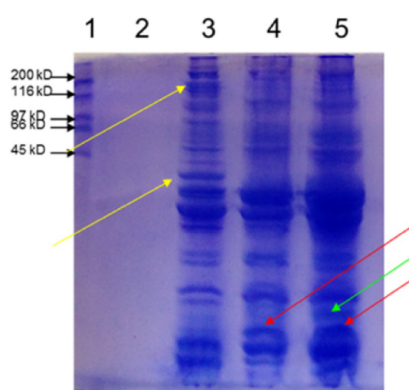

Fig. 3. Differentiation of meat origin using 15% SDS PAGE of meat proteins: #1 protein standards #2 empty #3 chicken meat #4 pork meat #5 beef meat

The influence of size on the muscle protein profile of Koi carp (Cyprinus carpio haematopterus)

The goal of this study was to show the eventual variations in the muscle protein gene expression in Koi carp (*Cyprinus carpio haematopterus*) caused by the fish size and living temperature differences. Urea PAGE methodology was used as standard procedure, where samples from 24 different fish were analyzed, separated into two groups, large and small fish. The results showed concentration differences in proteins located at the same region in the gels, present in some of the large fish, which points to the possible influence of the fish size on the muscle protein profile of the Koi carp (10). These types of variations were not recognized in the remaining analyzed samples (Fig. 4).
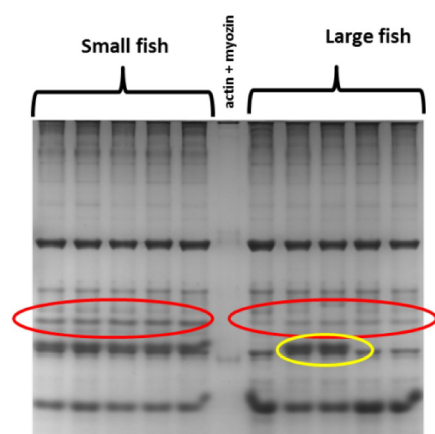

Fig. 4. Protein profiling of muscle proteins in large and small size koi carp fish on the same age grown in different conditions.

NUCLEIC ACIDS LEVEL

Molecular characterization of porcine stress syndrome in the Republic of Macedonia

Porcine stress syndrome (PSS) was a serious problem in swine breeding that has negative implications on the pork production. The cause for PSS is a point mutation in RYR – 1 gene and the aim of this task was to determine the presence of the PSS in swine population in the Republic of Macedonia in the different categories and breeds and to determine the influence of the PSS on some productive and reproductive attributes in the studied animals. Two hundred seventy eight animals were included in this study (11). The genotypization of PSS was performed on molecular level using PCR-RFLP technique. (Fig. 5)
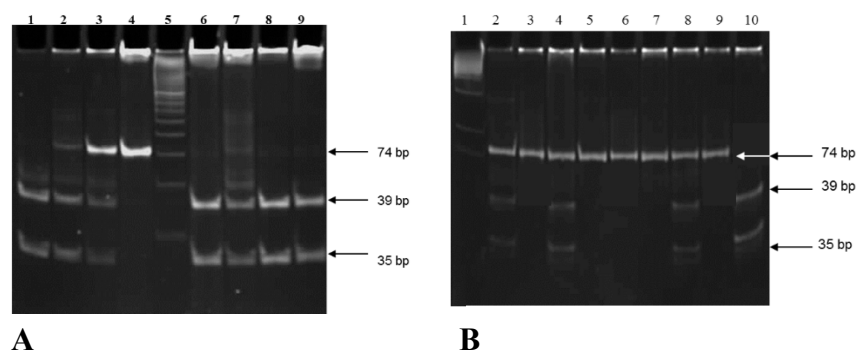

Fig. 5. Genotypization of PSS using PCR-RFLP with double digestion. A) with BsiHK1 and B) with Hha I restriction enzyme.

The results related to the influence of the genotype for PSS on some productive traits in boars showed that the stress sensitive animals have less food conversion, better daily growth, thinner back fat and higher percentage of meat, comparing with stress free animals. It is obvious that the stress syndrome has serious implications of the quality of the pork mainly because of the drastically decreased pH and higher percentage of pale, soft and exudative (PSE) meat in carcass of the stress susceptible animals. (Tab. 1)

Table 1. Association of carcass pH and temperature with PSS genotype

Genotype	pH of the *m.semimembranosus* after 1 hour		t		Genotype	Carcass temperature after 1 hour		T
	x	S_x				x	S_x	
NN	6,04	0,07		NN	30,95	0,4		
Nn	5,88	0,08		Nn	31,82	0,8		
nn	5,73	0.02	42.93**	Nn	29,90	0.7		1,24^{NS}
Genotype	pH of the *m.semimembranosus* after 24 hours (pHK)		t		Genotype	Carcass temperature after 24 hours		T
	x	S_x				x	S_x	
NN	5.85	0,04		NN	8,00	0,2		
Nn	5.58	0,02		Nn	7,89	0,2		
nn	4.99	0,02	76.79**	Nn	6,60	0.3		-1,22^{NS}
NS p>0,05; *p<0,05; **p<0,01								

□-casein polymorphisms in Holstein-Friesian cattle in the Republic of Macedonia and its association with milk traits

□□casein is used as an important marker in the selection of dairy cows. The aim of this task was to presume the presence of ꞁcasein genotypes in Holstein-Frisian cows in Macedonia and to check if any correlations exist between the genotype and some technological properties of milk from dairy cows with different genotype. The analysis was performed on 227 black-white Holstein-Friesian cattle. Genotyping of ꞁcasein polymorphisms was done by PCR-RFLP method using three different restriction enzymes. According to the electrophoretic analysis we found three different genotypes for ꞁcasein AA, AB and BB (12). The frequencies were 0.747:0.253 of the alleles A and B respectively, indicating dominancy of the allele A. (Fig.6)

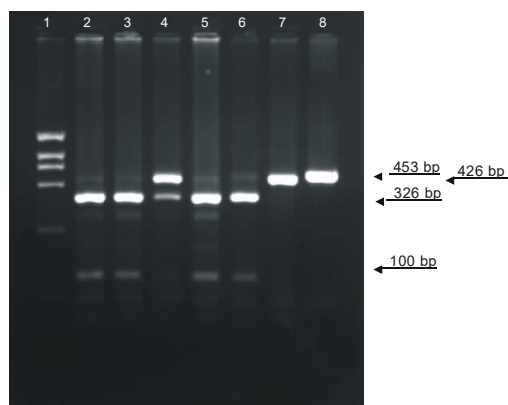

Fig. 6. 1.5% AGE of digested PCR products using *Hinf* I enzyme. #1 DNA ladder, #2, 3, 5 and 6 AA genotype #4 AB genotype; #7 BB genotype #8 undigested amplification.

Statistical analyses show that dairy cows with BB and AB genotype of κ-casein have 10% higher yield of milk than those with AA genotype. It was also shown that duration of the lactation was 12% longer in cows with BB genotype comparing with AA and AB genotypes. Milk yielded by cows with BB genotype need shortest time for the initial and final coagulation in the process of cheese making that is 30% shorter in contrast with AA genotype. Milk yielded by cows with AB genotype showed intermediate effect. During the cheese making process it was shown that syneresis was 14% higher in AB and BB genotypes in contrast with AA genotype. Considering these facts, in the future, the dairy cows with BB genotypes should be favorized in the reproduction to gain higher yields of milk and cheese (Tab. 2).

Table 2. Mean values of the parameters analyzed during the milk coagulation experiment associated with different genotype for κ-casein

Genotype	AA	AB	BB	p-value
Initial coagulation (min : sec)	11:35	10:30	9:30	0.008**
Final coagulation (min : sec)	46:15	44:06	33:30	0.009**
Extracted curd (ml)	264.50	301.25	307.50	0.027*
Curd fats (g/l)	0,465	0,285	0,260	0.034*
Curd proteins (g/l)	1,2850	1,2525	1,0450	0.23 NS
Dry content in curd (%)	7,2825	7,3250	7,2375	0.19 NS
NS p > 0.05; * p < 0.05, ** p < 0.01				

Determination of RNA/DNA ratio in white muscle samples of Koi carp using different techniques

The RNA/DNA (R/D) ratio is an indicator of muscle growth capacity in the fish. The amount of DNA in a cell is constant; while the amount of RNA indicates how actively the

cell is synthesizing proteins and growing (13). In Fig.7 are shown the results of simultaneously isolated RNA and DNA from the same muscle samples.
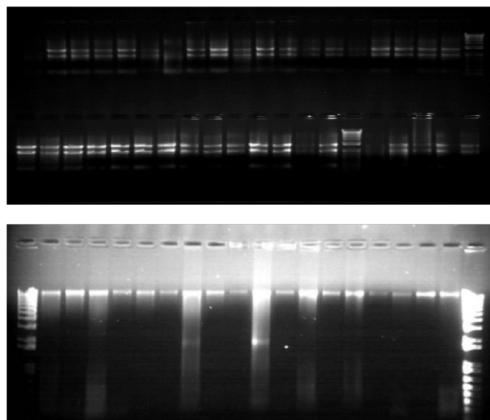

Fig. 7. Isolated DNA and RNA from same samples of white fish muscles.

An increase or decrease in the R/D ratio at a given temperature would indicate a change in growth rate. The results showed that the average values for the R/D ratio in large fish adapted to 25ºC was the highest, while the difference was shown in the samples from fish adapted to 5ºC and 30ºC. The obtained results are in accordance with the expected values where in the fish adapted to 5ºC were higher compared with those from the fish adapted to 30ºC (Graph. 1)
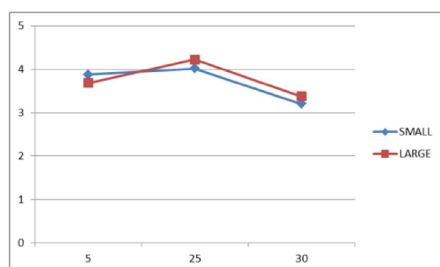
Graph 1. RNA/DNA ratio in large and small fish at the same age grown in same conditions.

Development of duplex PCR for detection of roundup ready GM crops

This experiment reports the screening of sausages that contain soy in a single step using duplex PCR. Before, the screening was performed in two steps, one for revealing the soy DNA, and the second for detecting the presence of the construct that is present in GM soy. An optimization of the PCR conditions was performed focusing on $MgCl_2$ concentration and primers annealing temperature. The collected data showed that concentration of 3.0 mM $MgCl_2$ and temperature of 60ºC are appropriate to amplify the both fragments in a single reaction (Fig. 7). The results did not show any false positive or false negative data. They were well-matched with those from the separately accomplished reactions. This kind of doubled PCR enabled faster detection of the presence of GM-soy (14). The method is

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shorter and cheaper. It gives a possibility to eliminate many negative samples before the quantification step with real-time PCR. (Fig.8)
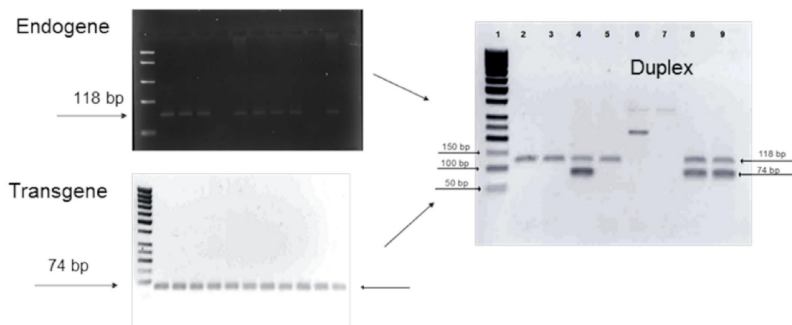

Fig. 8. Electropherogram of duplex PCR: #1 - 50 bp ladder; #2, #3 GMO-free soy, #4 positive control, #5 negative control, #6 nonspecific amplification, #7 chicken sausages without soy, #8, #9 chicken sausage containing soy.

Conclusion

Molecular approach in control of animal production is still far away from its routine application in the Republic of Macedonia, although there are technical and human capacities for wider use of these techniques.

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Oral presentation

EFFECTS OF VITAMIN E, SWEET CHESTNUT WOOD EXTRACT AND HOPS SUPPLEMENTATION ON PIG MEAT QUALITY AND OXIDATIVE STABILITY

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Abstract

The aim of the study was to investigate the effect of vitamin E and two natural additives with potential antioxidative activity (sweet chestnut wood extract – Farmatan, and hops) on growth performance, meat quality and meat oxidative stability of pigs. For this purpose, 41 crossbred pigs (20 gilts and 21 barrows) were assigned to four treatment groups. Control group (Control; N=11) was given a standard diet (13 MJ ME, 16% crude protein), while pigs in three treatment groups received the same diet supplemented with either 150 mg/kg α-tocopherol acetate (Vit E, N=7), 3% of tannin rich extract Farmatan (Tannin, N=12) or 0.4% of hop cones (Hop, N=11). Meat quality was evaluated in muscle *longissimus dorsi* by measuring pH at 24 h post-mortem, color (lightness, redness, yellowness), drip loss, thawing and cooking losses, and hardness. Meat chemical composition was assessed using NIRS (near-infrared spectroscopy). Oxidative stability of meat was studied by measuring concentrations of vitamin E, by total plasma antioxidant capacity of water-soluble compounds (ACW), fat oxidation by malondialdehyde (MDA) concentration and fatty acid composition and protein oxidation by concentration of carbonyl groups. Data was analysed using the General Linear Models (GLM) procedure of the SAS/STAT module. No differences in growth performance and meat quality were observed among experimental groups. Feeding the supplements also showed no influence on fatty acid composition, MDA and carbonyl groups concentration and on ACW. Concentration of α-tocopherol was higher in the group supplemented with vitamin E.

Keywords: nutrition, antioxidants, tannin, hops, vitamin E, oxidative stability, finishing pigs

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Introduction

Lipid oxidation is one of the main factors limiting the quality and acceptability of meat and meat products and is reflected in adverse changes in flavor, color, texture and nutritive value, and by possible production of toxic compounds. It can be reduced or inhibited by the use of antioxidants, the product quality and shelf-life can thus be improved (Shah et al., 2014). Vitamin E occupies an important position in the overall antioxidant defense; its supplementation above the requirements has been found to be effective in reducing lipid oxidation in meat and meat products (Morrissey et al., 1998). Several studies investigating the effect of natural antioxidants on oxidative stability of meat and meat products have been published. Tannins exert anthelmintic, antimicrobial and antioxidant properties (Mueller-Harvey, 2006). One of the main sources of hydrolysable tannins is sweet chestnut (*Castanea sativa* Mill.) wood extract (SCW). Hops (*Humulus lupulus* L) is also reported to be a source of polyphenolic substances and can act as a potent antioxidant (Krofta et al., 2008). As hops and SCW (commercial name Farmatan) are the two natural supplements available and economically relevant in Slovenia, their effect on meat quality and oxidative stability in comparison to control and vitamin E supplemented feed mixture was evaluated in the present study.

Material and Methods

At an average age of 140 days, 41 crossbred pigs (20 gilts and 21 barrows) were assigned to four treatment groups. Control group (Con; N=11) was given a standard corn based finishing diet (13.09 MJ ME, 16% crude protein; Table 1), while pigs in three treatment groups received the same diet supplemented with either 150 mg/kg *all-rac*-α-tocopherol acetate (VitE, N=7), 3% of Farmatan (Tannin, N=12) or 0.4% of hop cones (Hop, N=11). The diets were fed on the *semi ad libitum* basis. The number of pigs per group was different due to different size of pens and was adjusted to have the same space area per pig (1.2 m^2). After 69 days, the pigs were slaughtered and samples of *longissimus dorsi* (*LD*) muscle were collected to determine meat quality and oxidative stability.

Table 1. Chemical and fatty acid composition, and oxidative stability of experimental diets

		Supplemented		
	Control	VitE	Tannin	Hop
Dry matter (g/kg)	873.0	869.0	867.1	873.3
Crude protein (g/kg)	155.1	152.9	149.6	153.1
Crude fat (g/kg)	25.3	25.3	24.4	25.2
Crude fiber (g/kg)	49.6	50.7	51.0	49.9
Crude ash (g/kg)	59.4	48.2	48.9	47.4
Nitrogen-free extract (g/kg)	583.4	592.1	593.2	597.7
Fatty acid composition (wt % of fat)				
SFA	17.4	17.5	17.3	17.4
MUFA	24.2	24.0	25.0	24.2
PUFA	58.5	58.6	57.8	58.4
n-3 PUFA	3.38	3.43	3.49	3.40
n-6 PUFA	55.1	55.1	54.3	55.0
n-6/n-3 PUFA	16.3	16.1	15.6	16.2
Vitamin E (mg/kg)				
α-tocopherol	62.1	143.6	41.0	37.1
β+γ-tocopherol	19.6	19.1	17.5	16.5
δ-tocopherol	18.8	18.5	14.9	16.8
Oxidative stability				
MDA (μmol/kg)	2.23	3.04	3.21	3.89
ACW (mmol/kg)	26.8	33.7	50.2	36.8

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; MDA = malondialdehyde; ACW = antioxidant capacity of the water soluble compounds

Color was assessed using Minolta Chromameter and CIE (1976) L* (lightness), a* (redness) and b* (yellowness) color space. Muscle ultimate pH was determined in the central area of *LD*. Two 2.5 cm thick slices of *LD* were taken for the determination of drip loss (EZ method; Christensen, 2003), thawing and cooking loss, shear force and chemical composition. In order to determine thawing loss, the samples were thawed and reweighed. Cooking loss and shear force was determined in *LD* samples cooked in a thermostatic water bath (ONE 7-45, Memmert GmbH, Schwabach, Germany) until the internal temperature reached 72°C. Shear force was measured using a TA Plus texture analyzer (Ametek Lloyd Instruments Ltd., Fareham, UK) equipped with a 60° V-shaped rectangular-edged blade and a crosshead speed set at 3.3 mm/s. Chemical composition was determined according to Prevolnik et al. (2005) by near-infrared spectral analysis (NIR Systems 6500 Monochromator, Foss NIR System, Silver Spring, MD, USA).

Fatty acid (FA) composition of feed and *LD* samples was determined according to the method of Park and Goins (1994). Concentrations of vitamin E were analysed by Agilent 1260 Infinity HPLC system equipped with a 1260 Infinify Quaternary Pump (Agilent) according to the methodology of Abidi and Mounts (1997) and Rupérez et al. (2001). Concentrations of MDA in *LD* samples were analysed according to the methodology of

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Vilà et al. (2002) with slight modifications. An Agilent 1260 Infinity HPLC system equipped with a 1260 Infinify quaternary pump (Agilent) was used. Protein oxidation in myofibril isolates was measured according to the method of Oliver et al. (1987) as modified by Mercier et al. (1998). The protein and carbonyl concentrations were measured spectrophotometrically in the supernatant. The antioxidant capacity of the water soluble compounds (ACW) in *LD* samples was measured by the photo-chemiluminescence method by PhotoChem® (Analytik Jena, Jena, Germany).

Data was analysed using the General Linear Models (GLM) procedure of the SAS/STAT module (SAS 8e, 2000; SAS Inc., Cary, NC, USA) with fixed effects of treatment and sex. Differences between groups were tested using Tukey-Kramer multiple comparison test with significance at P<0.05. The results in the tables are presented as least square means (LS-means).

Results and Discussion

Supplementing pig diets with vitamin E is a conventional way to improve meat quality and oxidative stability (Jensen et al., 1998). However, in response to demand for natural products and consumers' willingness to pay significant premiums for natural foods, meat industry is seeking for natural solutions to minimize oxidative rancidity and increase products' shelf-life (Karre et al., 2013). Lipid oxidation namely leads to the formation of several compounds which affect the quality of meat and meat products (Shah et al., 2014). Due to high protein content in muscle tissues, oxidation of proteins can also be implemented to evaluate the quality of meat (Falowo et al., 2014). In the present study, no differences in performance, carcass and meat quality traits were observed between control group and groups supplemented with vitamin E, SCW extract or hops (Tables 2 and 3). This corroborates with studies where supplementation of vitamin E (Cannon et al., 1996) and SCW extract (Prevolnik et al., 2012) showed no effect on performance, carcass and meat quality of pigs. On the other hand, some studies demonstrated improved performance or carcass and meat quality traits of α-tocopherol (α-toc) supplemented diet; i.e. increased dressing percentage (Corino et al., 1999), improved water holding capacity (Guo et al., 2006) and delayed color deterioration (Waylan et al., 2002). Regarding natural extracts, pigs fed a diet containing 1% hops showed an improved gain to feed ratio (Fiesel et al., 2014), while supplementation with 3% of SCW extract resulted in reduced feed intake and daily gain of entire male pigs (Čandek-Potokar et al., 2015).

Table 2. The effect of dietary supplementation on growth performance and carcass traits of fattening pigs

| | | | Supplemented | | | |
	Control	VitE	Tannin	Hop	P-value	RMSE
Initial body weight (kg)	75.2	75.1	75.5	74.2	0.989	9.7
Final body weight (kg)	133.9	131.6	130.4	128.4	0.839	14.5
Average daily gain (kg)	0.851	0.819	0.796	0.786	0.765	0.154
Hot carcass weight (kg)	109.4	106.2	103.9	101.9	0.509	12.02
Lean meat %	62.1	61.3	62.2	61.7	0.947	3.6
Fat thickness (mm)	11.8	12.6	12.1	13.1	0.932	4.9
Muscle thickness (mm)	82.0	79.1	82.9	81.9	0.488	5.2

Table 3. The effect of dietary supplementation on meat quality traits of fattening pigs

| | | | Supplemented | | | |
	Control	VitE	Tannin	Hop	P-value	RMSE
pH 24h	5.37	5.36	5.34	5.37	0.525	0.07
L*	53.5	53.5	52.1	53.6	0.421	2.4
a*	8.5	8.5	9.0	8.6	0.768	1.3
b*	2.1	2.2	2.2	2.3	0.950	0.8
Drip loss 24h (%)	4.1	4.8	3.9	3.3	0.182	1.4
Drip loss 72h (%)	5.9	6.8	6.1	5.7	0.544	1.6
Thawing loss (%)	12.0	12.3	11.8	9.8	0.453	3.7
Cooking loss (%)	31.7	28.7	29.1	29.6	0.221	3.4
Shear force (WBSF; N)	135	128	124	125	0.602	21
Chemical composition						
Water (%)	74.1	72.9	73.7	74.3	0.213	1.34
Protein (%)	22.1	22.0	22.1	22.1	0.581	0.1
Intramuscular fat (%)	3.0	4.3	3.3	2.9	0.278	1.6

WBSF = Warner-Bratzler shear force

Interest in meat FA composition stems mainly from the need to find ways to produce healthier meat, i.e. with a higher ratio of polyunsaturated (PUFA) to saturated FA and a more favorable balance between n-6 and n-3 PUFA. The FA composition of animal products depends on tissue FA biosynthesis and the FA composition of dietary lipids (Mourot and Hermier, 2001). Experimental diets in the present study differed only in terms of supplementation; consequently, no differences in *LD* FA composition and n-6/n-3 PUFA ratio were observed (Table 4). As the concentration of α-toc was significantly higher in α-toc supplemented group, changes in the studied markers of oxidative stability were expected, but no effects on the formation of MDA, carbonyl groups, and ACW of *LD* were observed.

Table 4. The effect of dietary supplementation on *LD* muscle fatty acid composition (wt % of fat)

	Control	VitE	Supplemented Tannin	Hop
SFA	36.78	36.06	35.90	36.74
MUFA	43.56	43.82	43.42	43.21
PUFA	19.66	20.13	20.68	20.05
n-3 PUFA	0.99	1.04	1.06	0.99
n-6 PUFA	18.68	19.08	19.62	19.06
n-6/n-3 PUFA	18.93	18.29	18.58	19.30
LC PUFA	5.66	5.73	6.12	6.10
LC n-3 PUFA	0.61	0.68	0.68	0.64
LC n-6 PUFA	5.05	5.05	5.44	5.46
LC n-6/n-3 PUFA	8.23	7.42	7.96	8.48

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; LC = long chain

Table 5. Concentrations of α-tocopherol, antioxidant capacity of water soluble compounds (ACW), malondialdehyde (MDA) and carbonyl groups in *LD* muscle

	Control	Vit E	Supplemented Tannin	Hop	P-value	RMSE
α-tocopherol (mg/kg)	2.98[a]	3.79[b]	2.75[a]	2.69[a]	< 0.05	0.21
ACW (µmol/kg)	42.0	44.5	42.8	42.1	>0.95	0.49
MDA (nmol/g)	0.612	0.633	0.588	0.628	>0.95	0.014
Carbonyl groups (µmol/g protein)	0.82	0.70	0.85	0.83	0.20	0.15

Conclusion

Supplementing pigs with vitamin E, tannins and hops showed no effect on growth performance and meat quality. Besides, no influence on fatty acid composition, MDA and carbonyl groups concentration and on ACW was found. Supplementing animal feeds with vitamin E increased the vitamin E level in muscle *LD*.

Acknowledgments

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Poster presentation

INCREASE OF SELENIUM CONTENT IN TABLE EGGS

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Abstract

The aim of this research was to increase the selenium content in eggs, and to present how the different levels of selenium from feed affect the quality of fresh eggs. The experiment involved 90 layers divided into three experimental groups (A, B and C). Selenium content determined in the mixtures for laying hens was as follows: A=0.123 mg/kg, B=0.443 mg/kg and C=0.612 mg/kg. For analysis of selenium content in the edible part of egg 30 eggs was used (10 per group), while for the analysis of egg quality a total of 75 eggs were broken (25 pieces per group). Indicators of external and internal egg quality that were analyzed are: weight of eggs and main parts of eggs (g), shell firmness (kg/cm2) and thickness (mm), yolk color, pH value of yolks and albumen, albumen height (mm) and HU (Haugh units). The content of selenium in the edible part of egg increased in relation to its content in mixtures for laying hens. Statistically significantly higher (P<0.001) content of selenium in albumens and yolks of eggs was determined in the group C (albumen=0.209 mg/kg and yolk=0.863 mg/kg) compared to groups B (albumen=0.149 mg/kg and yolk=0.663 mg/kg) and A (albumen=0.053 mg/kg and yolk=0.387 mg/kg). Comparison of quality indicators results of tested eggs showed no statistically significant differences in weight of main parts of eggs, shell thickness, yolk color, pH values of albumen and yolk, albumen height and HU (P>0.05). A statistically significant difference (P=0.007) was found for egg weight. Laying hens of experimental groups C and A had heavier eggs in relation to the laying hens of group B (68.12 g and 67.72 g in relation to 65.96 g). In addition, statistically significantly firmer eggshell had eggs from group B in relation to group C (3.455 kg/cm^2 and 2.941 kg/cm^2; P=0.030). By analyzing the obtained results, it can be confirmed that the increase of selenium in feed for laying hens significantly affects the increase of mentioned microelement content in the edible part of eggs, and that addition of organic selenium to feed has no negative impact on quality indicators of eggs.

Keywords: organic selenium, table eggs, quality

Introduction

Selenium was discovered in 1818 by a Swedish chemist Berzelius. Back then, selenium was identified as a toxic element. However, in the middle of the 20th century, positive properties of selenium were determined (Ullrey, 1992). Selenium plays an important role in the regulation of various physiological functions in an organism, and it is an integral part of 25 selenoproteins and some enzymes, such as glutathione peroxidase and thioredoxin reductase (Gajčević et al., 2009; Kralik et al., 2016). Selenium enters the food chain through incorporation into plant proteins, such as selenomethionine and selenocysteine (Surai, 2006). The research results of some authors show that supplementation of selenium   into

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poultry feed can increase its content in meat and eggs. However, the facts is that the source of selenium is an important factor in the efficiency of enriching the stated products with this microelement. If compared to inorganic form, it has been proven that organic form of selenium is more effectively used as a poultry feed supplement (Payne et al., 2005, Kralik et al., 2009, Bennett and Cheng, 2010). More recent researches in the poultry production are focused on the use of cereals fortified with selenium in the poultry feed, in order to increase selenium content in meat and eggs (Hassan, 1990, Haug et al., 2008, Kralik et al., 2016). Nowadays, eggs enriched with selenium are produced in more than 25 countries around the world (Fisinin et al., 2009), and are proved to be a good source of selenium for humans (Surai et al., 2007). Moreover, eggs are a high quality foodstuff, affordable and cheap in many countries and cultures. Therefore, eggs enriched with selenium should be widely accepted by consumers, which is an incentive in Croatia to market this new brand of eggs.

Material and Methods

The experiment was performed on 90 laying hens of the Tetra SL hybrid, which were divided into three experimental groups. Group A (control) was fed a mixture supplemented with 0.15 mg Se/kg of feed. Group B was fed a mixture supplemented with 0.3 mg Se/kg of feed, and the group C was given feed containing 0.5 mg Se/kg of feed. Selenium used in this experiment was of organic origin Sel-Plex[1000]. Hens' diet was balanced at 17.50% crude protein and 11.40 MJ ME/kg of feed. Composition of feeding mixture is shown in the Table 1. Before starting the feeding treatment as a part of this experiment, the analysis of selenium contained in feed was performed to determine that selenium content in the feed of group A was 0.123 mg/kg, in the feed of group B = 0.443 mg/kg and in the feed of group C = 0.612 mg/kg. In order to determine the external and internal quality of eggs, there were 25 eggs analyzed per each group, while the content of Se in egg yolks and albumen was analyzed on 10 eggs from each group. Egg quality indicators analyzed in this experiment were: weight of eggs and main parts of eggs (g), shell firmness (kg/cm2) and thickness (mm), yolk color, pH value of yolks and albumen, albumen height (mm) and HU (Haugh units) as an indicator of egg freshness. Weight of egg, albumen and yolk, as well as of shell were determined by using the Mettler Toledo PB 1502-S scales. The shell firmness was measured by an automatic device Eggshell Force Gauge Model-II. Shell thickness was measured in the middle of egg shell by an electronic micrometer with an accuracy of 0.001 mm. Yolk color, HU and albumen height were measured by using an automatic device Egg Multi-Tester EMT-5200. The pH value of albumen and egg were measured by the pH meter Mettler Toledo MP 120. Concentration of selenium in feed, egg whites and yolks was analyzed by using a device Perkin Elmer Optima 2100 DV (Davidowski, 1993). The research results were processed in the Statistica for Windows, version 12.0 (StatSoft Inc., 2013).

Table 1. Composition of laying hens' feeding mixture[1]

Ingredient	Portion in feeding mixture
Corn	55.14
Soybean cake	11.23
Toasted soybean	15.00
Sunflower cake	8.30
Animal yeast	0.83
Limestone	6.93
Monocalcium phosphate	1.67
Animal salt	0.37
synthetic methionine	0.03
Premix	0.50
Total	100.00

[1]Feeding mixtures are composed to contain different amount of organic selenium, calculated as follows: A=0.15 mg/kg, B=0.30 mg/kg and C=0.5 mg/kg, and results of selenium content in feeding mixtures obtained by chemical analysis are: A=0.123 mg/kg, B=0.443 mg/kg and C=0.612 mg/kg.

Results and Discussion

The Table 2 overviews results related to weight of egg and of its main parts. Increased selenium content in hens' feed had statistically significant effect on egg weight (P=0.007). It was determined that hens in group B had statistically significantly lower egg weight (65.96 g) than laying hens in groups A and C (67.72 g and 68.12 g, respectively). Weight of albumen was the highest in the group C (41.95 g), followed by group A (41.25 g) and then by group B (40.71 g). Weights of egg yolk and shell were lower in the group B (17.00 g and 8.24 g, respectively) than in the groups A (17.75 g and 8.71 g, respectively) and C (17.40 g and 8.77 g, respectively). Increase of selenium content in hens' feed had no effect on weight of albumen, egg yolk and shell (P>0.05).

Table 2. Weight of eggs and main parts (g, $\bar{x}\pm s$)

Indicators / Groups	A	B	C	P-value
Egg weight	67.72±2.49[a]	65.96±1.66[b]	68.12±3.12[a]	0.007
Albumen weight	41.25±1.87	40.71±1.59	41.95±2.84	P>0.05
Yolk weight	17.75±1.21	17.00±1.03	17.40±1.28	P>0.05
Shell weight	8.71±0.76	8.24±0.71	8.77±0.99	P>0.05

$\bar{x}$=mean; s=standard deviation; Content of selenium in feeding mixtures: A=0.123 mg/kg, B=0.443 mg/kg and C=0.612 mg/kg.

The Table 3 presents some indicators of egg quality and freshness. The obtained data prove that the increase of selenium content in laying hens' feed had statistically significant influence on the egg shell firmness (P=0.030). Eggs of hens in the group B (3.455 kg/cm^2) had statistically significantly firmer shell than eggs of group C (2.941 kg/cm^2). Other indicators of egg quality and freshness did not exhibit statistically significant differences between groups (P>0.05). However, it is noticed that group C, with determined statistically significantly softer egg shell, had thinner egg shell and higher egg weight than eggs of groups A and B, which can also influence the values of shell firmness. Similar notices are

stated by Kralik et al. (2012) in their paper Comparison of table egg quality of different manufacturers.

Table 3. Egg quality and freshness

Indicators / Groups	A	B	C	P-value
Shell firmness (kg/cm$^{2)}$)	3.205±0.61^{ab}	3.455±0.76^{a}	2.941±0.61^{b}	0.030
Shell thickness (mm)	0.412±0.03	0.417±0.02	0.398±0.04	P>0.05
Albumen height (mm)	6.95±1.01	6.58±1.09	6.54±1.27	P>0.05
Yolk color	11.56±0.86	11.52±0.82	11.60±0.76	P>0.05
HU	80.74±6.56	78.51±7.88	77.02±10.73	P>0.05
pH of albumen	8.67±0.11	8.71±0.11	8.64±0.27	P>0.05
pH of yolk	5.99±0.09	5.98±0.06	6.03±0.06	P>0.05

$\bar{x}$=mean; s= standard deviation; Content of selenium in feeding mixtures: A=0.123 mg/kg, B=0.443 mg/kg and C=0.612 mg/kg.

The Graph 1 shows the content of selenium in edible part of egg. Increase of selenium contained in feed had statistically significant effect on the increase of selenium content in albumen and yolks (P<0.001). If compared to albumen of group B=0.149 mg/kg and group A=0.053 mg/kg, albumen of group C=0.209 mg/kg had statistically significantly higher content of selenium.

	A	B	C
albumen	0.053	0.149	0.209
yolk	0.387	0.663	0.863
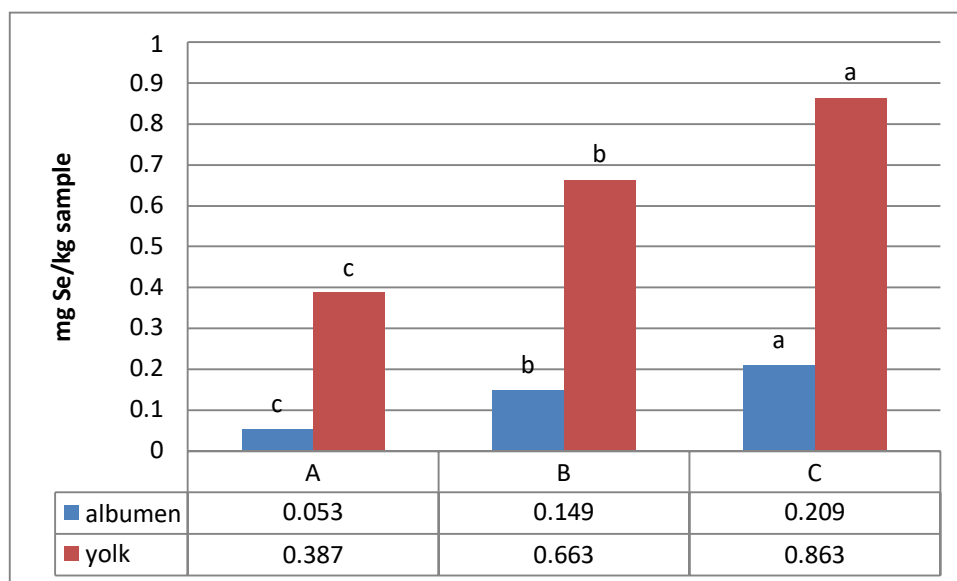
Values in rows marked with letters ^{a,b,c} for specific part of egg differ between groups at a significance level of P<0.001

Graph 1. Content of selenium in egg albumen and yolk

The least content of selenium was determined in yolks of the group A=0.387 mg/kg, followed by yolks of group B=0.663 mg/kg and then by yolks of group C=0.863 mg/kg. The obtained results are corresponding to results reported by Gajčević et al. (2009), and by Bennett and Cheng (2010), who also determined that the increase of selenium content in laying hens' feed influenced the increase of selenium content in eggs.

Conclusion

Upon analyzing the results obtained in this research, it is concluded that the level of selenium in feed statistically significantly influenced the egg weight (P=0.007) and shell firmness (P=0.030). Other indicators of egg quality and freshness (weight of albumen, yolk and shell, shell thickness, albumen height, yolk color, HU, pH of albumen and pH of yolk) were not statistically significantly influenced by the increased content of selenium in laying hens' feed and in the edible part of egg (P>0.05). Increased selenium content in feeding mixtures fed to laying hens had statistically significant (P<0.001) influence on the increase of selenium contained in albumen (A=0.052 mg/kg, B=0.149 mg/kg and C=0.209 mg/kg) and in egg yolks (A=0.387 mg/kg, B=0.663 mg/kg and C=0.863 mg/kg).

Acknowledgement

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Poster presentation

INFLUENCE OF DIFFERENT INCUBATION TEMPERATURE ON HISTOLOGICAL CHARACTERISTICS OF BREAST MUSCLE OF 42-DAY OLD BROILERS

Žikić D.[1], Stojanović S.[2], Jojkić Z.[2], Ušćebrka G.[2]

Abstract

Manipulations of incubation factors could cause alterations in development of muscle and connective tissue in chickens. In this paper, the histological characteristics of breast muscle of broilers incubated under increased temperature were examined.

Four groups of broiler eggs were incubated under different incubation temperatures. Incubation temperature in first group was 37.8°C, while in other groups, during the incubation temperature was increased in precisely defined intervals to 38.5°C (second group), 39.5°C (third group) and 41°C (fourth group). On the 42nd day of development, samples of breast muscles were taken from 10 chickens in all four groups. Diameter and nucleo-cytoplasmic ratio of muscle cells, as well as the volume density of connective tissue in muscle were examined. Results showed that diameter of breast muscle cells was higher ($p<0.01$) while nucleo-cytoplasmic ratio was lower ($p<0.01$) in groups in which incubation temperature was increased to 38.5°C and 39.5°C compared to all other groups. These results can be explained by influence of increased incubation temperature of up to 39.5°C on enhanced myoblast proliferation. Also, increased temperature has positive effect on muscle cells growth, which was detected in postnatal development.

It may be concluded that increased temperature during incubation could lead to enhanced myoblast proliferation and muscle cells growth, which in turn could cause higher breast and body weight in treated chickens.

Keywords: broiler, incubation, temperature

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Introduction

Changes of incubation factors can affect on development of broiler chickens (Halevy et al., 2006b). In studies so far, it was detected that manipulation of temperature (Yahav et al., 2004) and light (Rozenboim et al., 2004) during incubation have great influence on skeletal muscle development of chicken. Treating eggs with increased temperature and monochromatic green light in certain intervals during incubation could lead to changes in activity of myoblasts and satellite cells (Stojanović et al., 2013). These processes could cause greater development of skeletal muscle tissue and consequently higher meat weight (Ušćebrka et al., 2010a). Appliance of these treatments showed that chicken development can be affected not only by using different feed treatments, additives, etc. (Amad et al., 2011), but also by changing the incubation factors during their embryonic development.
The aim of this paper was to examine the influence of different incubation temperature on histological characteristics of breast muscle of 42-day old broilers.

Material and Methods

Four hundred broiler chicken eggs were divided in four groups of 100 eggs: control group (CG) and three thermal treated groups (TG1, TG2, TG3). Eggs were incubated under standard conditions (temperature 37.8°C, humidity 58%). Thermal treatments consist of temperature increase during the 16th, 17th and 18th day of incubation to 38.5°C (TG1), 39.5°C (TG2) and 41.0°C (TG3) for 3 hours each day. These days were chosen because in previous studies (Halevy et al., 2006a; Halevy et al., 2006b) it was detected that temperature treatments in these periods had the greatest influence on myoblast proliferation. After applied treatments, incubation was continued under standard conditions.

Samples of breast muscle (*M. pectoralis superficialis*) were taken from 10 chickens of each group during the 42nd day after hatching. From muscle tissue samples histological preparations were made according the standard procedure (Humason, 1972) which consist of tissue fixation in 10% formalin solution, dehydration, clearing, embedding in paraffin, cutting in 5μm thick sections and staining with hematoxylin-eosin (H&E). Analysis of histological preparations consists of determination of diameter and nucleo-cytoplasmic ratio of muscle cells, as well as volume density of connective tissue of muscles. For microscopic analysis of histological preparations light microscope Leica DM/LS equipped with digital camera Leica DC300 were used. Nucleo-cytoplasmic ratio of muscle cells and volume density of connective tissue of muscles were determined using the M42 testing system described elsewhere (Burity et al., 2004; Ušćebrka et al., 2010b) using following formulas:

$$Vd(ct) = \frac{P(ct)}{P(m)} \cdot 100 \ (\%)$$

where Vd(ct) – the volume density of connective tissue of muscle; P(ct) – the number of test points lying over the connective tissue of muscles; P(m) – the number of test points lying over the muscle;

$$N / C = \frac{P(n)}{P(c)}$$

where N/C – the nucleo-cytoplasmic ratio of muscle cell; P(n) – the number of test points lying over the cell nucleus; P(c) – the number of test points lying over the cytoplasm.

Statistical analysis was determined using ANOVA and followed by Tukey test for each of the parameter measured. The statistical significance of differences was expressed as significant at $p \leq 0.01$. Statistical analysis was carried out using Statistica for Windows ver. 10.0.

Results and Discussion

Results showed that diameter of breast muscle cells were higher ($p < 0.01$), while nucleo-cytoplasmic ratio was lower ($p < 0.01$) in groups which were thermally treated at 38.5°C and 39.5°C compared to all other groups. Between groups there were no differences in volume density of connective tissue (Figure 1, Table 1).
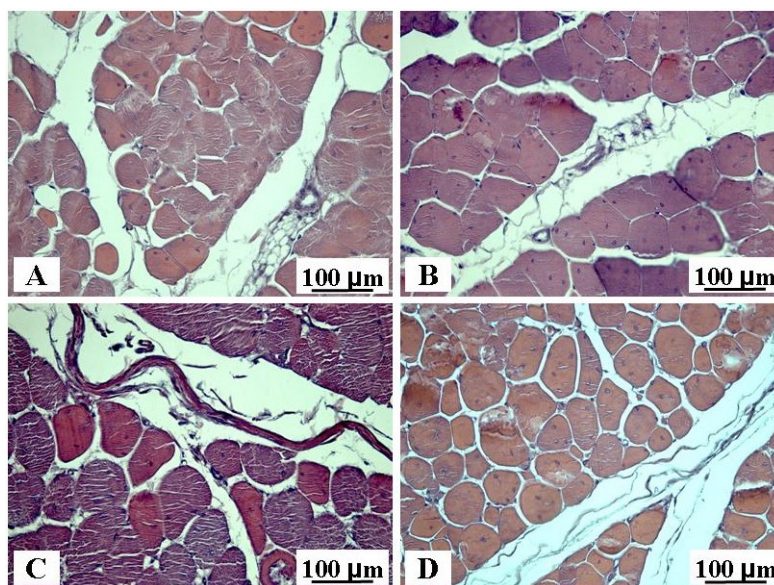

Figure 1. Breast muscle cells (*M. pectoralis superficialis*) in control group (A) and thermally treated groups at 38.5°C (B), 39.5°C (C) and 41.0°C (D); hematoxylin-eosin staining, bar = 100μm

Table 1. Examined parameters of breast muscle cells (*M. pectoralis superficialis*)

Diameter of muscle cells (μm)			
Control group	Treatment (38.5°C)	Treatment (39.5°C)	Treatment (41.0°C)
42.24 ± 0.59	45.52 ± 1.21*	45.00 ± 0.83*	42.14 ± 0.44
Volume density of connective tissue (%)			
Control group	Treatment (38.5°C)	Treatment (39.5°C)	Treatment (41.0°C)
19.76 ± 2.75	20.64 ± 1.44	20.27 ± 1.51	19.82 ± 2.80
Nucleo-cytoplasmic ratio of muscle cells			
Control group	Treatment (38.5°C)	Treatment (39.5°C)	Treatment (41.0°C)
0.11 ± 0.01	$0.07 \pm 0.02^{\#}$	$0.08 \pm 0.007^{\#}$	0.11 ± 0.01

Means in a row, for each parameter, with superscript asterisks (*) have higher ($p < 0.01$) values Means in a row, for each parameter, with superscript asterisks (#) have lower ($p < 0.01$) values

Higher diameter of breast muscle cells of broilers which during incubation were treated with 38.5°C and 39.5°C can be explained by influence of increased temperature on higher myoblast proliferation (Piestun, 2009). In addition to influence on myoblast proliferation, increased temperature during incubation also affect on higher growth of skeletal muscle cells in postnatal period of development (Žikić et al., 2013). Lower values of nucleo-cytoplasmic ratio of breast muscle cells of broilers which were incubated at 38.5°C and 39.5°C are related to higher diameter of these cells. As the muscle cells growth, the amount of cytoplasm increased (Stojanović et al., 2014), which cause the lower nucleo-cytoplasmic ratio.

In breast muscle cells of broilers which were treated with 41.0°C lower values of diameter were recorded compared to broilers which were treated with 38.5°C and 39.5°C. This can be due to narrow range of temperature manipulation which can have effect on increased myoblast proliferation and muscle cells growth. It seems that temperature increasing beyond certain limit has no further impact on higher myoblast proliferation. It can be assumed that exposing of eggs during incubation to certain temperatures which are higher than standard incubation temperature could have negative influence on some processes that occur during chick embryonic development. This is in agreement with observations of other authors (Yahav et al., 2004; Halevy et al., 2006a).

The same volume density of connective tissue in muscles can be explained by presence of muscle and connective tissue inside breast muscle. In the same area of breast muscles, in broilers which were treated with 38.5°C and 39.5°C smaller number of muscle cells of higher diameter was recorded, while in broilers which were treated with 41.0°C and in control group higher number of muscle cells with smaller diameter was recorded. Consequently, the amount of connective tissue is the same in all groups, which is similar to results previously reported (Ušćebrka et al., 2010a; Stojanović et al., 2013).

Higher myoblast proliferation and increased diameter of muscle cells during the postnatal development could be related to higher muscle tissue development, breast and body weight of chicken (Stojanović et al., 2011). This could be important for improvement of broiler production.

Conclusion

It can be concluded that increased temperature during chicken incubation can affect on higher diameter of muscle cells due to influence on higher myoblast proliferation and higher growth of muscle cells during the postnatal development. Temperature manipulation which cause the higher growth of muscle cells are restricted to narrow temperature range. Diameter increase could have positive effect on development of muscle tissue, as well as on higher meat quality and weight of broiler chickens.

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Poster presentation

INTERNAL AND EXTERNAL QUALITY OF EGGS FROM MONTENEGRIN DOMESTIC CHICKEN AND KOSOVO LONGCROWER

Đukić Stojčić M.[1], Marković B.[1], Radonjić D.[2]

Abstract

Domestic breeds of chicken populations in the area of Balkan countries are mostly of similar characteristics and therefore it is difficult to define it as a single race. These are identical races that differ only as certain varieties. This is especially typical for Montenegro, Republic of Serbia, Bosnia and Hercegovina, Macedonia and Albania.

In Montenegro, domestic chicken is now very difficult to be found in its original form, is represented only in the remotest mountain regions. Also in Montenegro indigenous breed Kosovo Longcrower is present. These two races are usually reared in traditional production in rural environment, around the house and with use pastures. However, little information has been published about the production and quality of eggs of domestic Montenegrin domestic chicken and Kosovo Longcrower.

The aim of this study was to determine the internal and external egg quality from the domestic Montenegrin domestic chicken and Kosovo Longcrower raised in traditional system. In a sample of 30 eggs we investigated internal and external egg quality. For the exterior egg quality, following traits were analyzed: egg weight, cleanness of egg, shell color, shape index, shell thickness and shell weight, and for the internal qualities: Haugh Unit (HU), yolk color and USDA. The breeds significantly influenced the egg weight, shell color, shape index and yolk color. The eggs from Montenegrin domestic chicken presented significantly darker shell color and higher shape index. The eggs from Kosovo Longcrower were significantly heavier and had darker yolk color.

Keywords: egg, quality, domestic, chicken, longcrower

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Introduction

Most of the countries in the world have their own domestic poultry, which is a product of local conditions. A small number of livestock are uniform by type and exterior, production characteristics and genetically consolidated. In some countries they are not defined as a race, but as local populations resulting from various unplanned crosses of local indigenous populations and a variety of imported breeds, and cultivated through a long period of time in special living conditions.

The largest number of poultry in Montenegro are domestic hens. Other poultry, like ducks, geese, or turkeys are symbolically represented. Faster development of poultry, especially poultry meat, has contributed to the completion of the production process through the development of the sector of animal feed, parental flock of hybrids and the opening of the hatchery and production of day-old chicks, that modern slaughterhouse for poultry processing facilities and development (AMIS, 2013). In Montenegro, production of eggs in the last ten years experienced growth. Current production is at the level of about 70 million pieces, of which approximately 60% originates from the purely commercial production. The rest of the production is mainly operated on households. Less than 1% of eggs in Montenegro, in intensive farming, are produced in line with the EU regulations on keeping laying hens. In intensive commercial farming, laying hens are kept in classical conventional cages. In extensive farming, at family farm holdings and for the purposes thereof, deep litter and free or semi free range methods of keeping laying hens are most frequently employed. (Marković et al., 2001; Boljević and Marković, 2012).

Domestic breeds and livestock chickens populations in the area of the Balkan countries have mostly similar characteristics. They are identical races that differ only as certain varieties. This is especially typical for Montenegro, Republic of Serbia, Bosnia and Hercegovina, Macedonia, and Albania.

There are several populations of domestic hens in the Balkans: crnogorska, bosanska, svrljiška, zagorska, lička, pogrmuša or živičarka, zaječarka and so on. All of them have mostly the same production characteristics, and are very similar in appearance.

Hens are small, with different colors of feathers. Original color was partridges and black. The domestic hen is rarely found in its native form, because of unplanned steam with various modern breeds of chickens such as Styrian, Rhode Island, Partridge Italian, *Andalusian* breed of chicken. In Montenegro, it is now very difficult to find domestic chicken in its original form, which is represented only in the remotest mountain regions. It has medium body size (1,5-2 kg), strong wings, long tail, strong and long legs with four fingers. Hens give 60-80 eggs a year of 50–55 g weight and has good quality of meat but its production is not efficient enough for the intensive farming. This breed is very immune and resistant. Reproductive characteristics of the breed are good. Hens start laying at the age of 8 to 9 months (Kuga, 2002; Milošević, 2003; Stojanović and Đorđević- Milošević, 2003).

Picture 1. Montenegrin domestic chicken

Kosovo Longcrower is a breed originated in the region of Kosovo and surrounding areas. From Kosovo this breed spread into other Balkan countries as well. Nowadays, it is found in Serbia (Kosovo), Bosnia and Herzegovina, Macedonia, Croatia, and Montenegro.

The birds are black. The rooster might have a few red or gold feathers on the wings. On the head there is a characteristic crest of black feathers. There might be two symmetrical short horns on its nose, but in most cases their V-shaped comb leans forward. Its beak is yellow and feet are mainly olive-green, as well as gray. The rooster's tail has some longer sword shaped feathers, and it is held straight. Unlike other breeds, hens lay eggs from time to time, but when the eggs are hatched, they manage to guide and protect their fledglings well. Roosters start singing by the 5th to 7th month of their life, while hens start laying eggs at the 8th month and give 160 eggs a year, of 55–60 g weight. The main feature of this breed is the long melodic singing, which lasts from 20 seconds to one minute (Kuga, 2002; Stojanović and Đorđević- Milošević, 2003; Lukanov, 2012)
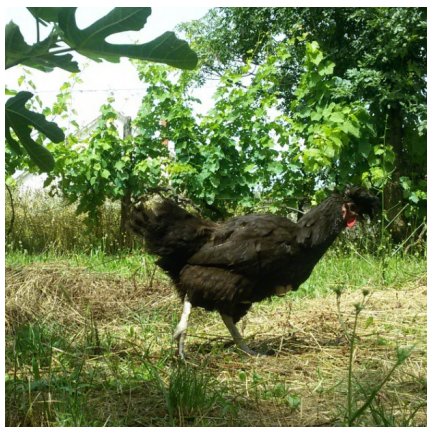
Picture 2. Kosovo Longcrower

However, little information has been published about the production and quality of eggs of domestic Montenegrin domestic chicken and Kosovo Longcrower.
The aim of this study was to determine the internal and external egg quality from the domestic Montenegrin domestic chicken and Kosovo Longcrower raised in traditional system.

Material and Methods

Sampling of eggs was carried out on a two farms with traditional production in rural environment, around the house and with use pastures. In a sample of 30 eggs of each breed we investigated internal and external egg quality. External egg quality characteristics: egg weight, shape index, shell cleanness, shell color, shell thickness, shell weight. Internal egg quality characteristics: egg yolk color, Haugh Unit (HU) and USDA.

Egg and shell weight ware measured by electronic digital balance. Shape Index is estimated using the following equation: Shape Index = [egg width / egg length] × 100 (Anderson et al., 2004). Shell cleanness was evaluated by scoring system on a scale from 1 (very dirty shell) to 5 (completely clean) (Mašić and Pavlovski, 1994.). Shell color was visually evaluated with points 1 (weight) – 5 (dark). Shell thickness (mm) was measured using a dial gauge micrometer. Egg yolk color was determined according to Roche yolk color fan (Vuilleumier, 1969). Albumen height was measured using tripod micrometer. On the basis of egg mass (M) and albumen height (H), device (Egg multi tester, model EMT 5200 Robotmotion Co. Japan) were calculated Haugh units according to following formula (Haugh, 1937):
HU = 100log (H+7.57-1.7M0.37)
H= albumen height, mm
M= egg weight, g

Data were analyzed by ANOVA followed by Duncan's post hoc test using StatSoft software (STATISTICA 12).The level of significance to indicate differences stated in the ANOVA model are P<0.05

Results and Discussion

The results for the external egg quality parameters are shown in Table 1. The Kosovo Longcrower laid eggs of greater weight compared to the eggs of the Montenegrin domestic chicken. The difference in egg weight was statistically significant.

In this study, both races produce heavier eggs than those eggs in investigation of Kuga, 2002; Milošević, 2003; Stojanović and Đorđević- Milošević, 2003, Lukanov, 2012. The results of Stojanović and Đorđević- Milošević, 2003 stated that domestic chicken has egg weight from 50 to 55 g, which was not the case in our research. The average weight of Kosovo singer eggs is 63 g, while Lukanov, 2012 said that the minimum weight for breeding is 55 grams, and average weight is between 55 and 60g.

Egg shells color was statistically darker in egg of domestic chicken compared to Kosovo Longcrower. In both breeds, statistically significant differences in shell cleanness, shell thickness, and shell weight were not found. Normally the outer cover of the egg, the shell comprises 10-11% of total egg weight. On an average the eggshell weighs 5-6g, as was the case in both the observed breed.

External characteristics of eggs from Montenegrin domestic chicken and Kosovo Longcrower are present in Table 2. The Haugh unit observed in the eggs from domestic chicken was higher than those from Kosovo Longcrower. But there was *no statistically*

significant difference between the two races studied. Albumen height and Haugh Unit (HU) are a major determinant of internal egg quality. The Haugh Unit (HU) is calculated from the height of the inner albumen and the weight of egg. In present study, the average HU value were for domestic chicken 81,65 and for Kosovo Longcrower 77,06. It is generally accepted that the higher the HU value than 70, the better freshness of eggs and egg quality are. According to USDA guidelines, eggs are graded and labeled as AA, A, and B. Grade AA eggs very good quality. The whites are thick and firm and the yolks are free from any defects. An AA quality egg has a HU greater than 72. In our research both breeds shown a very good quality of eggs.

Egg yolk color was statistically more intensive in the eggs from Kosovo Longcrower (10,38), compared to the domestic chicken yolk color (8,95). Most egg marketing authorities and some consumers may prefer eggs with deeper yolk colors in the range 9 to 12 on the Roche yolk color fan. In our investigation both races have an adequate color, and therefore might be preferred by consumers and marketing authorities.

Table 1. External characteristics of eggs from Montenegrin domestic chicken and Kosovo Longcrower

External characteristics	Egg weight (g)	Shell color (points)	Shell cleanness (points)	Shape index	Shell thickness (mm)	Shell weight (g)
MONTENEGRIN DOMESTIC CHICKEN	60.54[b]	2,75[a]	1,55	74.94[a]	0.37	5,85
KOSOVO LONGCROWER	63.38[a]	1,82[a]	1,68	70.93[b]	0.37	6,16

[a-b] Values within column with no common superscript are significantly different (P<0.05)

Table 2. Internal characteristics of eggs from Montenegrin domestic chicken and Kosovo Longcrower

Internal characteristics	HU	Yolk color	USDA
MONTENEGRIN DOMESTIC CHICKEN	81,65	8,95[b]	AA
KOSOVO LONGCROWER	77,06	10,38[a]	AA

[a-b] Values within column with no common superscript are significantly different (P<0.05)

Conclusion

In the present study we found that eggs from Montenegrin domestic chicken were characterized by lower total weight and shell weight. The eggs from Kosovo Longcrower had more intensive yolk color. Montenegrin domestic chicken and Kosovo Longcrower have eggs with good albumen quality traits. The results of our experiments indicate that both breeds have eggs with good quality parameters, and can be recommended for rearing in free range system.

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Poster presentation

SALMONELLA SP. AND CALCIUM CONTENT AS ONE OF THE MOST IMPORTANT MICROBIOLOGICAL AND CHEMICAL QUALITY PARAMETERS OF MECHANICALLY SEPARATED MEAT

Babić J.[1], Aleksić-Agelidis A.[2], Jakšić S.[1], Kartalović B.[1], Živkov- Baloš M.[1], Petrović J.[1]

Abstract

According to the Regulation (EC) No 853/2004, "Mechanically separated meat - MSM" means the product obtained by removing meat from flesh-bearing bones after deboning or from poultry carcasses, using mechanical means resulting in the loss or modification of the muscle fiber structure. Concerning public health risks related to MSM, the aim of this paper was to investigate the most important microbiological and chemical quality parameters of mechanically separated meat samples from January 2015 to December 2015, such as *Salmonella* sp. and calcium content. In 5% of the samples the presence of *Salmonella* sp. species was revealed. *S.* Infantis and *S.* Enteritidis were isolated in 4 out of 11 *Salmonella* positive samples (36.36%) and *S.* Typhimurium was isolated in 3 out of 11 *Salmonella* positive samples (27.27%). The calcium content was examined in 38 samples and 28.95% of samples were within the concentration range of 1000 – 1499 mg/kg, one of the samples had concentration even higher than 1500 mg/kg. According to our survey, the MSM is a food product with significantly high risk of the *Salmonella* presence and low compositional quality.

Keywords: MSM, *Salmonella*, calcium

Introduction

Changes of the poultry processing industry have begun in the late 1950s and early 1960s (Owens et al., 2010). At that time as a result of an increase in the consumption of poultry meat, more parts such as frames, backs, necks, drumsticks, wings, etc. became available for mechanical separation (Froning and McKee, 2001). After the removal of the usual meat cuts, a certain amount of meat is always firmly attached to the bones. In the process of mechanical separation, that firmly attached meat is removed by grinding the starting material and passing it through a sieve under low or high pressure. Low pressure MSM has the EU upper limit of 100 mg/100 g (1000 ppm) calcium. MSM with calcium concentration above this threshold is considered to be high pressure MSM (EFSA, 2013). If calcium content does not exceed EU upper limit, MSM may be treated as minced meat, but any MSM additives to the final meat product have to be declared (Branscheid et al., 2008).

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Until the end of last year the legislative act of the Republic of Serbia has identified two types of MSM - MSM produced using techniques that do not alter the structure of the bones with the upper limit of 100 mg/100 g and MSM produced using other techniques with the upper limit of 150 mg/100 g (Official Gazette RS, 31/2012). The legislative act the Republic of Serbia in force doesn't identify two types of MSM there is only one type with permitted level of 0.1% (100mg/100g) (Official Gazette RS, 94/2015).

MSM contains muscle, fat and connective tissue like minced meat and meat preparations, but MSM can also contain fine bone particles and bone marrow (James et al., 2013). According to Regulation (EC) No 853/2004, "Mechanically separated meat- MSM" means the product obtained by removing meat from flesh-bearing bones after deboning or from poultry carcasses, using mechanical means resulting in the loss or modification of the muscle fiber structure. MSM, which is relatively low in fat and low in cost, is widely used to manufacture further processed products such as bologna, salami, frankfurters, turkey rolls, restructured meat products, and soup mixes (Froning and McKee, 2001; Nagy et al., 2007).

Many researchers have remarked that MSM is "an excellent medium for bacterial growth". MSM also presents a high potential health risk to the consumers because MSM is usually contaminated with microorganisms such as *Salmonella* sp. *Campylobacter*, *Listeria* sp. and *Clostridium perfringens* that originate from the carcass raw material. These pathogens usually multiply thanks to the inappropriate storage history and the processing environment, mainly as a result of poor hygienic measures (environment, handlers, and equipment) (ICMSF, 2005). The risk of the risk of microbial growth increases with the degree of muscle fiber degradation, thus high pressure MSM may provide a more favorable substrate for bacterial growth compared with low pressure MSM (EFSA, 2013). Also, improper holding temperatures during the production and storage phases allow growth and multiplication of contamination (Yuste et al., 2002). Under the food safety criteria, MSM must also be tested for *Salmonella*. Five samples of 10g each must be taken from one batch per sampling period and *Salmonella* must be absent in all samples. In order to control MSM microbiological quality USDA/FSIS has established the HACCP procedure (USDA/FSIS, 1999).

Concerning public health risks related to MSM, the aim of this paper was to investigate the most important microbiological and chemical quality parameters of mechanically separated meat samples

Material and Methods

From January to December 2015, a total of 49 samples of MSM were analyzed. Samples were transported to the laboratory after being collected in a portable cooler at a temperature of 4°C and microbiological analysis were carried out immediately. 36 samples of MSM were analyzed for *Salmonella* sp. presence. Concentration of calcium was measured in 38 samples of MSM.

Isolation and identification of *Salmonella* sp. (in 10g of MSM) was performed according to standard methods (SRPS EN ISO 6579:2008). Calcium concentration was analyzed using flame emission spectrophotometry on flame photometer Jenway PFP7 ($\lambda = 422,7$ nm). Solution used for calibration was prepared from commercial stock standard solution

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--- with 1000mg Ca/L (*AccuTrace*^{TM} *Reference Standard* (USA)).

Results and Discussion

In 36 analyzed samples, *Salmonella* sp. was isolated from 11 samples or 30.6% from total number of analyzed samples. Three different serovars of *Salmonella* were found in 11 samples (Table 1.)

Table 1. *Salmonella* sp. isolated from MSM samples.

Salmonella	Number of positive samples	Percentage of total analyzed samples (%)	Percentage of contaminated samples (%)
S. **Infantis**	4	11.11	36.36
S. **Enteritidis**	4	11.11	36.36
S. **Typhimurium**	3	8.34	27.27
Salmonella **sp.**	11	30.56	100.00

The calcium content was examined in 38 samples and 29% of samples were with the concentration range of 1000 – 1499 mg/kg, one sample even had concentration higher than 150 mg/100 g (Table 2.)

Table 2. Calcium content in analyzed MSM samples.

Ca content (mg/kg)	Number of MSM samples	Percentage (%)
≤ 499	4	10.52
500-999	22	57.89
1000-1499	11	28.95
≥ 1500	1	2.63
Total	38	100.00

Salmonellae are one of the most important food-borne pathogens. Owing to a high prevalence in livestock, they tend to spread through the food chain, exposing humans to the risk of salmonellosis. *Salmonella* sp. are among the major causes of foodborne disease in the U.S. and worldwide (Alali et al., 2016). In France, salmonellosis is the main cause of foodborne bacterial infection and the consumption of poultry products is considered as a major source of salmonellosis (Poirier et al., 2008). Seven *Salmonella* serotypes were identified from spleen and mechanically separated ground chicken and *Salmonella* prevalence in MSC samples was 27.8%. About 2% of MSC samples had relatively high *Salmonella* numbers (i.e., 3.5–3.9 log MPN) which may pose a significant risk to human infection (Alali et al., 2016). Mattick and al. (2002) after investigation of the heat tolerance of *S*. Typhimurium in sausage-meat, concluded that *Salmonella* sp. are present in a significant proportion of sausages and are not always killed during the cooking process. Mechanically separated chicken meat is thought to be a major source of the contamination of sausage-meat with *Salmonella*. The products that are of a special

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concern are raw, smoked or lightly cooked products. *Salmonella* is usually killed by temperatures >50°C, with the death rate increasing as the temperature increases. As previously stated, the consumption of poultry products is a major source of *Salmonella* infections.

Jovanović at al. (2013) at the Institute for hygiene and technology of meat, Belgrade, Serbia, investigated 152 samples of MSM for *Salmonella* contamination. Eight samples (5,26%) out of 152 were contaminated with *Salmonella* sp. The latest available results in Serbia have shown that the prevalence of *Salmonella* sp. from MSM is only 4% (Kureljušić at al., 2016) which is lower than our results. MSM has higher bone content and consequently calcium content as compared to fresh meat. The calcium content is frequently used as one of the criteria to identify MSM (Branscheid et al., 2009). The determination of bones (or calcium content) in MSM is a form of controlling the yield of mechanical separation processes. A high bone content means that the pressure used in the deboning process was too high or that the meat to bone ratio was too low (EFSA, 2013).

Conclusion

The MSM is food product with significantly high risk of the *Salmonella* presence and low chemical quality due to high percentage of chemical nonconformity results.

Minimizing possibility for microbiological contamination and related hazards during production of MSM should be ultimate goal for poultry processing industry. That can be achieved through successfully implemented HACCP system built upon a solid foundation of prerequisite programs for good manufacturing practice and good hygiene practice. Holding proper temperature is also imperative during process of production, storage as well as transportation of MSM and products that contain MSM.

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Poster presentation

EFFECTS OF DIETARY MODIFIED DRIED VINASSE ON EGG QUALITY CHARACTERISTICS IN LAYING HENS

Yalçın S.[1], Yalçın S.[2], Onbaşılar İ.[3]

Abstract

The aim of this study was to determine the effects of the usage of modified dried vinasse on egg quality characteristics in laying hens. A total of 64 Brown Nick laying hens, 65 weeks old were used in this study. They were randomly allocated into one control group and three treatment groups of 16 hens each. The experimental period lasted 12 weeks. The first, second and third experimental diets were formulated to contain 3, 6 and 9% modified dried vinasse (LayMass, Integro Food and Feed Manufacturing Company, İstanbul, Turkey), respectively. The inclusion of modified dried vinasse at the level of 0, 3, 6 and 9% in the diets had no significant effect on the egg weight, egg breaking strength, shell thickness, albumen height, albumen index, yolk index, Haugh unit, the weight percentages of egg parts, egg yolk cholesterol and egg minerals. As a result of this study it was concluded that the use of modified dried vinasse can be considered as a safe and an alternative protein source for protein supplements.

Keywords: laying hen, vinasse, egg quality, egg yolk cholesterol

Introduction

Vinasse (condensed molasses solubles) is a by-product from industrial production of yeasts, alcohol, citric acid or other substances by fermentation of molasses. There are wide variations in nutrient composition of vinasse depending on the source of material, the manufacturing processes, the addition of auxiliary chemicals and depotassification (Stemme et al., 2005). The modified dried vinasse contains modified vinasse produced from bakers yeast industry. Vinasse is used in feed industry as a binder and dust reducer. It is very common to add up to 3% vinasse to the diets for monogastric animals and up to 5% to the compound feed for ruminants (Stemme et al., 2005). The modified dried vinasse contains 45-60% modified vinasse produced from bakers yeast industry. Modified dried vinasse contains less than 2% potassium and it is rich in protein and energy. The main sources of nitrogen are betaine (9-41%) and glutamic acid (15-26%) (Weigand and Kirchgessner, 1980; Yalçın et al., 2010). Researchers have been started to use it in many countries as a protein supplement in animal feeding during acute shortages. Therefore the aim of this study was to determine the effects of modified dried vinasse on egg quality characteristics in laying hens.

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Material and Methods

A total of 64 Brown Nick laying hens, 65 weeks of age were used in this study. They were randomly allocated into one control group and three treatment groups of 16 hens each. Each hen was housed in cages individually with a 16/8 h light/dark regimen. Feed in mash form and water were provided ad libitum during the 12 week experimental period.

The first, second and third experimental diets were formulated to contain 3, 6 and 9% modified dried vinasse (LayMass, Integro Food and Feed Manufacturing Company, İstanbul-Turkey), respectively. The control diet didn't contain LayMass. The diets were formulated to be isocaloric and isonitrogenous.

Nutrient composition of LayMass was determined according to the AOAC (2000). Mineral content was determined with Agilent 7500ce ICP-MS system (Yokogawa Analytical Systems, Yamanashi-Ken, Japan). To determine the internal and external egg quality characteristics 48 eggs per each group were collected during the last four consecutive day of the last week. Each egg was weighed, their shape index, egg hell breaking strength and egg shell thickness were measured. The length and width of the albumen and the diameter of the yolk were measured. By using these values yolk index, albumen index and Haugh units were calculated. All eggs were collected during the first two consecutive day of the last week to determine yolk cholesterol concentration (Waldroup et al., 1986). Cholesterol content was determined according to the enzymatic method of TECO (2001). The percentage values of shell weight, yolk weight and albumen weight were calculated. Calcium, phosphorus and magnesium levels in egg albumen and egg yolk were determined with Agilent 7500ce ICP-MS system.

Statistical analysis were done using SPSS program (SPSS INC., Chicago, IL, USA). The effect of graded levels of modified dried vinasse on different variables using polynomial contrasts. Statistical differences were considered significant at P≤ 0.05 (Dawson and Trapp, 2001).

Results and Discussion

Modified dried vinasse (LayMass) had 91.45% dry matter, 39.5% crude protein, 0.8% ether extract, 12.46% crude fiber, 11.8% crude ash, 2.36% calcium, 0.64% phosphorus and 0.36% magnesium. LayMass was high in crude protein. Vinasse is an effective additive in animal feeding due to probiotic properties (Hidalgo. 2009).

The inclusion of modified dried vinasse at the level of 0, 3, 6 and 9% in diets had no significant effect on the egg weight, egg breaking strength, shell thickness, albumen height, albumen index, yolk index, Haugh unit and the weight percentages of egg parts (Table 1). Egg yolk cholesterol and calcium, phosphorus and magnesium levels in egg albumen and egg yolk were not affected by dietary modified dried vinasse (Table 2). However Damron et al. (1978) reported that egg weight and Haugh unit scores were significantly reduced by the presence of condensed molasses solubles at the level of 2.5, 5.0 and 7.5%. In another study (Sattari Najafabadi et al., 2014), vinasse supplementation didn't affect egg weight and egg shape index but 3 and 6% vinasse increased Haugh unit and egg shell thickness significantly. Amjadian et al. (2016) observed that 4 and 8% molasses distillers condensed

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solubles had no negative effect on carcass traits and they concluded that molasses distillers condensed solubles can be included up to 8% without any adverse effects on broiler performance. The presence of yeasts and nutrients in the vinasse (condensed molasses solubles, molasses distillers condensed solubles) such as vitamin B complex, minerals especially available phosphorus, organic acid, glutamic acid and betaine are beneficial substances for laying hens (Hidalgo. 2009; Sattari Najafabadi). The differences among studies may be due to the composition and the manufacturing processes of condensed molasses solubles.

Table 1. Effects of dietary modified dried vinasse on egg internal and external quality characteristics

| | Modified dried vinasse (LayMass), % | | | | Pooled |
	0	3	6	9	SEM
Egg weight, g	70.22	69.95	69.47	69.54	1.004
Shell breaking strength, kg/cm^2	2.66	2.76	2.74	2.66	0.049
Shell thickness, μm	392.5	395.4	391.6	392.4	1.019
Shape index	77.03	76.25	76.10	76.97	0.199
Albumen height, mm	6.36	6.28	6.32	6.41	0.048
Yolk index, %	41.91	42.36	41.28	42.13	0.178
Albumen index, %	7.92	7.76	8.07	8.38	0.094
Haugh unit, %	76.14	75.87	76.24	77.27	0.365
Yolk weight percentages, %	29.64	29.75	30.02	30.42	0.115
Albumen weight percentages, %	61.58	61.55	61.26	61.17	0.144
Shell weight percentages, %	8.78	8.70	8.72	8.42	0.140

No significant differences among groups.

Table 2. Effects of dietary modified dried vinasse on egg yolk cholesterol and egg minerals

| | Modified dried vinasse (LayMass), % | | | | Pooled |
	0	3	6	9	SEM
Yolk cholesterol, mg/g yolk	12.56	12.79	12.34	12.79	0.166
Albumen Ca, mg/kg	76.69	78.94	78.69	81.00	1.153
Albumen P, mg/kg	147.13	155.63	142.88	141.31	2.550
Albumen Mg, mg/kg	117.69	121.56	121.50	123.31	1.676
Yolk Ca, mg/kg	1236.8	1264.4	1272.3	1197.4	12.665
Yolk P, mg/kg	4067.0	4072.3	4097.3	4091.2	168.984
Yolk Mg, mg/kg	53.56	58.19	57.94	54.44	0.856

No significant differences among groups.

Conclusion

Dietary modified dried vinasse at the level of 0, 3, 6 and 9% in the diets had no significant effect on the egg weight, internal egg and egg shell quality characteristics, egg yolk cholesterol and egg minerals. Therefore modified dried vinasse (LayMass) can be used up to 9% in the diets of laying hens without causing any adverse effect on egg quality characteristics. As a result of this study it was concluded that the use of modified dried vinasse can be considered as a safe and an alternative protein source for protein supplements.

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Invited paper

SMART EDUCATION FOR SMART AGRICULTURE: KEY COMPETENCES FOR THE 21ST CENTURY IN THE COURSES OF ANIMAL SCIENCES

Pešikan A.[1], Antić S[2], Poleksić V. [3]

Abstract

There are huge changes in modern economy that significantly influence today's life. These radical, rapid, and continual changes ask for the reconsideration of quality of knowledge, skills and values (learning outcomes) that are acquired in higher agriculture education. In modern times, education should be much more than transfer of knowledge. The modern economic climate prefers knowledge which can be used in unfamiliar circumstances and skills which are transferable. These skills are usually named as the key competences for the 21st century (KC21). In this paper, we want to point out the importance of KC21 and to refer to three studies that are looking for KC21 at the Animal science courses from three perspectives. First is the perspective of the curriculum, which competencies are intentionally incorporated in the curriculum? Second is the perspective of graduated zoo-technicians, how effective was their preparation for work during studying? And third perspective is the case study showing how to support the development of the KC21 in teaching at the Animal sciences courses.

Keywords: agriculture education, curriculum, key competencies for the 21st century, animal sciences education, quality of university teaching/learning

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1. *Why should we be concerned about teaching/learning of agriculture in higher education?*

There are two main forces that necessary ask for reconsidering quality of knowledge, skills and values (learning outcomes) that are acquired in higher agriculture education, and reconsidering of quality of teaching/learning at agriculture faculties which has to lead to these outcomes. First one is today's *demand for better quality workforce* motivated by the need for better developed economy (e.g., every $1 spent on the education program generates $22.96 of sales growth, Katz, 2007). But 'better quality' means educated differently, not just acquiring good technical subject knowledge but developing, now days unavoidable, so called key competences for the 21st century. The contexts in which work is performed in the 21st century are undergoing enormous, rapid and continual change. "Every aspect of work, from its practical everyday organization, its form and function in production and economy, to its meaning and value in individual and collective life, are affected by these changes." (Casey, 1999).

There are many significant changes in economy: 'knowledge explosion'; knowledge has become a primary productive force (knowledge-based economy); decline in traditional and craft skills, and rise of new forms of work in services and information; rapid development and expansion of ICT; advanced production technologies, bio-technologies and artificial intelligence (that include 'smart' machines and 'seeing' and 'sensing' robots), and their derivatives; decline in specialization and so on (US Department of Labor, 2005). All these changes profoundly affect industry and commerce, and social life entirely. New context and new jobs have emerged requiring different or completely new skills. A process of 'multi-skilling' and 'up-skilling' tends to occur and steady rise in 'soft skills', particularly social and emotional skills and many important transferable skills such as critical thinking, information literacy, ICT literacy, learning to learn, entrepreneurship, creativity, solving problems, etc. Workers must be willing and able to learn and perform new tasks, take on different roles and be easily redeployed in the new workplace.

Second force that necessary asks for reconsidering quality of teaching/learning at agriculture faculties is *the re-conceptualization of work and learning* in the new millennium. 'Work" and 'learning' are no longer so polarized, as previously were. On the one hand, learning was a preparation, training for work; and, on the other hand, work was about producing or doing things to earn a living. Learning has moved from something which happens before work and prepared people for employment "to the lifeblood which sustains them" (Boud and Garrick, 1999). There are few places left for employees at any level who do not continue to learn and improve their effectiveness throughout their working lives. In other words, lifelong learning (LLL) comes to the scene. A new focus on learning is changing the way the businesses are doing, and vice versa, employees with new competencies become more effective in their present responsibilities and help transform the nature of the work in which they are engaged creating new work practices and forms of production. Distinctions between learning and work, life and work, community and enterprise are becoming less strict. Shifting boundaries, changing values and purposes of work and learning affect the physical, emotional and cognitive demands on workers at all levels (Casey, 1999).

Under the economic pressure higher education (HE) becomes intimately linked with

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society, getting new responsibility for learning quality of their students and their competencies at workplace. It has to justify public investment in education, and to empower economic development of a country. Higher education institutions have to become focused not just on knowledge gathering but also on knowledge application and knowledge creation. No longer are the pools of knowledge acquired in HE sufficient for the work in the 21st. The modern economic climate prefers knowledge which can be used in unfamiliar circumstances and skills which are transferable (e.g., communication skills, effective teamwork, problem solving, critical thinking, creative thinking, ICT literacy, decision making).

Hence, these imply two novelties: *outcomes-oriented higher education*, and close *collaboration between those who are experts in teaching/learning and subject-experts* (experts in agriculture), in joint work to create learning environment which would enable learning to occur and to be fostered (Poleksić, Pešikan, Pekić-Querrie and Antić, 2016). Universities in Serbia, in general, have a delayed response to the new circumstances, despite the adoption of the Bologna Declaration in 2003. Most of them are living in 'ivory tower' neglecting importance of teaching/learning strategies, and over-emphasizing importance of the content. Of course, no one can be a professional without solid content knowledge. However, as we have seen previously, today it's not enough – profound using of the subject content is *condition sine qua non* for efficient work.

Learning in HE has to be concerned not only about immediate work competencies, but about future competencies both the subject-specific and the general ones (Pešikan, 2005; Pekić Quarrie et al., 2006; Antić, Ivić and Pešikan, 2008). Students beginning their careers are facing with demands to innovate solution of vague problems, work in interdisciplinary teams and different environments, and effectively communicate their knowledge to colleagues and clients with diverse socio-culture backgrounds. Traditional animal science curricula pay no attention to the key competencies for the 21st century that our students need in their careers (Maguire, 2004). Curriculum is mainly focused on the content that has to be accepted by students without critical evaluation. Teaching method used to offer 'one right answer' to professional questions and dilemmas losing sight of promotion questioning, exploration, teaching students to think analytically and to make independent judgments about scientific claims, and to be aware and take into account the values lying behind the knowledge (Antić and Pešikan, 2015; Antić, (2007); Wals & Jickling, 2002). Learning environment in which students feel comfortable taking risks and developing the courage to make and defend judgments will enable the development of needed competencies. This teaching approach empowers not just intellectual but also social, emotional and ethical development of students, allowing them to serve themselves and society in responsible ways (Shillo, 1997).

2. *What are the key competencies for the 21st century?*

Diverse terms are used in the literature for the 21st century competencies (KC21), such as: *transferable knowledge, key skills, core skills, soft skills, generic skills, deeper learning, college and career readiness, student-centered learning, next-generation learning, new basic skills, higher-order thinking, twenty-first century skills*. Levy and Murnane (2004) emphasized a crucial component of what constitutes KC21 "expert thinking or complex communication—tasks that computers cannot do" (pp. 53–54).

The first conceptual frameworks for KC21 appeared during the final years of the 20th

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century (Salas-Pilco, 2013). Diverse institutions (international organizations, private consortia, and governments, etc.) have ordered elaboration of the frameworks with different purposes. Consequently, this led to different KC21 classification categories. Current conceptual frameworks for KC21 include (Dede, 2010; Salas Pilco, 2013): UNESCO's Report (Delors, 1996); the DeSeCo[146]/OECD, 1997-2003 (OECD, 2005); 21st century skills and competencies for new millennium learners in OECD countries (Ananiadou & Claro, 2009); the Partnership for 21st Century Skills (2009); the framework build by Trilling and Fadel (2009); National educational technology standards for students (NETS•S/ISTE), 1998-2007 (2007); the Metiri Group and NCREL (2003); Key competencies in Europe (European Community, 2006); the American Association of Colleges and Universities (2007); Assessment and teaching of 21st century skills (ATCS) 2010, Cisco, Intel and Microsoft (Binkley et al., 2010); 21st-century competencies – Hewlett Packard 2010 (Finegold & Notabartolo, 2010); and 21st century competencies – Singapore, 2010; and National Research Council, Committee on Defining Deeper Learning and 21st Century Skills (Pellegrino & Hilton, 2013).

We have analyzed these frameworks, and made our model which is appropriate for the curriculum analysis (Pesikan and Lalovic, 2016). The model consists of: (1) Socio-emotional skills (Knowing self and others; Self-regulation; Social consciousness; Social skills; Cooperation; Responsible decision making), (2) Problem solving, (3) Critical thinking, (4) Creativity, innovations, (5) Information literacy, (6) ICT literacy, (7) Learning to learn, and metacognition, (8) Working skills, entrepreneurship, and (9) Responsibility toward self and others (development of healthy life style; and Ecological awareness and responsibility).

3. Key competencies for the 21st century in the Animal sciences courses in Serbia

The Panel discussion at this Symposium will be introduced by three abstracts dealing with smart education for smart agriculture. Here, our aim is to point out the logic connecting these studies. We were interested in three perspectives: First is the perspective of the curriculum, which competencies are intentionally incorporated in the curriculum (planning phase)? Second is the perspective of graduated zootechnicians, how they see their education and what competencies they need at work (test of practice)? And third perspective is the look forward, i.e., the case study showing how to support the development of the KC21 in teaching at the Animal sciences courses (model for teaching/learning improvement).

Because of the relevance of the KC21 in agriculture education we wanted to find out which of the KC21 are included in curricula of Animal sciences in Serbia. In order to achieve this goal, we have analyzed the curricula of the Animal sciences at three Faculties of Agriculture in Serbia: Belgrade, Novi Sad and Čačak. The curricula were analyzed by the PL KC21. Content analysis was used as a method and the unit of the analysis was each statement in the curriculum. The findings of the study are presented in Table 1.

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[146] *Definition and Selection of Competencies: Theoretical and conceptual foundations (DeSeCo),* Organization for Economic Cooperation and Development

Table 1: Key competencies for the 21st century in the curriculum of animal sciences courses in Serbia

Competences		Curriculum elements			
		Purpose, mission	Goals	General outcomes	Specific outcomes
1. Socio-emotional competences	1.1. Knowing self and others				
	1.1. Self-regulation				
	1.2. Social consciousness			x	
	1.3. Social skills			x	x
	1.3.1. Cooperation	x		x	x
	1.4. Responsible decision making				
2. Problem solving	2.1. Problem solving		x	x	xx
	2.1. Competencies for undertaking small research		x		
3. Critical thinking		x	x	x	x
4. Creativity, innovations		x	xx		
5. Information literacy		x	x	x	xxx
6. ICT literacy					x
7. Learning to learn, metacognition		x	xxxx	x	xx
8. Working skills, entrepreneurship			x		
9. Responsibility toward self and others	9.1. Healthy life style				
	9.1. Ecological consciousness and responsibility	xx	x		

The result shows that surveyed curricula contained none of the competencies aimed at personal qualities development such as self-awareness, self-regulation, development of healthy life styles and decision making. Just 3 out of 16 competencies: learning to learn, information literacy and critical thinking are emphasized. Analysis indicates lack of internal cohesion or alignment among curriculum elements (from mission through specific outcomes): 2 out of 16 competencies are presented in the aims but not in the outcomes (social awareness and social skills), and vice versa, 4 out of 16 are given in the outcomes but not in the aims (entrepreneurship, ecological awareness and responsibility, research competencies and creativity and innovations).

Therefore, these findings indicate that the KC21 are planned in the curricula of the Animal sciences in Serbia, but they are not included fully (many important working skills are omitted) and not consistently performed. However, it should be noted that the Agriculture faculties are among the rare faculties in Serbia that introduced the KC21 in their curriculums. Second is the perspective of graduated zootechnicians, and their evaluation on how well their studies equipped them with skills necessary for work in the industry? In the study, the education process is said to *provide necessary* subject knowledge and the development of KC21. The mix method was used. The questionnaire was constructed for the purpose of this study and the data were collected online. The questionnaire covered three groups of indicators: assessment of subject knowledge; assessment of skills (specific and general, transversal) and professional values (healthy style of life, ecological responsibility, ethical values, etc.). At the same time, the questionnaire was the base for carrying out a focus group with selected professionals. In the focus group the zootechnicians working in various professional environments were able to give elaborated comments on the quality of knowledge and skills they had developed during studying. Online questionnaire was answered by 61 zootechnicians -

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63.3% of them were studying before Bologna reform and 26.7% were studying under the reformed course. Most of them have studied at the University of Belgrade (82%), then at the University of Novi Sad (9.8%), Priština (4.9%), and Kragujevac (3.3%). The items were 5-grades Lickert scale type. Main findings are presented in the Table 2.

Table 2: Quality of studying Zootechnics from the perspective of graduated zootechnicians

	Question (Q):	1 Very unsatisfactory	2 unsatisfactory	3 Average satisfaction	4 satisfactory	5 Very satisfactory
What is your satisfaction with:						
1.	Quality of knowledge you have got on animal science studies	1.6%	6.6%	24.6%	52.5%	14.8%
2.	General preparation for professional work	13.1%	18%	34.4%	31.1%	3.3%
3.	Quality of specific subject knowledge necessary for professional practice	1.6%	16.4%	36.1%	32.8%	13.1%
4.	Quality of specific skills necessary for professional practice	9.8%	16.4%	45.9%	19.7%	8.2%
In what degree studying animal science develop these specific competence:						
5.	Professional patterns of behavior	11.5%	36.1%	29.5%	19.7%	3.3%
6.	Responsibility and ethics	4.9%	14.8%	29.5%	37.7%	13.1%
7.	Communication skills	8.2%	14.8%	32.8%	31.1%	13.1%
8.	Constructive conflict resolution	11.5	27.9%	32.8%	23%	4.9%
9.	Cooperation and team work	9.8	19.7%	26.2%	27.9%	16.4%

10.	Application of knowledge	3.3%	14.8%	44.3%	29.5%	8.2%
11.	Problem solving	6.6%	18%	45.9%	23%	6.6%
12.	Competencies for undertaking small research	9.8%	34.4%	26.2%	23%	6.6%
13.	Responsible decision making	8.2%	18%	29.5%	36.1%	8.2%
14.	Critical thinking	4.9%	24.6%	26.2%	24.6%	19.7%
15.	Initiative and entrepreneurship	11.5%	37.7%	21.3%	18%	11.5%

The results indicate that although most professionals are generally satisfied with knowledge quality (52.5% choose grade 4 on Q1); they estimate that the faculty preparation with necessary professional skills were only moderate quality (45.9% choose grade 3, on Q 4). The development of the ethical values (37.7%) and responsible decision making (36.1%) are on satisfactory level (grade 4). On the other hand, they are unsatisfied (grade 2) with the support of the development of other competencies, such as: professional patterns of behavior (36.1%), ability to undertake research (34.4%), and initiative and entrepreneurship (37.7%). In the focus group the competences related to management and entrepreneurship were stressed as the least supported through studying, as well as, complex communication skills. They found themselves completely unprepared for the situations where it is needed to communicate, argue, and lead unprofessional workers on farms. The answers are equally distributed among all grades for questions related to development of critical thinking, standing for the profession in society, and agriculture legislation. We hypothesized that when answering these questions the zootechnicians have had different aspect in mind.

The results showed certain discrepancy between shaping of future professionals and the requests of the practice in today's circumstances. The faculty should be more oriented toward building all competencies compared to the contemporary state of affairs.

In the third study, where we applied a multidisciplinary approach and active learning in the class with students in the 4th year of study, the potential of this type of teaching for development of the KC21 is discussed. The instruction consists of 5 scenarios that are connected and contribute to the development of the following competencies: linking previous knowledge from various subjects; knowledge application in real life situations; critical reading of the text and formulating arguments; problem solving of a socio-scientific issue; responsible decision making; understanding different views on the problem through the role-playing; skillful presentation of the project results tailored to the audience; effective cooperation on task in team work; self-awareness and meta-cognition – the ability of self-evaluation and evaluation of the working process and its effectiveness; communication skills, argument dialog and discussion; ecological awareness and responsible behavior toward environment; awareness of issues related to the position of

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agriculture in the society.

This pattern of lesson scenarios was replicated two school years in a row (2014-15 and 2015-16) with different generations of student. After the lessons we evaluated student's reflections on process and their attitude on the lesson potentials to support development of the KC21. The questionnaire consisted of open questions related to student's general impression of lessons, evaluation of the learning benefits, advantages and weak sides of such teaching/learning method, etc. The same questionnaire that consisted of open questions was applied in the first and the second year.

The findings in the first year suggest that students have never been asked to evaluate lessons, to be reflective and metacognitive. So, their answers were short, poorly elaborated and many questions were left unanswered. Because of that we treated all answers as the answer on only one question: *What are your comments on these lessons?* Unit for analysis were each student's comment (f=149). Results are presented in Table 3.

Table 3: Student's evaluation of model lessons in Zootechnics

Student's comments	frequency
Benefit for professional development	18.12%
Benefit for learning (getting new knowledge; activating old knowledge; connecting different subject knowledge; connecting theory and practice, better memorization, etc.)	16.78%
Benefit for cooperation, team work and development communication skills	16.78%
Benefit for personal growth (new experience, creativity, decision making, self-regulation)	9.4%
Development of thinking and critical thinking and problem solving	8.7%
Benefit for motivation for learning	15.44%
Pleasant atmosphere on lessons	5.37%
General benefit to teaching (*it should be more lessons like this*)	7.38%

In second part of questionnaire students were asked to grade the statements about quality of lessons from 1 to 5 (*not at all related to* these lessons to *very related to* these lessons). In total, 31 students fulfilled questionnaire. The finding shows that the best were: Pleasant working atmosphere (100%); cooperation, team work (96.77%); connecting different subject knowledge (93.55%); and developing professional competences (93.55%). It can be concluded that from students' perspective this teaching strategy has great potential for the development of KC21.

The results in the second year (26 students responded) indicate higher student's competences to evaluate their learning experience. The results indicate that the majority of the students (80.8%) found that these lesson scenarios have great potential in development of professional ethics, professional patterns of behavior, knowledge application, etc. The same amount of students considers that there should be more lessons like these during studying. Students appreciated especially following aspects of lessons, e.g. they gave the highest grade to: stimulating work atmosphere during lessons (79 %): development of professional competences (75%) and development of problem solving skills (70%).

Conclusion

The results of these studies are the solid base for an improvement of the quality of curriculum and teaching/learning methods at Zootechnics and can be used in coming accreditation of the Faculties of Agriculture in Serbia. The opinion of stakeholders and Zootechnics alumni and their active contribution is also indispensable in this process. Finally, it should be noted how important it is that all teachers participate in a discussion on development of the KC21 in the Animal Sciences courses with the joint aim to educate high quality agriculture - animal science professionals.

Acknowledgement

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Abstract

SMART EDUCATION FOR SMART AGRICULTURE: QUALITY OF STUDYING ZOOTECHNICS FROM THE PERSPECTIVE OF GRADUATED ZOOTECHNICIANS

Antić S.[1], Poleksić V.[2], Pešikan A.[3]

Abstract

For the effective professional role nowadays is not enough to equip students with quality content knowledge. Modern professional knowledge consists of both subject knowledge and development of the key competencies for the 21st century: problem solving, critical thinking, creative thinking, learning to learn, entrepreneurship, socio-emotional competencies (self-awareness, self-regulation, social awareness, social skills, responsible decision making, and environmental awareness).

The subject of the study is the research of quality of acquired competencies of graduated students of Zootechnics at the Faculty of Agriculture. In the study, quality of education is defined as subject knowledge and the key competencies for 21st century required by Zootechnic's practice in the complex, demanding and rapidly changing today's society. The mix method is used. The questionnaire is constructed for the purpose of this study and the data were collected online from the professionals. The questionnaire was the base for carrying out a focus group with selected professionals.

The results showed a discrepancy between shaping of future professionals and the requests of the practice in today's circumstances. The faculty should be more oriented toward building of all competencies that consist of subject knowledge, skills, attitudes and professional values compared to the contemporary state of affairs. Obtained results are the solid base for the improvement of the quality of curriculum and teaching/learning methods at Zootechnics and could be used for the purpose of coming accreditation of Zootechnics at the faculties of agriculture in Serbia.

Keywords: quality of zootechnic' s education, key competencies for the 21st century, zootechnicians, Zootechnics, curriculum development, agriculture education

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Abstract

SMART EDUCATION FOR SMART AGRICULTURE: A MODEL OF TEACHING/LEARNING FOR DEVELOPMENT OF THE KEY COMPETENCIES FOR THE 21ST CENTURY

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Abstract

In the paper the contribution of a multidisciplinary approach and active learning in the class with students in the 4th year of study for the development of the key competencies for the 21st century (KC21) is analyzed. The aims and students' activities are analyzed by content analysis. The results show that this model of teaching/learning significantly contributes to:

- the linking previously acquired knowledge in various subjects; application of academic knowledge in life situations; and the evaluation of knowledge and skills;
- the development of the KC21 (15 out of 16): decision making and taking responsibility for the chosen; critical reading of the text and formulating arguments; learning to learn; self-awareness and meta-cognition – the ability of self-evaluation and evaluation of the working process and its effectiveness; problem solving of a "socio-scientific issue"; effective cooperation on task through team work; understanding different views on the problem through the role-playing; communication skills; information literacy; ecological awareness and taking activities to preserve environment; and the awareness of issues related to the position of agriculture in the society.

The results also indicated that the majority of the students (80.8%) found that such interactive classes have great potential in development of professional ethics, professional patterns of behavior, knowledge application, and that there should be more lessons like these. Following aspects were appreciated by the students, i.e. they gave the highest grade to: pleasant atmosphere for work – stimulating learning environment (79%): development of professional competences (75%) and development of problem solving skills (70%).
In addition, the answer to the question: what should be learned during studies, the most frequently choosen answer was that students should be trained how to anticipate consequences of decisions, pointing out that the multidisciplinary approach contribute to better learning of both professional and K21 skills.

Keywords: quality of university teaching/learning, key competencies for the 21st century, cooperative learning, multidisciplinary approach

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Abstract

SMART EDUCATION FOR SMART AGRICULTURE: KEY COMPETENCIES FOR THE 21st CENTURY IN THE CURRICULUM OF ANIMAL SCIENCES COURSES IN SERBIA

Pešikan A.[1], Poleksić V.[2], Antić S.[3]

The main purpose of the paper is to evaluate current curricula of the courses in Animal Sciences to ensure that key competencies for the 21st century (KC21) are included. The focus of the study is on the *soft skills* that are needed for employability and adequate response to the complex demands of nowadays jobs. The sample consists of the curricula of the Animal sciences at three faculties of Agriculture in Serbia: Belgrade as a one of the leading faculty in the field of teaching/learning and curriculum development in higher education in Serbia, and Novi Sad and Čačak where animal sciences are also taught. The content analysis is used and the unit of the analysis is each statement in the curriculum.

The Belgrade and Novi Sad curricula are the same and Čačak has no general statements on aims and outcomes of the course. The study showed that surveyed curricula specifically contained none of the competencies aimed at developing personal qualities such as self-awareness, self-regulation, development of healthy life styles, and decision making; while 3 out of 15 competencies: learning to learn, information literacy and critical thinking are emphasized; there is no alignment in curriculum: 2 out of 16 competencies are presented in the aims but not in the outcomes (social awareness and social skills) and vice versa, 4 out of 16 are given in the outcomes but not in the aims (entrepreneurship, ecological responsibility, research competencies and creativity). The analysis of the books of subjects at the Animal sciences course in Čačak showed fewer KC21 and less elaborated.

The findings may serve as a tool for an improvement of the curricula of faculties of Agriculture.

Keywords: curriculum, key competencies for the 21st century, agriculture education, animal sciences education, quality of university teaching/learning

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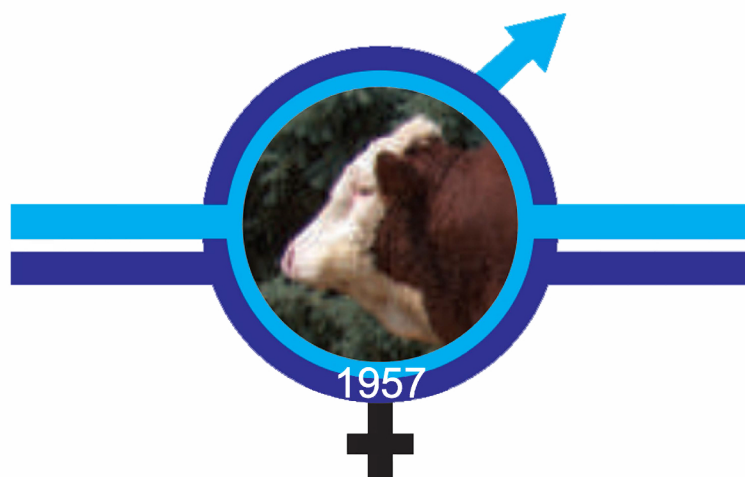Fax: +381 11 63 555 84
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