

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

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CARCASS CHARACTERISTICS OF BROILERS FED ENZYME COMPLEX

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Abstract: Enzyme supplementation in diets based on corn and soybean meal can improve the productive performance of broilers. Thus, we aimed to evaluate the effect of the inclusion of different levels of an enzyme complex consisting of phytase, protease, xylanase, β -glucanase, cellulase, amylase, and pectinase, for diets based on corn and soybean meal, on the parameters of carcass yield and meat quality of broilers. Six hundred broiler chicks were used, and the animals were females with one day of age, from the Cobb 500 strain, and distributed in a completely randomized design, with five levels of inclusion of the enzyme complex (0, 100, 200, 300 and 400), and six repetitions, with twenty animals each. The carcass yield and meat quality were evaluated at 35 and 42 days of age. We evaluated the characteristics of weight loss by cooking (WLC), shear force (SF), water holding capacity (WHC), pH, lightness (L*) and color (a* and b*). The parameters of performance, carcass yield and carcass parts, and meat quality were not affected by the enzyme supplementation of diets fed to broiler chickens ($P > 0.05$), except for the performance characteristics of the breast and the wings at 42 days of age ($P < 0.05$).

Key-words: poultry, enzymes, meat quality, carcass yield.

Introduction

Broiler rations in Brazil are, almost entirely, formulated from two basic ingredients: corn, which is an excellent energy source, and soybean meal, which contributes with high-quality proteins and with great amino acid availability (*Opalinski et al.*; 2006). However, it is known that the nutrients originated from these foods are not properly absorbed, mainly because of the presence of anti-nutritional factors, such as NAPs (non-amylaceous polysaccharides) and phytic

acid. Therefore, mechanisms to enhance the performance of foods given to animals were made necessary. Aiming to increase the efficiency of rations, the usage of exogenous enzymes in the feeding of broilers is gaining more space and has become a great alternative, since it enhances food digestibility, minimizing the anti-nutritional effects and promoting the productivity indices (*Hooge et al.*; 2010). The use of enzyme complexes is effective, since the wide range of enzymes present in this type of product allows for greater action in different types of substrates and, or, foods utilized in the process of ration fabrication.

Factors that influence meat quality can mostly be controlled at various stages of setting up the chicken or during slaughter and processing. The carcass yield is closely linked to adequate food and nutrition of broilers. After all, animals with adequate supply of nutrients will deposit effectively muscle. The main meat quality measurement parameters are: pH, color, water-holding capacity and weight loss to cooking (*Mendes et al.*, 2003). The final pH measured 24 hours post-mortem, it is decisive for quality meat, because it is directly related proteins and meat pigments. Thus, stabilization of the pH value influences the characteristics color, water-holding capacity, cooking weight loss, juiciness and softness (*Qiao et al.*, 2001).

In light of this, this study aimed at evaluating the carcass yield and the carcass parts, and the quality of the meat for broilers that were submitted to the diets based on corn and soybean meal with different levels of the SSF (solid state fermentation) enzyme complex.

Materials and Methods

This experiment was conducted in the facilities of the broiler sector of the Animal Science Department of University Federal of Vales of Jequitinhonha and Mucuri (UFVJM). Six hundred broiler chicks were used, and the animals were females with one day of age, from the Cobb 500 strain. This design was completely randomized with five treatments and six replications with 20 broilers each. The treatments consisted of five inclusion level of enzyme complex (0, 100, 200, 300, 400 g/ton). The enzyme complex SSF is composed of seven distinct enzymes: phytase, protease, xylanase, β -glucanase, cellulase, amylase, and pectinase.

The diets were formulated according to the adaptations by *Rostagno et al.* (2011). The percent composition and the calculated levels of nutrients for the control diets for the initial stage (1 to 21 days of age), the growing stage (22 to 35 days of age) and the final stage (36 to 42 days of age) are presented on Table 1.

Table 1. Percentage composition and calculated nutrient levels of experimental diets.

Ingredients	Initial	Growth	Final
Corn	61.110	64.026	66.659
Soybean meal	33.413	30.255	26.619
Soybean oil	1.169	2.636	3.169
Limestone	0.925	0.816	0.000
Dicalcium phosphate	1.490	1.156	2.040
Common salt	0.456	0.443	0.418
L-lysine HCl 99%	0.245	0.136	0.104
DL-methionine 99%	0.289	0.209	0.159
L-threonine 98%	0.073	0.005	0.000
Mineral supplement ¹	0.050	0.050	0.050
Vitamin supplement ²	0.100	0.100	0.100
Salinomycin 12%	0.055	0.055	0.055
Antioxidant BHT	0.010	0.010	0.010
Choline chloride 60%	0.100	0.100	0.100
Enzyme complex ³	0.000	0.000	0.000
Inert ⁴	0.040	0.040	0.040
Total	100.0	100.0	100.0
Metabolizable energy, MJ/kg	12,56	12,98	13,19
Crude protein (%)	20.400	19.000	17.500
Calcium (%)	0.809	0.683	0.759
Available phosphorus (%)	0.386	0.319	0.264
Digestible lysine (%)	1.165	1.005	0.892
Digestible methionine (%)	0.559	0.467	0.403
Methionine+digestiblecystine (%)	0.839	0.733	0.651
Sodium (%)	0.200	0.195	0.185

¹Safety levels per kg of the product (Min): Folic acid 750 mg, Pantothenic acid 12g, B.H.T. 1.000 mg, Biotin 25 mg, Niacin 35g, Vitamin A 8.000.000 UI, Vitamin B1 1.500mg, Vitamin B12 12.000 mg, Vitamin B2 5.000 mg, Vitamin B6 2.800 mg, Vitamin D3 2.000.000 UI, Vitamin E 15.000 UI, Vitamin K3 1.800 mg.

²Safety levels per kg of the product (Min): Copper 20 g, Iron 96 g, Iodine 1.400 mg, Manganese 156 g, Selenium 360 mg, Zinc 110g.

³Allzyme SSF – Alltech Ind.: minimal levels of enzyme activity: phytase 300 UF/g; protease 700 UI/g; xylanase 100 UI/g; β -glucanase 200 UI/g; cellulase 40 UI/g; α amylase 30 UI/g and pectinase 4000 UI/g.

⁴Caulim.

At 35 and at 42 days of age, two animals of each repetition were selected by the average weight of the group ($\pm 5\%$) for evaluation of the performance for the carcass, breast, leg quarter, wing and abdominal fat. After eight hours of fasting, the animals were packed in boxes and transported to a room lit by artificial blue light. All the slaughtering procedures were approved by the Ethic Committee of UFVJM, process n° 034/12.

After the evisceration, the carcass yield was obtained in relation to the body weight: % CY = (carcass weight x 100/body weight). The performance for the breast, the leg quarter and the wing were calculated in function of the carcass weight: % BP = (weight of the part x 100/carcass weight). The performance for the abdominal fat was calculated in function of the body weight of the animals. For the evaluation of meat quality, cooled, skinless, boneless breast meat. The pH was standardized at room temperature, 25° C, by means of a pH meter (Tecnopon mPA210) attached to the penetration electrode (Hanna HI 8314) and introduced directly in the muscle “Pectoralis major”. The method described by *Hamm (1960)* was utilized in order to determine the water retention capacity (WRC).

Weight loss by cooking was achieved with the methodology proposed by *Cason et al. (1997)*. The analysis of the shear force was made by a StableMicroSystems TAXT 2 PLUS texturometer attached to a blade set V Wanner Bratzler probe. It was considered the force peak of the analysis, therefore determining necessary force for the cuts. Color analysis was conducted with a raw meat sample, with longitudinal cuts in the breast portion made by a Minolta CR 400 colorimeter, with a CIELAB system (L^* , a^* and b^*), where L^* = luminosity, a^* = red content and b^* = yellow content.

The statistical analysis of the data was carried out by the GLM procedure of the SAS program (SAS, 2002), the data were submitted to a regression analysis, with the significance rate at 5%.

Results and Discussion

The inclusion of the SSF enzyme complex did not influence ($P > 0.05$) the parameters of carcass yield of broilers with 35 days of age ($P < 0.05$) (Table 2).

Table 2. The parameters of carcass yield of broilers with 35 days of age

Variables	Levels of EC addition (g/ton) – 35 days					CV (%)	P value
	0	100	200	300	400		
CY	73.54	74.32	74.17	74.04	73.60	1.39	0.9344
BP	38.61	38.07	38.12	38.71	38.67	3.70	0.6733
WP	10.47	10.34	10.18	10.32	10.43	4.49	0.8695
LQP	27.04	26.57	26.60	26.88	26.53	2.49	0.4168
FP	1.88	1.62	1.41	1.70	1.67	27.33	0.5648

CV = coefficient of variation (%); P value = significance rate of the regression analysis.

The inclusion of the SSF enzyme complex did not influence ($P > 0.05$) the parameters of carcass yield of broilers with 42 days of age, but did have an influence on breast and wing performance ($P < 0.05$) (Table 3).

Table 3. Average values for carcass yield (CY), breast performance (BP), wing performance (WP), leg quarter performance (LQP) and fat performance (FP), of broilers of 42 days of age, submitted to diets containing different levels of enzyme complex (EC).

Variables	Levels of EC addition (g/ton) – 42 days					CV (%)	P value
	0	100	200	300	400		
CY	74.06	73.25	73.21	72.70	75.36	2.53	0.2838
BP	40.05	40.11	40.41	39.73	38.24	3.83	0.0417
WP	9.73	10.44	10.22	10.84	10.43	5.28	0.0228
LQP	26.46	26.74	26.85	27.21	27.50	6.75	0.3225
FP	1.76	1.67	1.64	1.98	1.58	26.60	0.8591

CV = coefficient of variation (%); P value = significance rate of the regression analysis.

Broiler meat, according to *Petracci and Baéza (2011)*, has the following as its main intrinsic attributes: appearance, texture, succulence, flavor, and functionality; coloring is the most important factor that affects the choice of

consumers. They also state that the pH is closely related to all the factors that affect meat quality, although this effect is complex. This complexity is due to the many reaction associated with the heme factor, which depends on pH (Werner *et al.* 2009). However, the inclusion of the SSF enzyme complex did not influence ($P > 0.05$) the parameters of meat quality or meat color, carcass yield of broilers with 42 days of age (Table 4).

Table 4. Average values for weight loss by cooking (WLC), shear force (SF), water retention capacity (WRC), hydrogenionic potential (pH), luminosity (L*), red content (a*) and yellow content (b*) of the breast of broilers with 42 days of age, submitted to diets containing different levels of enzyme complex (EC).

Variables	Levels of EC addition (g/ton) – 42 days					CV (%)	P value
	0	100	200	300	400		
WLC (%)	30.54	26.40	29.54	31.48	26.03	16.79	0.5510
SF (kgf.cm ⁻²)	3.16	3.07	3.33	2.45	3.20	18.61	0.4969
WRC (%)	44.98	47.13	44.2	48.01	46.71	10.51	0.4850
pH	5.72	5.67	5.68	5.69	5.71	0.93	0.8317
L*	49.79	49.37	50.40	49.6	48.22	5.23	0.3751
a*	3.16	3.07	3.33	2.45	3.20	21.78	0.3226
b*	7.66	6.72	7.67	7.22	7.82	15.10	0.5782

CV = coefficient of variation (%); P value = significance rate of the regression analysis.

Cardoso *et al.* (2011) also did not verify any differences ($P > 0.05$) on carcass yield for broilers with 42 days of age. Regarding abdominal fat, Souza *et al.* (2008) observed an increase in the carcass of broilers at 42 days of age. Kessler *et al.* (2000), state that the most efficient way to avoid fat excess in the carcass is the approximation between energy and protein. This fact can be explained by the increase in food digestibility to the level recommended for the addition of the complex, which overestimates the energy values of the ration. Thus, with the energy excess, there is the possibility of greater accumulation of abdominal fat, a fact that was not identified within this study.

This study verified a significant effect ($P < 0.05$) for breast performance and wing performance at 42 days for broilers fed with diets supplemented with SSF EC. The equation for breast performance was: $BP = 40.355 - 4.057EC$ ($r^2 = 0.15$), evincing the lack of concrete explanation of the effect of the inclusion of SSF EC on breast performance. Similar results were found by *Soto and Salanova et al. (1996)*, who verified an effect ($P < 0.05$) of enzyme supplementation in diets based on corn and soybean meal on the augmentation of the breast muscle in broilers with 42 days of age. The equation estimated from the significance of the regression regarding wing performance was: $WP = 9.953 + 1.729EC$ ($r^2 = 0.18$). It is possible to observe that a small variation on wing performance can be explained by the supplementation with SSF EC. This variance may have occurred due to an error inherent to the cutting that was carried out. It was executed by the collaborators of the activity, who may not have observed the necessary standardization and accuracy.

The greatest percentage increase, an average of 2.15% in relation to the other treatments, of the breast, with the enzyme levels recommended by the manufacturer (200 g/ton), may have occurred due to the fact that this level provides a better digestibility of the ingredients and, therefore, increases the amount of nutrients available for breast growth, since this cutting represents about 40% of the total carcass yield. For every other level over the recommended value for EC, this response may have not existed due to the lack of substrate available after the addition of an amount of enzymes greater than the recommended number without considering the nutritional energy matrix and, or, the diet proteins. It may also be due to the low fiber content present in low viscosity diets (*Soto and Salanova et al., 1996*). Therefore, regarding the rations with enzyme supplementation in the “on top” form, with supplementation of enzyme levels without the reduction of the total metabolizable energy, it is verified that the enzyme does not produce any beneficial effects above the recommended level, which happens due to the quality of the foods used in the formulation and to the meeting of the nutritional demands of the animals.

According to *Werner et al., (2009)*, the addition of enzymes does not affect quality parameters of the meat; they are interconnected with color and pH, which are mainly hampered by the loss of exudate and temperature pitches. *Zakaria et al., (2010)*, while working with diets based on corn and soybean meal supplemented with the SSF enzyme complex, also did not observe any effects ($P > 0.05$) regarding the parameters pH, WLC, WRC, color and luminosity for broilers at 42 days of age supplemented with EC (xylanase, protease and amylase).

Conclusion

The inclusion of the SSF enzyme complex in diets based on corn and soybean meal for broilers in the levels recommended by the manufacturer, 200 g/ton, enhanced the efficiency of the breast and the wing at 42 days and did not significantly influence the carcass yield and the quality of the meat.

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Osobine trupa brojlera hranjenih enzimskim kompleksom

F. S. Dalólio, D. P. Vaz, J. Moreira, L. F. T. Albino, L. R. Valadares

Rezime

Dodavanje enzima obrocima koji se zasnivaju na kukuruzu i sojinoj sačmi može poboljšati proizvodne performanse brojlera. Stoga, naš cilj je bio da se proceni efekat uključivanja različitih nivoa kompleksa enzima koji se sastoji od fitaze, proteaza, ksilanaza, β -glukanaze, celulaze, amilaze i pektinaze za obroke na bazi kukuruza i sojine sačme, na parametre prinosa trupova i kvalitet mesa brojlera. Šest stotina brojlerski pilića je korišćeno u ogledu, ženskog pola u uzrastu od jednog dana, hibrida Cobb 500, distribuirano u potpuno slučajnom dizajnu, sa pet nivoa uključivanja kompleksa enzima (0, 100, 200, 300 i 400), i šest ponavljanja, sa dvadeset životinja u svakoj. Prinos trupa i kvalitet mesa su procenjeni na 35 i 42 dana starosti. Ispitivali smo sledeće karakteristike: kalo kovanja (WLC), silu kidanja (SF), sposobnost zadržavanja vode (WHC), pH vrednost, jačinu boje (L *) i boju (* b *). Parametri performansi, prinosa trupa i delova trupa i kvalitet mesa nisu bili pod uticajem enzimskih dopuna u ishrani brojlera ($P > 0,05$), osim osobine performansi grudi i krila u uzrastu od 42 dana ($P < 0,05$).

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EFFECT OF SEX-LINKED DWARF GENE ON EXTERIOR APPEARANCE, PRODUCTIVE PERFORMANCE AND EGG CHARACTERISTICS IN A COLORED BROILER DAM LINE

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Original scientific paper

Abstract: The effect of sex-linked dwarf gene was investigated through comparison of dwarf hens with their full-sib normal sisters obtained by mating heterozygous males (DW/dw) to normal females (DW/_) from line F (used as maternal form for production of slow-growing colored chickens) with respect to the following traits: body weight, shank and keel length at 40 weeks of age, age of sexual maturity (at 50 % production), egg production, egg weight, feed intake, feed utilization, livability, fertility, hatchability and egg quality characteristics. The results demonstrated that the dw gene caused statistically significant reduction of body weight by 29.15 %, shank length by 20.17 %, keel length by 7 % and egg weight by 5.72 % ($p < 0.001$). The hens with normal genotype attained sexual maturity 7 days earlier ($p < 0.001$), but nevertheless, rate of lay was similar to that of mini forms. There were no considerable differences between both genetic groups with respect to livability percentage over the production cycle. Dwarf hens consumed by 23.38 % less feed ($p < 0.01$) than normal sized hens and converted nutrients more efficiently by 12.69 % ($p < 0.05$). The presence of dw gene in hen genotype increased the eggshell percentage, reduced egg yolk and albumen weights and had no effect on their quality. The positive effect of the sex-linked dwarf gene on economically important traits – feed intake and feed conversion, hatchability of eggs set, is a prerequisite for the development of more efficient broiler breeder hens for production of slow-growing chickens.

Key words: Sex-linked dwarf gene, broiler breeder hens, production traits, reproductive fitness

Introduction

Efficiency is a key factor in poultry breeding. One of the possible ways for its improvement is the utilization of mini-hens, carriers of a recessive gene located in the sex chromosome. On the basis of extensive literature data, *Merat (1990)* outlines that the so called dwarf gene, described by *Hutt (1959)*, results in reduction of body weight of birds by 33 % and feed consumption by 20-25 %. The existing interest towards the practical application of that gene is due to several advantages of mini hens: higher stocking density per square meter (*Charpentier, 2009*), better utilization of dietary nutrients for egg production (*Galal and Younis, 2006; Galal et al. 2007*), no need from application of restricted feeding programmes (*Decuyper et al., 2006; Dawkins and Layton, 2012*), higher survival rate (*Garcês et al., 2001*), better reproduction ability (*Decuyper et al., 2012*), better resistance to heat stress (*Gowe and Fairfull, 1995; Rashid et al., 2005; Islam, 2005*). These advantages resulted in using the dwarf gene in some broiler breeder maternal lines for production of standard broilers, chickens with medium growth or slow growth such as the Label rouge type. Data reported by *EFSA (2010)* showed that in Europe, female dwarf broiler breeders are 18-20 %, whereas in France, constitute the major part of broiler hens.

The production of broilers from mini maternal forms is based on the acknowledged fact that when the sire is from the standard DW/DW genotype, the progeny is of normal body size.

The most commonly used breeding scheme for production of dwarf maternal form is through crossing homozygous recessive dwarf male *dw/dw* with a standard female (*DW/_*). A second alternative is to use heterozygous *DW/dw* cocks, but this is related to extra costs and time.

The economically relevant differences between normal and mini-hens vary depending on the production type and genetic diversity of the population, in which the *dw* gene is introduced. The introduction of the gene in light populations leads to delayed sexual maturity and lower egg production (*Merat, 1990; Horst and Becker, 1991; Merat and Bordas, 1991; Merat et al., 1994; Garcês et al., 2001; Missohou et al., 2003*), whereas in broiler hens, this negative effect is less pronounced or absent (*Marks, 1981; Kousiakis et al., 1985*). *Anonymous (2003)* reports about relatively higher egg production and higher number of eggs fit for incubation in dwarf breeders consequently to lower number of double-yolk eggs, eggs with soft or thin shell, irregular shape or other defect. After introduction of *dw* gene in broiler lines selected for high egg production, *Leenstra et al. (1986)* established that hens with normal genotype laid about 17 % of all ovulated follicles in defective eggs while for dwarf hens this percentage was only 2 %. The economic analysis of results from using a mini-maternal form for production of broiler chickens showed that financial costs for rearing of the breeder flock were reduced

by 15 %, and for production of one day-old chick – by 20 % (Charpentier, 2009; Tudik *et al.*, 2011).

The objective of the present study was to evaluate the effect of sex-linked dwarf gene on exterior appearance and productive performance of hens of a colored broiler dam line used for production of slow-growing chickens.

Materials and methods

Stock, Husbandry and Traits measured

The experiment was performed in the Selection Base of the Poultry Breeding Unit, Agriculture Institute – Stara Zagora from December 2013 to June 2014. Dwarf hens and their normal sibs were used for the current study. They were obtained by mating heterozygous cocks (DW/dw) and hemizygous normal hens ($DW/_$) from line F which was developed in the Selection Base and used as maternal form for production of colored slow-growing broiler chickens. Dwarf and normal genotype birds were separated by visual appraisal on the basis of body size and shank length. At 20 weeks of age, two groups were formed - with normal ($DW/_$) and dwarf genotype ($dw/_$). Each group consisted of five replicates of 20 hens and 2 cocks, housed in floor pens. The birds were reared until 50 weeks of age under identical conditions, in the same laying house, on wooden shavings litter as required by the production system used in the Selection base, with free access to feed and water. They were fed a diet containing 16 % crude protein and 2750 kcal/kg metabolizable energy.

During the experiment the following parameters were observed:

Body weight and body measurements: Body weight was determined individually at 40 weeks of age for each genotype using a technical balance with precision of 5 g. At the same age, keel length (distance between the anterior and posterior end of the sternum) and shank length (from the top of hock joint to the foot pad) were determined with a measuring tape.

Egg production traits: The number of eggs laid and dead birds was recorded daily for each replicate. On this basis, the hen day egg production (%) was calculated for the entire experimental period. Sexual maturity was determined when 50 % egg production has been reached. Mean egg weight was monitored at 2-week intervals from the beginning of lay to the end of the trial via weighing the daily yield of all replicates of both genotypes. Egg mass was calculated on the basis of hen day egg production and mean egg weight.

Feed intake was recorded at the end of each week. Feed conversion ratio (FCR) was expressed as kg of feed consumed per kg of egg produced.

Egg quality traits were evaluated at 40 weeks of age. For this purpose, 30 eggs from each group (six eggs per replicate), laid within a day were examined.

The weights of the whole egg, albumen, yolk and eggshell (together with membranes) were determined with precision of 0.01 g. Eggshell weight, albumen weight and yolk weight were expressed as a percentage of the egg weight. The length and breadth of eggs were measured using a digital caliper with precision of 0.01 mm. Egg volume and egg surface area were calculated on the basis of exterior egg dimensions (Narushin, 2005). Albumen and yolk heights were measured with Ames micrometer (precision 0.01 mm), albumen and yolk diameters – with digital caliper (precision 0.01 mm), eggshell thickness together with the inner membrane – with micrometer (precision 0.001 mm) at three different points (top, middle, and bottom) and presented as average of the three measurements. Egg albumen quality was assessed through albumen index and Haugh units; yolk quality – via the yolk index.

For determination of the reproduction potential of dwarf hens and their full-sib normal sisters, 400 eggs from each group collected over 7 consecutive days at the end of the experiment, were set for incubation. The fertility rate, hatchability of eggs set and hatchability of fertile eggs were calculated on the basis of obtained results.

Statistical Analyses

The analysis of data was performed with Statistica software (Stat Soft), using one-way analysis of variance and the following linear model:

$$Y_{ij} = \mu + g_i + e_{ij}, \text{ where}$$

Y_{ij} – j^{th} observation of the respective trait

μ - grand mean of the trait

g_i – fixed effect of the i^{th} genotype ($i=1,2$)

e_{ij} – random error

Results and discussion

The data presented in Table 1 show that under the influence of the *dw* gene, the body weight of mini-forms was reduced by 29.15% as compared to those of their full-sib normal sisters. The dwarf gene does not reduce uniformly the growth of the different body parts. The length of metatarsus was most substantially reduced (20.17%), a reason for the so-called shortlegness of dwarf birds. Hussain *et al.* (1982) have established that the shank length in mini hens was 7-8 cm, while in those of normal size: 9.5-10.5 cm. This finding is in agreement with our results. The keel length changed less markedly (-7%) and the differences between both genotypes were proportional to the change in body weight. The reduction of body weight, shank length and keel length in the present study was confirmed by Missohou *et al.* (2003), Chen *et al.* (2004) Younis and Galal (2006).

Hens with normal genotype attained sexual maturity 7 days earlier than birds carrying the dwarfing gene (Table 2, $p < 0.001$). This difference is limited by the reduction of body weight and feed intake (*Renden and Marple, 1986*), as well as by the body fat deposition level (*Zelenka et al., 1986*) and the related functional ovarian activity in maturing mini-hens. In their experiments with different polygenic combinations, *Khan and Verma (1983)* demonstrated ages of sexual maturity of 152 and 145 days for (dw) and (DW) broiler breeders, respectively. According to *Sharifi et al. (2010)*, the sex-linked recessive gene prolonged this age by about two weeks. Opposite data are communicated by *Marks (1981)* and *Sadjadi et al. (1983)*, which stated the lack of statistically significant difference between both genotypes. The advantage of mini-breeders vs normal breeders was also reported by *Anonymous (2003)*, accounting for a difference of 14 days.

Table 1. Body weight, shank length and keel length of hens as affected by dwarf (dw) gene.

Trait ¹	Genotype		Reduction, %	Significance ²
	DW/_	dw/_		
BW 40-wk, g	2701.82±69.90	1914.29±68.33	-29.15	***
ShL(cm)	10.36±0.12	8.27±0.10	-20.17	***
KL (cm)	12.86±0.09	11.96±0.15	-7.00	***

¹BW= body weight, ShL= shank length, KL= keel length;

² Significant difference between normal and dwarf hens: *** $p < 0.001$

There were no statistically significant differences between both genotypes with respect to the egg production rate and egg mass, which ranged between 57.23 - 61.63 % and 32.08 - 36.57 g, respectively. The lack of differences in egg production between normal and dwarf sibs is supported by *Marks (1981)*. Presented by *Islam (2005)* reports showed that laying rate decreased by about or more than 10% in strains with low body weight, while this parameter was unchanged or improved in heavy-type birds.

The data for egg weight (Table 2) showed that the presence of dw gene in the genotype of hens reduced considerably the weight of laid eggs by 3.4 g (-5.72%). This could be explained by the existing high positive correlation between body weight and egg weight, as well as the smaller reproductive tract of dwarf hens (*Katongole et al., 1990*). The effect of sex-linked dwarf gene on this trait is confirmed by *Missohou et al. (2003)*, *Galal et al. (2007)*, whose results provide evidence for lower egg weight by -9% and -4.8%, respectively. According to *Sadjadi et al. (1983)* the reduction of egg weight in birds from the egg-laying type was greater and ranged between -9.6 to -12.6%.

Feed intake and conversion are economically important traits. Under the conditions of this experiment, the presence of the dw gene, determining the dwarfism expression in birds, reduced substantially feed consumption by 23.38 %

($p < 0.01$) as compared to their normal sibs (Table 2). Mini forms converted feed energy by 12.7% more efficiently ($p < 0.05$) for egg mass synthesis. The effect of the gene with respect to these parameters is explained not only by the lower body weight, but also by reduced biochemical activity of the basic metabolism, the higher ratio between anabolic and catabolic processes compared to those in normal sibs, resulting in lower energy demands and more efficient dietary energy utilisation by mini-hens (*Kiselev and Nadalyak, 1985*). Our results are comparable to those reported by *Missohou et al. (2003)*, *Galal and Younis (2006)* and *Galal et al. (2007)*, demonstrating lower feed intake and better feed utilisation under the influence of the dwarf gene.

Table 2. Egg production, feed intake and feed conversion ratio of hens as affected by dwarf gene

Trait	Genotype		Reduction, %	Significance ¹
	DW/	dw/		
Age (d) at 50 % lay	180.80±1.07	187.80±0.66	+3.87	***
Hen day egg production (%)	61.63±3.48	57.23±4.13	-7.14	NS
Mean egg weight (g)	59.25±0.13	55.86±0.39	-5.72	***
Egg mass (g/d/hen)	36.57±2.09	32.08±2.42	-12.28	NS
Feed intake (g/d/hen)	163.40±5.35	125.20±8.97	-23.38	**
Feed conversion ratio (kg/kg egg mass)	4.49±0.12	3.92±0.19	-12.69	*
Livability (%)	99.05±1.00	95.00±5.00	-4.09	NS

¹ Significant difference between normal and dwarf hens: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS=Not significant

The presence of the *dw* gene in hens' genotype did not have a statistically significant effect on the livability within the duration of the present experiment, as the differences were insignificant. A similar conclusion was drawn by *Ipek et al. (1999)*. In the view of *Garcês et al. (2001)* birds carrying the *dw* gene exhibited higher livability percentages than their normal sibs, and *Kousiakis et al. (1985)* reported a high mortality in dwarf hens during the production period.

The data from Table 3 present the results related to reproductive performance in both genotypes. It showed a statistically significant difference ($p < 0.05$) only in hatchability from eggs set, higher in mini-forms (18.03%), as confirmed by the studies of *Ipek et al. (1999)*. The fertility and hatchability of eggs set percentages varied within similar ranges, 86-88 % and 88-91%, respectively. The results of *Kousiakis et al. (1985)* showed a superiority of hens with normal genotype with regard to the fertility and hatchability of eggs set percentages although the hatchability of fertile eggs between the groups was not generally different. On the other hand, *Islam (2004)* and *Tahir et al. (2011)* observed a positive effect of the *dw* gene on fertility and hatchability of eggs. Investigating the fertility rate between dwarf hens and their normal sibs, *Marks (1983)* established identical values of both genotypes. The hatchability of fertile eggs according to the author was by about 10% higher in dwarf hens compared to those of normal sized.

Table 3. Hatching results as affected by dwarf gene

Trait	Genotype		Reduction, %	Significance ¹
	DW/_	dw/_		
Fertility (%)	85.81±0.81	88.00±0.47	+2.55	NS
Hatchability (%)				
- from set eggs	67.68±1.20	79.88±1.42	+18.03	*
- from fertile eggs	87.91±2.89	90.79±2.09	+3.28	NS

¹ Significant difference between normal and dwarf hens: * p<0.05; NS=Not significant,

The data about egg quality traits presented in Table 4 showed that the reduction of egg weight in mini-forms was accompanied by a proportional change in external egg quality characteristics – egg length and breadth, egg volume and egg surface area by -4.10% (p<0.001), -2.27% (p<0.01), -7.97% (p<0.001) and -5.53% (p<0.001) respectively. A marked and statistically significant tendency towards lower egg albumen and yolk weights – by -7.38 and -4.84 % respectively (p<0.05), was present. This was confirmed by *Garcês and Casey (2003)*. The presence of *dw* gene had a substantial effect on eggshell weight and thickness, as well as on egg quality parameters related to yolk (yolk index) and albumen (Haugh units, albumen height and albumen index). Published data about the influence of the *dw* gene on internal egg quality traits are controversial. Comparing the eggs of hens of a normal genotype with eggs produced by dwarf dams *Islam (2005)* did not found differences in albumen height, similar to our results. *Garcês and Casey (2003)* reported about lower albumen height in eggs of dwarf hens, whereas *Galal et al. (2007)* affirmed that the egg albumen quality of mini-forms was higher.

Table 4. Egg quality traits as affected by dwarf gene

Trait ¹	Genotype		Reduction, %	Significance ²
	DW/_	dw/_		
External egg quality				
EW (g)	59.75±0.80	54.84±0.89	-8.22	***
B (mm)	43.22±0.24	42.24±0.25	-2.27	**
L (mm)	56.82±0.46	54.49±0.34	-4.10	***
V (cm ³)	56.06±0.72	51.59±0.81	-7.97	***
S (cm ²)	70.68±0.61	66.77±0.70	-5.53	***
EST (mm)	0.304±0.006	0.311±0.005	+2.30	NS
ESW (g)	6.40±0.14	6.31±0.08	-1.41	NS
ESR (%)	10.72±0.20	11.55±0.15	+7.74	**
Internal egg quality				
AH (mm)	6.40±0.23	5.87±0.25	-8.28	NS
AW (g)	35.35±0.77	32.74±0.76	-7.38	*
AR (%)	58.94±0.73	59.44±0.57	+0.85	NS
AI	0.079±0.004	0.076±0.004	-3.79	NS
HU	78.70±1.65	76.52±1.72	-2.77	NS
YI	0.43±0.01	0.42±0.01	-2.33	NS
YW (g)	18.18±0.24	17.30±0.31	-4.84	*
YR (%)	30.47±0.48	31.55±0.51	+3.54	NS

¹EW= egg weight; B=egg breadth; L=egg length; V=egg volume; S=egg surface area; EST= egg shell thickness; ESW= egg shell weight; ESR= egg shell ratio; AH= albumen height; AW= albumen weight; AR= albumen ratio; AI=albumen index; HU= Haugh unit; YI= yolk index; YW= yolk weight; YR= yolk ratio

² Significant difference between normal and dwarf hens: * p<0.05; ** p<0.01; *** p<0.001; NS= Not significant

The analysis of data about egg part proportions in normal and mini-hens, showed similar values for albumen (58.94-59.44 %) and yolk (30.47-31.55 %) proportions, whereas eggshell percentage changed considerably under the influence of the dwarf gene by 7.74 % (p<0.01). *Hussain et al. (1982)* and *Islam (2005)* also outlined on influence of the dwarf gene on albumen and yolk percentages, while *Galal et al. (2007)* found out that the presence of the *dw* gene increased considerably the proportion of the yolk and decreased that of albumen. According to the data of authors, the *dw* gene resulted in higher eggshell percentage similar to our findings, whereas *Kousiakis et al. (1985)* established no statistically significant difference between hens with or without the *dw* gene with respect to eggshell quality.

Conclusion

The positive effect of the sex-linked dwarf gene on economically important traits – feed intake and feed conversion, hatchability of eggs set, is a prerequisite for the development of more efficient broiler breeder hens for production of slow-growing chickens.

Uticaj polno vezanog gena za patuljavnost na eksterijerne osobine, produktivnost i osobine jaja ženske linije brojlera obojenog perja

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Rezime

Uticaj gena za patuljavnost ispitivan je poređenjem patuljaste kokoši sa normalnim sestrama dobijenim parenjem heterozigotnih muških grla (DW/dw) sa normalnim ženskim (DW / _) iz linije F (koristi se kao majčinska forma za proizvodnju sporo rastućih obojenih pilića), a u vezi sa sledećim osobinama: telesna masa, dužina golenjače i kobilice u uzrastu od 40 nedelja, uzrast polne zrelosti (u 50% proizvodnje), proizvodnja jaja, težina jajeta, konzumacija hrane,

korišćenje hrane, vitalnost, plodnost, procenat izleganja i osobine kvaliteta jaja. Rezultati su pokazali da dw gen izaziva statistički značajno smanjenje telesne težine od 29,15%, dužine golenjače od 20,17%, dužine kobilice za 7% i težine jajeta za 5,72% ($p < 0,001$). Ženke sa normalnim genotipom dostigle su polnu zrelost 7 dana ranije ($p < 0,001$), ali ipak, stopa izleganja bila je slična onoj kod mini formi. Nije bilo značajne razlike između obe genetske grupe u odnosu na procenat vitalnosti u odnosu na proizvodni ciklus. Patuljaste kokoši troše 23,38% manje hrane ($p < 0,01$) u odnosu na kokoške normalne veličine i pretvaraju hranljive materije efikasnije za 12,69% ($p < 0,05$). Prisustvo dw gena u kokošijem genotipu uticao je na povećanje procenta ljuske jajeta, smanjenje težine žumanca i belanca i nije imao nikakvog uticaja na njihov kvalitet.

Pozitivan efekat polno vezanog gena za patuljavost na ekonomski važne osobine - konzumaciju hrane i konverziju hrane, procenat izleganja hatchability postavljenih jaja, je preduslov za razvoj efikasnijih brojlerskih roditelja za proizvodnju pilića sporijeg rasta.

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CORRELATION BETWEEN SOME INDICATORS OF BROILER CARCASS FAT

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Original scientific paper

Abstract: Fifty-two (26 males and 26 females) week old broilers were examined for carcass fat characteristic. The phenotypic correlation coefficients between abdominal fat and other traits were: 0.65 – left external sartorial; 0.51 – right external sartorial fat; 0.65 – total external sartorial fat; 0.51 – left internal sartorial fat; 0.46 total internal sartorial fat; 0.66 – total left sartorial fat; 0.49 – total right sartorial fat; 0.63 – total sartorial fat; 0.37 – back skin pinch thickness (under wings).

Key words: broiler, carcass fat, correlation

Introduction

The increased fat content in carcass of broilers, as a result of long-term selection for rapid weight increase, represented, and still represents one of the biggest problems of this type in poultry production (*Lin, 1981; Soller and Eiten, 1984; Leclerq and Guy, 1991; Leenstra, 1986*). Increased fat content is not favorable both for producers (chickens have poor utilization of food) and for consumers (negative attitude to animal fats in the human diet).

Numerous tests were performed in order to establish criteria for assessing the amount of carcass fat tissue. Most of them paid special attention to measuring the presence of abdominal fat (*Pym and Thompson, 1980; Miroch and Becker, 1984; Sonaiya, 1985; Milošević et al., 1987*). In contrast, there are few studies that use some other indirect methods as an indicator of carcass fat (*Miroch et al., 1981; Burgener et al., 1981; Antonijević et al., 1988*). *Zerehdaran et al. (2004)* have found high genetic correlation between abdominal fat amount/mass and skin mass (0.54), whereas the genetic correlation between abdominal and percentage of fat within the muscle (intramuscular fat) is very low, almost zero (0.02). A positive genetic correlation was determined between weight gain (7 weeks) and indicators

of fat of broiler chickens, which was high for the percentage of intramuscular fat (0.87), medium for the percentage of skin (0.17) and share of abdominal fat (0.13).

Kleczek et al. (2010) indicate that the determination of the best indicators of fat tissue and the share of meat in carcass of broiler chickens have been the subject of numerous studies. The most accurate data are obtained by dissection of certain body parts. In this study, the authors have used mass of different muscles of the carcass as indicators in the assessment.

With this in mind we felt justified to investigate the correlation between some indicators of broiler carcass fat and the amount of abdominal fat.

Materials and Methods

In comparative testing of broilers, which lasted 6 weeks (42 days) and carried out with three hybrids, and between which no significant differences in the amount of abdominal fat were established, a random sample of carcasses of 26 males and 26 females, i.e. 52 chickens of both sexes was taken. On each carcass characteristics were measured as indicators of the presence of fat tissue. The following properties were measured on each carcass:

- Abdominal fat, g,
- External sartorial fat, g (mast extracted from the surface of *m. sartorius*),
- Internal sartorial fat, g (fat extracted below the caudal edge of *m. sartorius*),
- Sartorial fat, total, g (calculated based on previous two measures),
- Back skin pinch, mm (measured using the caliper over the first dorsal vertebra with accuracy 0.1 mm),
- Wingweb thickness, mm (measured using the slide rule on the inner side of the elbow joint with accuracy 0.1 mm),
- Caudal skin pinch (under wing) thickness, mm (measured using the slide rule on the inside in front of the shoulder joint with accuracy 0.1 mm).

All results were processed using conventional variation-statistical methods, while phenotypic correlation coefficient was calculated between the abdominal fat and other studied parameters.

Results and Discussion

The results of measurements of selected indicators of broiler carcass fat are shown in Table 1.

It can be seen from the presented data that the highest amount of fat tissue is deposited in the abdominal cavity, which was in average of 18.25 g, and in a rather wide range of 5.86 to 37.05 g. Other indicators of carcass fat expressed through internal and external sartorial fat on the left and right limb (thigh) had significantly lower average values. External sartorial fat, both from the right side (1.46 g) and the left (1.48 g) and total (2.92 g) was significantly higher than the internal (1.09 g; 1.10 g; 2.20 g). Between sartorial fat from the left and right sides of the body there were no significant differences. Thus the total sartorial fat from the left thigh was 2.58 g, and 2.53 g from the right.

The average value of the back skin pinch the back was 3.40 mm, wingweb thickness of 1.72 mm, a caudal skin pinch thickness under wings of 0.58 mm.

All investigated parameters showed a high variability (CV 30 - 40%).

Table 1. Indicators of broilers carcass fat

Trait	x	Sx	CV
Abdominal fat, g	18.25	1.00	39.34
Left external sartorial fat, g	1.48	0.01	37.84
Right external sartorial fat, g	1.46	0.07	37.00
Total external sartorial fat, g	2.92	0.15	36.30
Left internal sartorial fat, g	1.10	0.04	29.09
Right internal sartorial fat, g	1.09	0.05	30.27
Total internal sartorial fat, g	2.20	0.09	28.64
Total left sartorial fat, g	2.58	0.11	31.01
Total right sartorial fat, g	2.53	0.11	30.43
Total sartorial fat, g	5.12	0.21	29.87
Back skin pinch thickness, mm	3.40	0.14	29.52
Wingweb thickness, mm	1.72	0.07	28.23
Caudal skin pinch (under wing) thickness, mm	0.58	0.03	41.03

Coefficients of phenotypic correlations between selected indicators of carcass fat and abdominal fat are shown in Table 2.

According to Roemer - Orphal classification for the strength of correlation between characteristics, established correlation coefficients (Table 2) showed mainly medium and strong link between abdominal fat and sartorial fat. This is in line with the results of *Burgener et al. (1981)*, who point out that, given that the external sartorial fat can be separated on live chickens through biopsy, this analysis provides an opportunity for assessing the amount of abdominal fat without slaughtering chickens. On the other hand, based on such analysis, it may be possible at an earlier age to estimate the amount of abdominal fat that will be deposited at a later age.

Table 2. Correlations between abdominal fat and selected indicators of broiler carcass fat

Trait	xy
Abdominal fat, g	1.00
Left external sartorial fat, g	0.65
Right external sartorial fat, g	0.51
Total external sartorial fat, g	0.65
Left internal sartorial fat, g	0.51
Right internal sartorial fat, g	0.34
Total internal sartorial fat, g	0.46
Total left sartorial fat, g	0.66
Total right sartorial fat, g	0.49
Total sartorial fat, g	0.63
Back skin pinch thickness, mm	0.37
Wingweb thickness, mm	0.11
Caudal skin pinch (under wing) thickness, mm	-0.01

It is particularly interesting, as our results show, that in this respect it may be sufficiently reliable to extract external sartorial fat from only one side of the body, in this case the left side.

The relatively good correlation (0.37) between the back skin pinch thickness and amount of abdominal fat is in accordance with the results of *Petersen and Horst (1983)*. In contrast, the correlation between abdominal fat and wing skin pinch thickness and skin pinch thickness under the wings was not established, as confirmed by the results of *Miroch et al. (1981)*. The fact that the problem of broiler carcass fat still has not been resolved, is proven by research of *Bosho et al. (2013)*. According to this group of researchers, until today, numerous studies have been carried out in order to determine the most accurate indicator of the share of meat and fat in the carcass of broilers. They have made a modification of the method of removing the skin with subcutaneous fat on the carcass. They found that the weight of the removed skin, with the subcutaneous fat from the whole carcass (without the parts of the wings and legs) are highly significantly correlated with the total subcutaneous and intramuscular fat of broiler chickens. Carcass weight without (removed) skin is a good indicator of total meat in the carcass.

Conclusion

Based on the results obtained the following can be concluded:

- Between the amount of abdominal and sartorial fat and thickness of the skin on the back there is a medium and strong correlation.

- Between the amount of abdominal fat and thickness of the skin on the wings and under the wings there is no correlation.
- All indicators of carcass fat of broiler chickens belong to the group with high variability.

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Korelacije između nekih pokazatelja masnoće trupa brojlera

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Rezime

Ispitivanja osobina masnoće trupa obavljena su na 52 pileta (26 petlića i 26 kokica) u uzrastu 42 dana. Koeficijent fenotipske korelacije između abdominalne masti i ostalih osobina bili su: 0,65 leva spoljašnja sartorijalna mast; 0,51 – desna spoljašnja sartorijalna mast; 0,65 – ukupna spoljašnja sartorijalna mast; 0,51 – leva unutrašnja sartorijalna mast; 0,34 - desna unutrašnja sartorijalna mast; 0,46 - ukupna unutrašnja sartorijalna mast; 0,66 - ukupna leva sartorijalna mast; 0,49 – ukupna desna sartorijalna mast; 0,63 – ukupna sartorijalna mast; 0,37 – leđni kožni nabor; 0,11 – krilni nabor i 0,01 - kožni nabor ispod krila.

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SEASONAL VARIATION IN EGG PRODUCTION AND MORTALITY OF MUSCOVY DUCKS (*CAIRINA MOSCHATA*)

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Original scientific paper

Abstract: Seasonal variation is one of the principal non-genetic factors influencing performance of poultry in tropical environment. This study was conducted to investigate influence of seasonal variation on egg production and incidence of mortality in intensively-reared non-descript Muscovy ducks in Nigeria. Egg production and incidence of mortality in sixty two (62) female Muscovy ducks was studied in a 12-month trial divided into two major seasons: wet (April – September) and dry (October – March) and four sub-seasons: early rainy season (April – June), late rainy season (July – September), early dry season (October – December) and late dry season (January – March). Student's t-test and Completely Randomized Design was used to analyse seasonal and sub-seasonal effect on performance, respectively. Season and sub-season significantly ($P < 0.05$) affected egg production; higher egg production was recorded in wet season compared with dry season (16.18% vs. 1.32%). Among sub-seasons, highest egg production was recorded in late rainy season (20.92%) while the least (0.00%) was obtained in late dry season. Conversely, there was no significant ($P > 0.05$) effect of season and sub-season on mortality. It is evident that seasonal variation had no effect on incidence of mortality but significantly affected egg production of Muscovy duck and the adverse effect is more pronounced in dry season most especially in late dry season.

Key words: Ducks, poultry, late dry season, non-genetic factors, tropical environ

Introduction

Poultry is now by far the largest livestock species world-wide (FAO, 2000), accounting for more than 30 % of all animal protein consumption (Permin and Pedersen, 2000). In Nigeria, poultry are the most numerous class of animal.

Members of this class include chicken, turkey, duck, guinea fowl and pigeon, however; preponderance of them is chicken (*Nwanta et al., 2006; Dafwang et al., 2010*). It is worthy of note that Nigerian poultry sector is dominated by local/indigenous breeds. These local avian species are bred under traditional breeding systems and constitute a fast means of bridging the protein deficiency gap in most developing countries (*Jibir and Usman, 2003*).

Muscovy duck is commonly referred to as local duck in Nigeria. It is an integral part of local poultry sector in Nigeria and are concentrated mostly in rural areas in the hands of small-holder farmers (*Oguntunji and Ayorinde, 2014*). They are estimated to be approximately 11million and were reported to be distributed all over the agro-ecological zones particularly in village settings (*FLDPCS, 1992*). Muscovy duck constitutes about 10% of local poultry sector in Nigeria (*Oluyemi and Ologhobo, 1997*) representing 74% of ducks reared in Nigeria (*Adesope and Nodu, 2002*).

This waterfowl is also one of the least exploited and underutilized locally adapted poultry species in Nigeria in spite of its innate potential for meat and egg production (*Oguntunji, 2013*) and adaptability to different climatic conditions. Dwindling population of Muscovy duck in the recent years attests further to its utter neglect.

Commercial egg production in Nigeria and other developing nations in warm and hot tropical environments is dominated by exotic strains that were developed and evaluated in temperate regions under optimal rearing conditions (*Oguntunji and Salako, 2012*). Over reliance on exotic commercial layers for internal egg production has not only led to the neglect and relegation of local poultry species to the background but has also served as a clog in the wheel of accelerated all-encompassing improvement of local poultry. Exploration of potentials of alternative poultry species such as Muscovy ducks for egg production is long overdue and will go a long way in boosting internal egg production and reducing foreign exchange on exotic chickens.

Seasonal variation is one of the major non-genetic factors affecting poultry production most especially in tropical environment. There are two major seasons in Nigeria, wet and dry seasons; each of these seasons is identified principally by change in ambient temperature, relative humidity and amount of rainfall (*Oguntunji et al., 2008*). Season has been identified as one of the most important factor adversely affecting poultry production in the tropics, not only in those reared extensively, but also in those intensively-reared without artificial regulation of microclimatic conditions (*Mahmoud et al., 1996; Ayo et al., 2007; Obidi et al., 2008*). The principal meteorological element commonly implicated with the adverse effect of seasonal variation on performance of poultry is ambient temperature, most especially in tropical and sub-tropical regions of the world.

Synthesis of literatures demonstrated that high environmental temperature commonly called heat stress adversely affected egg production performance of

commercial layers (Oguntunji and Salako, 2012; Yakubu et al., 2007; Mashaly et al., 2004; Rozenboim et al., 2007; Shitu et al., 2014), fertility (McDaniel et al., 1995; 1996; Obidi et al., 2008) and hatchability (Lourens et al., 2005) of breeders, immunoresponse of chickens (Mashaly et al., 2004; Tirawattanawanich et al., 2011) and increases incidence of mortality in chickens (Mashaly et al., 2004; Yakubu et al., 2007; Oguntunji and Salako, 2012; Shittu et al., 2014).

Literatures abound on effects of season on egg production performance and incidence of mortality in chickens; however, related studies on influence of seasonal variation on egg production and mortality of Muscovy duck in tropical environment is scarce. To the best knowledge of the authors, there is dearth of empirical studies on seasonal effect on egg production and mortality of local Muscovy ducks reared intensively or extensively. In view of the foregoing, the present study was conducted to investigate influence of seasonal variation on egg production and incidence of mortality in intensively-reared unselected Muscovy ducks in a Derived Savanna environment in Nigeria.

Materials and Methods

Study area

This study was conducted at the duck unit of the Teaching and Research farm of Bowen University, Iwo, Osun State, Nigeria. The study area is located in Derived Savanna Agro-Ecological Zone characterized with double maxima of annual rainfall.

Experimental animals

Sixty two (62) sexually matured pullets of local Muscovy ducks were purchased at Shasha poultry market, Ibadan, Oyo State, Nigeria. These experimental animals originated from the northwest region of the country. They were non-descript, unselected and were reared primarily on extensive system. Since the age of the birds could not be ascertained, efforts were made to buy only those that have not commenced laying. This was achieved through visual examination of caruncles and vents.

Management of the experimental animals

The birds were reared in deep litter and were also provided with fresh drinking water and wallowing trough for their water-related activities like preening, bathing, e.t.c. They were also fed *ad libitum* with commercial layer feed throughout the experimental period.

Data collection and analyses

Though the birds were sexually mature and were purchased in October 2011, they did not commence laying until April 2012 (six months after) when rain commenced. Therefore, data on egg production and mortality were taken between April 2012 and March 2013. Besides, data on meteorological elements {Ambient

temperature (AT) and Relative humidity (RH)} were collected from the meteorological station of Folawiyo Farms Limited, Iloro, Oyo State, Nigeria. The farm is a reputable commercial poultry farm and is about 45km to Iwo, and is also located in the same agro-ecological zone (Derived Savanna) with Iwo. Data collected on production performance and meteorological indices were categorized into two seasons: wet (April – September) and dry (October – March) and four sub-seasons: early rainy season ERS (April–June), late rainy season LRS (July–September), early dry season EDS (October–December) and late dry season LDS (January–March). Student's-t test at 5% probability level was used for testing significant differences between seasonal performance while data on sub-seasons were analysed with analysis of variance procedure:

$$Y_{ij} = \mu + S_i + e_{ij}$$

Y_{ij} = individual observation;

μ = fixed overall mean;

S_i = effect of sub-season (ERS, LRS, EDS, LDS);

e_{ijk} = experimental error, assumed to be independently and identically normally distributed, with zero mean and constant variance, i.e. i and $(0, \sigma^2)$.

Significant differences between sub-seasons were separated with Duncan Multiple Range Test at 5% probability level.

Besides, regression analysis model was used to investigate relationship existing between egg production of Muscovy ducks and meteorological indices (Ambient temperature and Relative humidity).

The regression model used was of the form:

$$Y = a + b_1X_1 + b_2X_2 + e$$

Where,

Y = Dependent variable (egg production)

a = Constant/intercept

b_1 = Regression coefficient of temperature

b_2 = Regression coefficient of relative humidity

X_1 = Ambient temperature

X_2 = Relative humidity

e = Error term

All statistical analyses were carried out with *SPSS (2001)*.

Results and Discussion

Egg production

Average monthly and seasonal records of egg production, incidence of mortality, ambient temperature and relative humidity are presented in Table 1. There was significant ($P < 0.05$) higher egg production in wet season compared with dry season (16.18% vs. 1.32%).

Analysis of sub-season effect (Figure 1) on egg production revealed significant ($P < 0.05$) differences. Highest (31.17%) and least (0.00%) egg productions were recorded in LRS and LDS, respectively.

Mortality

Zero mortality was recorded throughout the experimental period and there was no significant ($P > 0.05$) effect of season (Table 1) and sub-season (Figure 1) on incidence of mortality.

Regression analysis

Regression analysis of meteorological indices (AT and RH) on egg production revealed significant ($P < 0.05$) relationship. Besides, R^2 representing coefficient of determination was 0.854 while the generated regression equation using standardize coefficients of the two climatic factors was:

$$Y = 83.06 - 0.70X_1 + 0.248X_2 + e$$

Related researches on seasonal influence on egg production of non-descript intensively-reared Muscovy ducks are very scarce to validate result obtained in this present study.

Table 1. Seasonal variation in performance of Muscovy ducks and prevailing meteorological indices

Season	Month	Egg production (%)	Mortality (%)	Ambient temperature (°C)	Relative humidity (%)
Wet					
	April	4.34	0.00	34.70	50.10
	May	10.25	0.00	30.60	58.30
	June	19.73	0.00	28.20	74.30
	July	29.84	0.00	28.40	72.04
	August	18.99	0.00	29.70	68.74
	September	13.92	0.00	31.30	63.84
	Mean (±SD)	16.18 ±8.80^a	0.00 ±0.00	30.48 ±2.39^a	64.55 ±9.13^a
Dry					
	October	5.15	0.00	33.50	56.60
	November	1.77	0.00	33.50	56.80
	December	1.01	0.00	34.20	41.60
	January	0.00	0.00	33.41	30.03
	February	0.00	0.00	36.51	29.90
	March	0.00	0.00	35.10	31.30
	Mean (±SD)	1.32 ±2.01^b	0.00 ±0.00	34.37 ±1.23^b	41.02 ±12.87^b

^{ab}Means along the same column with different superscripts are significantly ($P < 0.05$) different

The egg production of the studied population is higher than values reported for two ecotypes of local Muscovy ducks reared intensively in north-central Nigeria (Ogah et al., 2011). The low egg production of Muscovy ducks reported by Ogah et al. (2011) compared with the result of the present study could probably be attributed to differences in prevailing climatic conditions and management systems.

Significant higher egg production in wet season in compared to dry season agrees with the earlier reports on seasonal effect on egg production of commercial egg layers (Guobadia, 1997; Yakubu et al., 2007; Oguntunji et al., 2008). These investigators attributed poor egg production in dry season to the adverse effects of high ambient temperatures. The observed seasonal variation in egg production of the studied population is consistent with the report of Sauveur and DeCarville (1995) that Muscovy ducks are seasonal breeder and express seasonal character in egg laying which in most cases is dependent on the season and genetics.

This submission agrees with the report of Ola (2000) that Muscovy ducks have limited breeding capacity during the dry season. This submission was buttressed further that though Muscovy ducks attained sexual maturity at 27 weeks of age in dry season; oviposition was delayed for about 20 weeks later when ducks reached 47 weeks of age at the onset of the wet season (Ola, 2000). It is noteworthy that the trend in egg production of Muscovy ducks in the present study aligns with the report of Ola (2000). The population under study were purchased in October 2011 but failed to lay eggs until April 2012 following year (6 months after) when rain started. In addition, the author observed similar trend in the first set of 100 adult female Muscovy ducks bought in November 2009 and were reared in the same farm but egg production did not commence until April 2010 (5 months after) when rain started (*Personal observation, data not published*).

Highest egg production in LRS (July – September) compared with ERS (April – June) is in agreement with the report of Ogah et al. (2011) on egg production performance of two ecotypes of Muscovy ducks reared in north-central Nigeria. Besides, the trend of egg production performance in the studied production whereby highest egg production was recorded in LRS, followed by ERS, EDS but least in LDS is consistent with previous reports on seasonal effect on egg production of commercial egg layers in Derived Savanna agro-ecological zone (Oguntunji, et al., 2008; Oguntunji and Salako, 2012) in Nigeria.

Though heat stress impairs the physiological mechanisms connected with egg production in poultry; however, neither the remote nor the immediate reasons for the negligible and cessation of egg production in EDS and LDS, respectively is clearly understood in spite of the fact that they are all-year-round breeder under extensive management system in Nigeria.

It could be inferred from Figure 1 that meteorological indices under investigation influenced egg production of Muscovy ducks. Highest egg production was recorded in LRS having the least ambient temperature and highest relative

humidity. Conversely, least egg production (0.00%) was recorded in LDS, the sub-season with highest ambient temperature and lowest relative humidity. Increased relative humidity and decreased environmental temperature was accompanied with 45.32% increase in egg production between ERS and LRS. Conversely, significant increase in temperature and decreased relative humidity resulted in 87.38% drop in egg production between LRS and EDS.

Relationship between egg production and weather elements as depicted in Figure I suggest further that climatic factors (low AT and high RH) in LRS is more conducive and favourable to the optimum physiological activities connected with the egg production in Muscovy ducks. Putting into consideration the highest and lowest egg production recorded in seasons with lowest and highest ATs, respectively, It is suggestive that this waterfowl could probably perform better at lower AT while higher AT is detrimental to their egg production ability.

Furthermore, the highest ambient temperature and lowest relative humidity in LDS resulted in cessation of egg production in the studied population. Poor egg production recorded in sub-seasons (EDS and LDS) with higher environmental temperatures is consisted with the recent reports on influence of environmental temperature on egg production by *Nickolova (2004)* on Muscovy ducks and *Ma et al. (2014)* on egg-laying Fujian ducks in China.

Studies had revealed adverse relationship between high ambient temperatures, plasma reproductive hormonal levels and potency of reproductive hormones regulating egg production mechanisms in female poultry (*Oguntunji and Alabi, 2010*). *Ayo et al (2011)* corroborated this that stress due to adverse effect of HAT disturbs the pulsative gonadotrophin-releasing hormone generator frequency, which in turn compromised reproduction functions of the axis, due to heat-induced impairment in the secretion of follicle stimulating and luteinizing hormones in laying birds (*Ayo et al., 2011*). In similar vein, *Oguntunji and Alabi (2010)* attributed low egg production of heat-stressed female poultry to the fact that attempts by egg-type poultry to offset the physiological stress induced by high environmental temperature is accompanied by alteration and disruption of hormonal equilibrium of laying hens thereby resulting in inefficient and impairment of the entire mechanism involved hence; poor egg production of heat-stressed hens.

Regression analysis of climatic factors on egg production

The coefficient of determination (R^2) generated from the regression analysis is very high (0.854). This implies that synergistic effects of AT and RH were responsible for 85.40% of the observed variations in egg production of the studied population while the remaining 14.60% could be adduced to other extraneous factors such as feed intake, age of the ducks, management system among others which are not considered in the present study. Putting into consideration high R^2 of the two meteorological elements, it is instructive that they

are important environmental factors affecting egg production of Muscovy ducks in the study area.

In addition, comparison of degree of contribution cum relative importance of the two independent variables (AT and RH) to egg production through the absolute values of their beta coefficients indicated higher contribution of AT (0.700) compared to RH (0.248). It could be inferred further from their beta coefficients that AT exerts greater influence on physiological mechanisms connected with egg production of Muscovy ducks than RH.

Further investigation into the nature (direct or inverse) of relationship between climatic factors under consideration and egg production of the studied population using standardized coefficients revealed that inverse and direct relationship exists between AT and Muscovy duck egg production, and RH and Muscovy duck egg production, respectively. The trend observed in the nature (direct and inverse) of the relationships between meteorological indices and egg production corroborates Figure 1 in the present study; whereby egg production declines and increases as AT and RH increases, respectively. The reported direction of relationship indicates that AT impacted negatively on egg production and vice versa for RH.

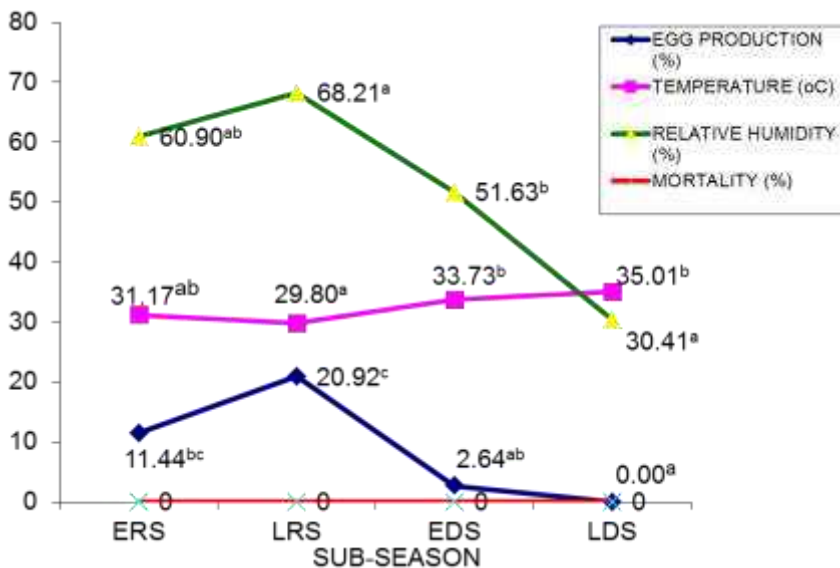


Figure 1. Sub-season effect on performance of non-descript Muscovy ducks

Mortality

Dearth of related studies on seasonal influence on incidence of mortality of non-descript Muscovy ducks and other local poultry species reared intensively did not permit head-to-head comparison with the result of the present study.

Zero mortality throughout the trial period (12 months) contradicts reports of *Ogah et al (2011)* who reported 20% and 24.14% mortality in Guinea Savanna and Rain Forest ecotypes of local Muscovy ducks, respectively, in a six-month trial in north-central Nigeria. The observed differences in viability of Muscovy ducks in the two studies might be attributed to different management practices adopted and prevailing environmental factors.

The reported zero mortality and absence of seasonal influence on mortality of local Muscovy duck in the present study also contradicts reports of related studies on commercial egg layers whereby significant differences were observed in the incidence of mortality between seasons (*Oguntunji et al., 2008; Oguntunji and Salako, 2012; Yakubu et al., 2007; Shitu et al., 2014*). The remote and immediate underlying factors responsible for zero mortality throughout the 12-month trial period are not clearly understood. A possible reason for the absence of mortality throughout the study period could be attributed to the improved immunity enhanced by routine medication.

Absence of seasonal and sub-seasonal influence on mortality in spite of significant differences in ambient temperature, relative humidity and egg production suggests that physiological mechanisms responsible for egg production and livability/survival ability of Muscovy duck are different. It implies further higher sensitivity of physiological activities connected with egg production to adverse meteorological elements than livability trait in Muscovy duck.

Conclusion

It is evident that seasonal variation affects egg production of Muscovy ducks conversely; season had no influence on incidence of mortality.

It could be inferred further that AT is an important climatic factor adversely affecting egg production of Muscovy ducks in the study area. Adoption of improved management systems to mitigate adverse effects of heat stress most especially in dry season months would enhance optimal performance of laying ducks and all-year-round production of eggs of Muscovy ducks reared intensively.

Further investigation of seasonal influence on reproductive hormones of this waterfowl is recommended to give insight to relationship between the studied meteorological indices and reproductive hormones connected with egg production.

Sezonsko variranje u proizvodnja jaja i smrtnost mošusne patke (*Cairina moschata*)

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Rezime

Sezonsko variranje je jedan od glavnih ne-genetskih faktora koji utiče na performanse živine u tropskom okruženju. Ova studija je sprovedena kako bi se ispitalo uticaj sezonskih varijacija na proizvodnju jaja i učestalost smrtnosti mošusnih pataka u intenzivnom odgoju u Nigeriji. Ispitivana je proizvodnja jaja i učestalost smrtnosti u šezdeset dve (62) mošusne patke u periodu od 12 meseci koliko je trajao ogled - podeljen u dve glavne sezone: sa padavinama (april - septembar) i sušni period (oktobar - mart) i četiri pod-godišnja doba: rano kišna sezona (april - jun), kasno kišna sezona (jul - septembar), rano sušna sezona (oktobar - decembar) i kasno sušna sezona (januar - mart). Student t-testa i potpuno slučajan dizajn su korišćeni za analizu uticaja sezone i pod-godišnjih doba na performanse, respektivno. Sezona i pod-godišnja doba su značajno ($P < 0,05$) uticali na proizvodnja jaja; veća proizvodnja jaja je zabeležena u vlažnoj sezoni u poređenju sa sušnom sezonom (16,18% prema 1,32%). Među pod-godišnjim dobima, najviša proizvodnja jaja je zabeležena krajem kišne sezone (20,92%), dok je najmanja (0,00%) bila zabeležena krajem suve sezone. Obrnuto, nije bilo značajnih ($P > 0,05$) uticaja sezone i pod-godišnjih doba na mortalitet. Evidentno je da sezonsko variranje nije imalo efekta na učestalost smrtnosti, ali značajno je uticala na proizvodnju jaja mošusne patke i da je neželjeno dejstvo izraženije u suvoj sezoni najviše u kasno sušnom periodu.

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QUANTITATIVE-GENETIC ANALYSIS OF INTENSITY GROWTH OF GILTS FERTILE BREED AND THEIR HYBRIDS IN THE NUCLEUS FARM

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Original scientific paper

Abstract: The paper analyzes the 2760 gilts four different genotypes, two of which are pure bred Landrace (429 gilts) and Yorkshire (421 gilts) and two hybrid $F_{1(Y \times L)}$ (999 gilts) and $F_{1(L \times Y)}$ (911 gilts), tested in the period from 2010 to 2011. Analyzed by the following traits of intensity growth: weight at weaning (WW), daily gain at suckling (DGS), weight in rearing (WR), daily gain at rearing (DGR), weight in test (WT), daily gain on test (DGT), weight of gilts (WG) and life gain (LG). Due to the manifestation of heterosis effect, hybrid gilts in rearing made any higher body weight of about 3 kg, while the age of 160 days on average had a higher body weight by 7.0 kg compared to the pure breed gilts, which resulted in higher daily gain in different phases of rearing. Degree of heritability for analysis traits of intensity growth is of medium to high. Heritability (h^2) for daily gains were larger (0.640 for DGS, 0.858 for DGR and 0.859 for DGT) in relation to the heritability for achieved body weight (0.584 for WW, 0.558 for WR and 0.816 for WT) in different phases of rearing. Between the most observed traits were found positive genetic and phenotypic correlations. The negative correlation found between WR, DGR and WT, DGT ($r_g = -0.055$ to -0.108 ; $r_p = -0.010$ to -0.033), between WW, DGS and DGR ($r_g = -0.301$ respectively -0.466 ; $r_p = -0.234$ respectively -0.271).

Key words: intensity growth, gilts, heritability, genetic and phenotypic correlation

Introduction

Modern breeding animals involve genetic improvement of animals by applying the basic principles of quantitative genetics. In order to achieve this genetic improvement, it is necessary to properly select the superior parents of future generations. For all this it is necessary a good knowledge of genetic parameters heritability, correlation, covariance and variance (*Thompson et al., 2005*). Knowledge of the genetic parameters for economically important traits of animals is necessary, is essential in order to evaluate the breeding values of individuals, made an effective plan and program breeding, and evaluate effects of selection

Today we have specialized pig farms, commercial and the nucleus farms. The nucleus of the farms are grandfather, grandparents and parents, were strict biosecurity regulations. There are only healthy animals with a minimum number of vaccinations. Repair of sows on these farms is about 150% and 300% boar. Commercial farms with slightly weaker biosafety regulations, higher number of vaccinations used for the production of hybrid pigs with a minimum expenditure of labor and cost price (*Vidović et al., 2011*). At European proportions, and in our crystallized are fertile breeds, Landrace and Yorkshire (*Bidanel 2010; Bergsma et al., 2010*). They are used for the production of F₁ mothers that crossing with the terminal boar breed Duroc, Hampshire and Pietrain as well as their F₁ product (synthetic boars that containing recombination of favorable genes for the most important traits) whose descendants are the final product.

The most significant intensification factors influencing production potential are growth intensity, food utilization and slaughter value. These traits pose major influence on the effectiveness of breeding and selection herds (*Brzobohaty et al., 2012*). *Serrano et al. (2009)* states that like any other characteristic, the growth intensity is the result of both internal (breed, sex, age) and environmental factors working together (nutrition, feeding technique, technology). Out of the external conditions, the nutrition of pigs was found to be of the greatest importance (*Bee et al., 2007*). Considering that quantitative traits and their expression are under the influence of several genes, they are under strong influence of environment factors. This shows the significance of accurate and precise assessment of these traits, as well as of the breeding value of the animal.

On the basis of the above, the objectives of this research was determine the intensity growth Landrace and Yorkshire gilts and and their hybrids, and evaluation of genetic parameters heritability, correlations and (co) variances examined traits.

Materials and Methods

Animals and studied traits

The paper, for quantitative-genetic analysis of intensity growth gilts were used results of a Nucleus farm capacity 400 sows pure breed Landrace and Yorkshire, which produces and hybrid gilts F₁ generation for other farms. Analyzed in total 2760 gilts four different genotypes, two of which are pure bred Landrace (429 gilts) and Yorkshire (421 gilts) and two hybrid F₁(YxL) (999 gilts) and F₁(LxY) (911 gilts), from 2010 to 2011. Analyzed by the following traits of intensity growth: weight at weaning (WW), daily gain at suckling (DGS), weight in rearing (WR), daily gain at rearing (DGR), weight in test (WT), daily gain on test (DGT), weight of gilts (WG) and life gain (LG).

Statistical analysis

The significance of the fixed effects and inclusion in the models were determined for each trait using the general linear model (GLM) procedures in software package Statistica 12. In order to examine the influence of the season, the year is divided into three seasons: Season I (November, December, January, February); Season II (March, April, September, October); Season III (May, June, July, August). How would we examined the effects of weight at birth, piglets were divided into six groups: group I (from 1000 to 1200 g), group II (from 1200 to 1400 g), group III (from 1400 to 1600 g), group IV (from 1600 to 18000 g), group V (from 1800 to 2000 g), group VI (> 2000 g).

To estimate genetic parameters, constructed the following model:

$$Y_{ijklmn} = \mu + A_i + Y_j + S_k + B_l + BW_m + e_{ijklmn}$$

where Y_{ijklmn} = phenotypic values of traits; μ = average mean; A_i = random influence of animal; Y_j = fixed influence of year; S_k = fixed influence of season; B_l = fixed influence of breed; BW_m = fixed influence of weight at birth; e_{ijklmn} = random error

Genetics parameters (heritability, correlations) including variance components, were estimated using the restricted maximum likelihood (REML) procedure based on an animal model using the Wombat program (Meyer, 2007) with multivariate analyses. The model can be represented in matrix terms by:

$$y = Xb + Za + e$$

where y is the vector of observations; X is the incidence matrix of fixed effects; b is the vector of fixed effects; Z is the incidence matrix of random effects; a is the vector of random effects; e is the vector of residuals.

Result and Discussion

Table 1 shows the adjusted mean (LSM) and standard error of the adjusted mean (SE_{Lsm}) intensity growth gilts four genotypes. From the table we can see that the average weight at birth (WB) and weaning (WW) was slightly larger (100g respectively 800g) in hybrid gilts in relation to gilts pure breed. In the lactation period, which lasted for all 28 days, hybrid gilts have achieved higher average daily gain (DGS). A substantial advantage of the increase in the intensity growth of hybrid gilts can be seen in rearing, which lasted 52 days. In that period, hybrid gilts are achieved higher body weight (WR) for about 3 kg and daily gain (DGR) for about 100 g compared to gilts pure breed, so that F_1 gilts in the performance test entered with body weight of about 32 kg and gilts pure breed with about 28 kg. In the performance test, which lasted 80 days, the highest body weight (WT) of 73 kg had $F_{1(Y \times L)}$ gilts, then $F_{1(L \times Y)}$ gilts about 71.5 kg, and the lowest body weight (WT) of 66.8 kg had Yorkshire gilts, while Landrace gilts in performance test which lasted 82 days archived body weight (WT) of 71.2 kg. Daily gain in the test (DGT) ranged from 0.832 in Yorkshire to 0.905 in $F_{1(Y \times L)}$ gilts. Finally, with a total age of 160 days, $F_{1(Y \times L)}$ gilts achieved the body weight (WG) of 104.6 kg, $F_{1(L \times Y)}$ gilts 102.4 kg, 99.1 kg Landrace gilts and 93.7 kg Yorkshire gilts.

Many authors have found significant differences in the intensity growth between gilts of different genotypes on commercial farms. Thus, the research *Brkić et al. (2001)*, gilts $F_{1(L \times Y)}$ achieved better results for the traits of intensity growth compared to Landrace gilts. With the age of 209 days, archived an WG 103.81 kg and LG 491 g. The study *Gjerlaug-Enger et al. (2011)* DGS in Landrace was 390g, and DGT 100 kg 905 g. *Vuković et al. (2007)* are in hybrid gilts with the age of 190 days recorded WG of 99.83 kg, and LG 526 g. *Szyndler-Nedzi et al. (2010)* are in gilts with ages from 150 to 210 days, recorded WG of 104.7 kg and LG of 630 g in Yorkshire, 105.5 kg WG and LG of 633 g in Landrace, 111.9 kg WG and LG 658 g in Duroc, 116.9 kg WG and LG of 655 g in Pietrain gilts. *Kawecka et al. (2009)* are in gilts aged 180 days, recorded WG 120 kg and 701 g DGT. *Szostak (2011)* notes that now gilts intended for reproduction much sooner gain the weight of 110–120 kg, which has often been, and still is, the criterion for taking the decision concerning the time of the first mating. This too rapid growth rate and a small amount of fat may have a negative effect on the reproductive functions of primiparous gilts (*Matysiak et al. 2010; Amaral Filha et al., 2010*).

Table 1. Intensity growth of gilts

Traits	Landrace		Yorkshire		F ₁ (YxL)		F ₁ (LxY)	
	LSM	SE _{LSM}	LSM	SE _{LSM}	LSM	SE _{LSM}	LSM	SE _{LSM}
Lactation length, days,	28.00		28.00		28.00		28.00	
Weight at birth, kg (WB)	1.344	0.010	1.380	0.010	1.443	0.006	1.415	0.007
Weight at weaning, kg (WW)	7.030	0.088	6.722	0.089	7.810	0.058	7.665	0.060
Daily gain at suckling, kg (DGS)	0.201	0.002	0.191	0.002	0.220	0.001	0.220	0.001
Rearing length, days	51.00		52.00		52.00		52.00	
Weight in rearing, kg (WR)	20.829	0.276	20.228	0.278	23.798	0.180	23.330	0.189
Daily gain at rearing, kg (DGR)	0.262	0.005	0.252	0.005	0.313	0.003	0.302	0.003
Duration of test, days	82.00		80.00		80.00		80.00	
Weight in test, kg (WT)	71.191	0.648	66.802	0.654	72.993	0.424	71.433	0.444
Daily gain on test, kg (DGT)	0.865	0.006	0.832	0.06	0.905	0.004	0.886	0.004
Age of gilts, days	161.00		160.00		160.00		160.00	
Weight of gilts, kg (WG)	99.109	0.711	93.693	0.718	104.608	0.466	102.411	0.488
Life gain, kg (LG)	0.601	0.003	0.573	0.003	0.644	0.002	0.630	0.002

Residual, direct additive genetic variance components and phenotypic, residual and direct heritability with standard errors for the intensity growth of gilts are shown in Table 2. From Table 2 it can be seen that all traits intensity growth of medium to high degree of heritability. Heritability (h^2) for daily gains were larger (0.640 for DGS, 0.858 for DGR and 0.859 for DGT) in relation to the heritability for achieved body weight (0.584 for WW, 0.558 for WR and 0.816 for WT) in different phases of rearing. Less additive genetic variance (V_a) were recorded in daily gain compared to additive genetic variance archived body weight. Larger heritability estimates but lower than our for growth traits were given by other authors. *Gjerlaug-Enger et al. (2011)* found heritability for DGR 0.25 in Landrace and 0.48 in Duroc, for DGT 0.41 in Yorkshire and 0.42 in Duroc. Heritability for DGT in the range of 0.27 to 0.58 in its research were given *Szynder-Nedzi et al. (2010)* in Yorkshire (0.29), Landrace (0.39) and Duroc (0.58) gilts, *Hoque and Suzuky (2008)* of Duroc (0.38) and Landrace (0.47) gilts, *Imboonta (2007)* in Landrace (0.38) gilts, *Gilbert et al. (2007)* in Yorkshire gilts (0.35). Slightly lower heritability for DGT (0.19) and LG (0.16) found *Nguyen and McPhee (2005)* for Yorkshire gilts, *Szynder-Nedzi et al. (2010)* for the DGT (0.16.) in Pietrain gilts. *Chimonyo and Dzama (2007)* for DGR obtain heritability from

0.15 to 0.27 in Landrace, and *Hermesch et al. (2000)* for the DGR 0.10 and for DGT 0.48 in Landrace.

Table 2. Variance and heritability for intensity growth of gilts

Traits	V_e	V_a	V_p	h_e^2	SEh_e^2	h^2	SEh^2
WB	0.180	4.070	4.250	0.042	0.055	0.958	0.055
WW	0.196	0.275	0.471	0.416	0.056	0.584	0.056
DGS	13.456	23.897	37.354	0.360	0.056	0.640	0.056
WR	0.574	0.724	1.299	0.442	0.056	0.558	0.056
DGR	19.102	115.569	134.671	0.142	0.052	0.858	0.052
WT	0.358	1.594	1.952	0.184	0.057	0.816	0.057
DGT	27.025	164.576	191.601	0.141	0.055	0.859	0.055
WG	0.205	0.500	0.705	0.291	0.057	0.709	0.057

V_e – residual variance; V_a – additive genetic variance; V_p – phenotypic variance; h_e^2 – heritability of residual variance; h^2 – heritability; SEh^2 – standard error of heritability

Genetic and phenotypic covariances and correlations between traits are presented in Table 3 and 4. Negative genetic and phenotypic covariances were obtained between DGS and WR, DGR, between WR and WT, DGT, between DGR and WT, DGT, between WW and DGR. Between the most observed traits were positive genetic and phenotypic correlations. Very strong positive genetic and phenotypic correlations were found between WT, DGT and WG, LG ($r_g = 0.709$ to 0.953 ; $r_p = 0.810$ to 0.942), while weak negative correlation was found between WR, DGR and WT, DGT ($r_g = -0.055$ to -0.108 ; $r_p = -0.010$ to -0.033) and medium negative correlation between WW, DGS and DGR ($r_g = -0.301$ respectively -0.466 ; $r_p = -0.234$ respectively -0.271). Positive genetic correlation between the DGR and DGT are obtained *Hermesch et al (2000)* ($r_g = 0.32$), *Gjerlaug-Enger et al. (2011)* in Landrace ($r_g = 0.15$) and Duroc ($r_g = 0.40$).

Table 3. Genetic (above the diagonal) and phenotypic (below the diagonal) covariance between traits

Traits	WW	DGS	WR	DGR	WT	DGT	WG	LG
WW	1.000	0.736	0.547	-0.456	3.931	0.519	8.644	0.371
DGS	1.086	1.000	-0.185	-0.208	1.072	0.136	1.666	0.115
WR	0.335	-0.254	1.000	0.882	-6.072	-0.055	22.219	0.133
DGR	-0.523	-0.211	6.161	1.000	-0.797	-0.115	1.733	0.681
WT	4.258	1.096	-0.795	-0.422	1.000	11.506	141.924	6.099
DGT	0.508	0.127	0.147	-0.521	14.703	1.000	14.063	0.832
WG	8.708	1.998	38.085	4.772	149.600	16.301	1.000	8.242
LG	0.469	0.138	1.605	0.241	7.688	0.705	10.280	1.000

V_e – residual variance; V_a – additive genetic variance; V_p – phenotypic variance; h_e^2 – heritability of residual variance; h^2 – heritability; SEh^2 – standard error of heritability

Table 4. . Genetic (above the diagonal) and phenotypic (below the diagonal) correlation between traits

Traits	WW	DGS	WR	DGR	WT	DGT	WG	LG
WW	1.000	0.796	0.052	-0.301	0.162	0.231	0.326	0.295
DGS	0.810	1.000	-0.185	-0.466	0.192	0.206	0.248	0.304
WR	0.026	-0.061	1.000	0.882	-0.096	-0.055	0.322	0.133
DGR	-0.234	-0.271	0.885	1.000	-0.088	-0.108	0.157	0.113
WT	0.170	0.138	-0.010	-0.032	1.000	0.953	0.899	0.883
DGT	0.186	0.133	0.017	-0.033	0.942	1.000	0.901	0.709
WG	0.302	0.210	0.435	0.302	0.879	0.853	1.000	0.933
LG	0.285	0.027	0.312	0.252	0.810	0.821	0.891	1.000

V_e – residual variance; V_a – additive genetic variance; V_p – phenotypic variance; h_e^2 – heritability of residual variance; h^2 – heritability; SEh^2 – standard error of heritability

According to *Rosycki et al. (2003)* and *Nowachowicz et al. (2012)* any genetic progress in growth traits that occurs in a population is the result of breeding those animals that have achieved significant results in a performance test and had a good breeding values. Good selection of gilts with knowledge of quantitative genetic parameters, can be achieved faster genetic progress and faster to improve the desired traits.

Conclusions

Based on these results we can conclude that the hybrid gilts had higher intensity growth in rearing and later in the performance test in relation to gilts pure breed, which resulted in higher body weight at the end of the test, which can be explained by the manifestation of heterosis effect in hybrid gilts. Degree of heritability for traits analysis of intensity of growth is medium to high and is slightly larger than the results of other authors, which can be explained by the fact that gilts from nucleus farms, which originate from genetically quality parents and where apply a high selection criteria. The most traits intensity growth of medium to high degree of heritability. Between the most observed traits were positive genetic and phenotypic correlations. Obtained results of intensity growth should be monitored and analyzed at all times, because the analysis of the results of intensity growth gilts, in terms of heritability and correlations that arise between traits, it is possible to estimate the changes that occur in the population, which can be used in pig breeding programs.

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Kvantitativno-genetska analiza intenziteta porasta nazimica plodnih rasa i njihovih hibrida u Nukleus zapatu

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Rezime

U radu je analizirano 2760 nazimica četiri različita genotipa, od kojih su dva čiste rase landras (429 nazimica) i jorkšir (421 nazimica) i dve hibridne $F_{1(Y \times L)}$ (999 nazimica) i $F_{1(L \times Y)}$ (911 nazimica), u periodu od 2010 do 2011 godine. Analizirane su sledeće osobine intenziteta porasta: masa na zalučenju (WW), dnevni prirast na sisi (DGS), masa u odgoju (WR), dnevni prirast u odgoju (DGR), masa u testu (WT), dnevni prirast u testu (DGT), ukupna masa nazimica (WG) i životni prirast (LG). Usled manifestacije heterozis efekta, hibridne nazimice su u odgoju ostavile veću telesnu masu za oko 3 kg, dok su su sa istom starosti od 160 dana prosečno imale veću telesnu masu za 7.0 kg u odnosu na nazimice čiste rase, što je rezultiralo i većim dnevnim prirastima u pojedinim fazama odgoja. Analizirane osobine intenziteta porasta su imale srednji do viskog stepen heritabiliteta. Heritabilnosti (h^2) za dnevne priraste su bile nešto veće (0.640 za DGS, 0.858 za DGR i 0.859 za DGT) u odnosu na heritabilnosti za ostavrene telesne mase (0.584 za WW, 0.558 za WR, i 0.816 za WT) u pojedinim fazama odgoja. Između većine posmatranih osobina zabeležene su pozitivne genetske i fenotipske korelacije. Negativne korelacije ustanovljene između WR, DGR i WT, DGT ($r_g = -0.055$ to -0.108 ; $r_p = -0.010$ to -0.033) i između WW, DGS i DGR ($r_g = -0.301$ odnosno -0.466 ; $r_p = -0.234$ odnosno -0.271).

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EFFECTS OF DIFFERENT PRODUCTION SYSTEMS ON CARCASS AND MEAT QUALITY OF SHEEP AND LAMB FROM WESTERN BALKAN AND NORWAY

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Abstract: The identification of meat quality characteristics from selected breeds grazing in specific regions is particularly relevant to achieve a marketing advantage. *Longissimus thoracis at lumborum* (LTL) from the indigenous Western Balkan (WB) sheep - VlašićkaPramenka (VP) sheep and lambs, and Pivska Pramenka (PP) sheep grazing in Bosnia & Herzegovina (B&H) and Montenegro (MN), respectively, was compared regarding carcass and meat qualities to the crossbred Norwegian white sheep (NWS) - sheep and lambs, grazing in wide Hardangervidda and Jotunheimen regions where the lamb meat is marketed as gourmet meat. The WB sheep had lower average carcass weights and antioxidant capacity, higher ultimate pH, intramuscular fat and *n-6/n-3* ratio, but better tenderness and color stability compared to NWS. The WB lambs were lighter, had higher *n-6/n-3* ratio, lower antioxidant capacity and became more easily rancid despite a higher fat α -tocopherol content. The marketing advantage of WB meat is its tenderness properties while NO's NWS lambs displayed a better nutritional profile.

Key words: production system, sheep meat quality, physical and chemical traits, meat color, fatty acid composition.

Introduction

The consumers' have an increasing interest in more healthy meat products and lower production costs. EU's Common Agricultural Policy stimulates

at the same time pasture-based production systems resulting in meat with higher content of omega 3 polyunsaturated fatty acids (PUFA) (Enser et al., 1998; Carrasco et al., 2009). The consumers in Western Balkan (WB) are becoming more aware of claimed organic meat advantages, but prefer domestic meat from non-conventional production systems. The purchase motives for such meat are safety, "natural" content, health, good meat quality and a distinctive taste (Vukasović, 2013). The Norwegian consumers also prefer domestic meat from mountain pastures with perceived elements of naturalness, healthiness and environmental friendly production combined with good meat quality (Hersleth et al., 2012).

Meat quality differ among animal species (Guerrero et al., 2013), and can be used to promote sheep and lamb sale, such as done for the Texel sheep (Cockett et al., 2004) and lamb from Aragosa (Martinez-Royo et al., 2008). The producers in EU were encouraged to continue producing lamb meat according to the traditional methods (Texiera, 2005) in agreement with consumers' requirements and acceptance. In Europe the Spanish scientists have carried out a substantial amount of research on their autochthonous breed Aragonese in order to obtain the PGI (Protected Geographical Indication) label (Martínez-Cerezo et al., 2005).

The predominant sheep breed in the WB is the Pramenka sheep (PS). It makes up 80 to 90% of the sheep population and belongs to indigenous primitive sheep type (Robić, Liker, and Rupić, 1992). In the 20th century, most PS types were crossed with different exotic breeds, mostly Merino, but the last indigenous PS types remain in the high mountain regions of the Balkan Peninsula, where the environmental conditions and quality of pastures are less favorable for conventional sheep grazing (Cinkulov et al., 2008).

In B&H, the dominant sheep is Vlašićka Pramenka (VP) (synonym Dubska) with female adults weighing 60-70 kg (Porcu and Marković, 2006), while PP (synonym Jezeropivska) is the predominant sheep in MN, with female adults weighing 51-54 kg (Marković, Marković, and Adžić, 2007). Farming in WB is done semi extensively, oriented towards utilization of grassland and pasture areas.

A predominant sheep breed in Norway is the Norwegian White Sheep (NWS). It constitutes 76.2% of all sheep flocks in Norway (Domke et al., 2011). NWS is a crossbreed composed of Dala, Rygja, Steigal and Texel breeds selected for fast growing lambs, good reproduction and high meat yield (Boman, et al., 2010). NWS rearing is intensive, but lamb and sheep graze outdoors during the summer. An adult sheep can reach up to 100 kg live weight. Norwegian lambs grazing in specific regions are marketed by origin (e.g. Gourmet lamb from the mountains in Central Norway; Lofot-lamb from the mountainous islands of North Norway).

The research on NWS meat quality began in 1990, but is still not extensive. Meat quality characteristics such as typical EU grade scores, fat content, fatty acid composition (only adipose tissue), color, flavor and sensory traits have

been reported to depend on grazing regions (*Ådnøy et al., 2005; Lind et al., 2009*). The fattening of lambs on nutrition rich pastures lowered n-6/n-3 FA ratio, while fattening on a concentrate-based diet lowered the content of C18:3 (n-3) fatty acids and intensity of acid taste (*Lind et al., 2009*).

The aim of this study was to: 1) describe the meat quality characteristics of Western Balkan PP and VP breeds grazing in typical regions; 2) compare sheep and lamb meat quality from WB regions with a crossbreed NWS from Norwegian mountains developed for intensive meat production; 3) describe the meat quality variations within each meat production group.

Materials and Methods

Grazing regions

All three grazing regions are characterized by a complex, but different floristic composition.

WB: PP animals were collected in 2012 from the grazing region Ljubišnja, at an altitude of 900-1300m. The MN pastures are unique areas of fragmented mountain grasslands with trees and bushes. *Poetum violaceae*, *Festucetum ovinae*, *Festucetum rubra-falax*, *Festucetumvalesiaca*, *Nardetum strictae*, *Brometum erectistrictae* predominate the floristic composition of the grasslands up to 1200 m (*Dubljevic, 2009*). VP animals were collected in 2012 from the Vlašić grazing region, at an altitude of about 1500 m. The grazing region of VP is characterized by fragmented mountain grasslands, separated by trees and bushes. *Poa pratensis*, *Bromus racemosus*, *Dactylis glomerata*, *Briza media*, *Lotus corniculatus*, *Trifolium pratense*, *Trifolium repens*, *Vicia sativa* and *Pteridium aquilinum* dominate floristic composition (*Alibegovic-Grbic, 2009*).

Norway: NWS animals were collected in 2012 from grazing regions in central and southeast Norway at an altitude 500-1700 m. The region is about 40 000 km², and covers the production of Gourmet lamb. At an elevation of 500-900 m, the grazing area is characterized by spruce and pine forests, while at an elevation of 900-1700 m by scarce birch forests with little grass. *Avenella flexuosa*, *Luzula pilosa*, *Festuca ovina*, *Anthoxanthum odoratum*, *Agrostisca pillaris*, *Deschampsia cespitosassp.cespitosa*, *Carex spp.* are floristically predominant (*Lunnan and Todnem, 2011*).

Only the 4 years old NWS were fed indoor their last 3 months after the outdoor grazing period on the concentrate and local grass silage.

Slaughtering

Totally 92 *Longissimus thoracis at lumborum* (*LTL*) sheep/lamb samples were collected from 3 countries.

B&H: *LTL* was collected at “BB” Kotor Varoš, a traditional slaughterhouse, from 15 female sheep (age 4-5 years) and 15 lambs (age 5-6 months). Traditional slaughtering without stunning was used. The handling of *post mortem* (*pm*) was set up to reduce the effect of cold shortening, i.e. by a controlled temperature drop. **MN:** *LTL* was collected from 15 female sheep (age 4-5 years) at the meat production company Franca, Bijelo Polje. We were not able to collect the lambs from MN, because there was not a sufficient number of female lambs ageing 5-6 months from the same herd in a small production area. In addition, lambs are not commonly raised to age 5-6 months to be slaughtered for meat consumption. **Norway:** *LTL* from 14 female sheep (age 4-5 years) and 15 female sheep (age 2 years) as well as from 18 lambs in an early fattening phase (9 ecologically fed) were collected at the Nortura Gol slaughter plant. The only difference between ecological and conventional production was the lower level of the fatty acid C22:6 (*n*-3) in ecological lamb, and therefore these two groups were merged into a single group in all analysis.

The carcasses in Norway and MN were exposed to low electrical stimulation, and then returned to the chiller (4°C). All *LTL* samples were cut along the carcass length and vacuum-packed in the cutting room ≤ 5 h at 10°C, before being returned to the chiller. The vacuum packaged samples were transported on ice to the laboratories 24 h *pm*.

One *LTL* from each animal was stored at 4°C for 7 days and then sliced, vacuum-packed and frozen. The second *LTL* was cut in pieces suitable for the intended measurements, vacuum-packaged and stored at -80°C, for tenderness measurements at -40°C.

Meat quality assessments

pH: In Norway and MN, the pH value was measured 24 h *pm* (pH₂₄) using the Knick Portamess Model 913 (Knick, Berlin Germany), while in B&H using the HANNA Model 99161 (Cluj-Napoca, Romania). Both instruments were calibrated with commercial standard solutions.

Color stability: Fresh meat samples (24 h *pm*) were sliced into 2 cm thick cuts, and placed on trays (Polystyrene Weigh Boats 85x85x24mm, VWR International, Darmstadt, Germany) over-wrapped with oxygen-permeable polyvinyl chloride film (PVC) and stored at 4°C. One hour after slicing was denoted as time zero. The meat color was determined in triplicates on slices after 4, 72 and 144 h chill storage. The meat surfaces were turned up, towards the cling wrap, during measurements at a temperature of 19°C. **Norway:** Konica Minolta Spectrophotometer CM 700d (Konica Minolta Sensing Inc., Osaka, Japan)

calibrated by a white ceramic calibration cap (CM-A177) was used. The light source was a pulsed xenon lamp. Illuminant D65 (Daylight, color temperature 6504 K) with a 10° observer (CIE Konica-Minolta 1964) was used. **B&H**: Konica Minolta Spectrophotometer CM 2600d (Konica Minolta Sensing Inc., Osaka, Japan) calibrated by a white ceramic calibration plate (CM-A145). The light source, standard illuminant and observer was the same as in Norway. **MN**: Color-Tec PCM+ (ColorTec, Clinton-New Jersey, USA) 20 mm reflectance colorimeter was used. The light source was a light emitting diode (LED) array.

To secure that the measurements were comparable in the 3 countries, seven paint codes (black, white and 5 shades of red) from "JOTUN" A/S (Sandefjord) were measured in Norway, B&H and MN and used to calculate and correct for instrumental differences.

Warner Bratzler tenderness measurements: Slices (4 cm), thawed overnight and heated at 72°C in the core of the samples, were cooled on ice up to approximately 20°C. Sensors inserted in dummy samples recorded internal temperatures. Muscle samples (1×1×4 cm) were cut in parallel to the fiber direction, and sheared across the fiber direction. **Norway**: shear cell HDP/BSK Warner Bratzler, load cell 25 kg, TA-HDi Texture Analyser, Stable Micro Systems, Godalming, UK. **MN/B&H**: Shear cell HDP/BS Warner Bratzler, load cell 25 kg, TA.XT, PLUS, Texture Analyser, Stable Micro Systems, Godalming, UK. The number of replicates was 6-8. In order to transfer data between labs, a rubber was split in two and each half was measured in each country, and a factor was calculated to transfer data from one instrument to another one.

Cooking loss (% weight loss): Cooking loss (%) was calculated as a percent difference between the fresh and heated samples weights.

Chemical composition

Protein Content: Nitrogen content was determined using the Kjeldahl method as described by ISO 937:1992 (ISO, 1992). Total Kjeldahl nitrogen was converted to protein by conversion factor 6.25.

Water content: Water content in meat samples was determined, according to the AOAC Official Method (AOAC 950.46, 1950) in three replicates.

Fat content and fatty acid composition: Fat content was determined according to the AOAC Official Method (AOAC 991.36, 1996), and fatty acid composition according to the O'Fallon method (2012).

Vitamin E content: The measurements were carried out by applying the procedure of *Triumpf et al. (2012)*, with modification of the centrifugation time.

2,2-diphenyl-1-picrylhydrazyl (DPPH), total antioxidant capacity: The antioxidant capacity was determined by using DPPH, according to the procedure

described by *Brand-Williams et al. (1995)*, with some modifications. Meat pieces (0.5 g) were added to 4 ml of DPPH in ethanol (0.050mg/ml). The homogenates were incubated (50 min) in the dark at room temperature. Trolox solutions were used as a standard. The samples were shortly vortexed and centrifuged at 2534 x g for 5 min. The reduction of DPPH was measured by Synergy H4, Hybrid Multi-Mode Microplate Reader from BioTek Instruments Inc., P.O. Box 998 (Highland Park, Winooski, Vermont 05404-0998 USA) at 515 nm after 60 min incubation (until stable absorptions values were obtained). The percentage of DPPH-scavenging activity was calculated as $(A_o - A_t) / (A_o) \times 100$, where A_o was the absorbance of the control and A_t was the absorbance in the presence of the sample after 1 h of incubation.

Cathepsin B analysis: The assay was based on the procedure of *Barret and Kirschke (1981)*, with some modifications. The frozen meat was pulverized (IKA 11 basic Analytical mill, Germany). Meat (1 gram) was mixed with 10 ml extraction buffer (containing 0.25 M of sucrose and 1 mM EDTA in 0.2 M KCL; pH 6.0, adjusted with NaOH). After adjusting the pH of the extraction buffer 0.2 (w/v) Triton X100 was added. The meat homogenates were vigorously shaken and centrifuged (VWR by Hitachi Koki, CT 15E, Japan) at 1946 x g for 20 min at 4°C. The supernatant was mixed with 100 µl buffer, 50 µl Milli-Q water and 100 µl stock solution (15mM Z-Arg-Arg-AMC in 100% DMSO). The blank sample contained 150 µl Milli-Q water, 100 µl assay buffer (containing 0.2 sodium acetate, 4mM EDTA and 8 mM DTT, the final pH 6.0 was adjusted with NaOH) and 50 µl supernatant.

The stock solution of the standard contained Milli-Q water, 7-methylcoumarin amide MCA (1mM MCA in 100% DMSO) and assay buffer. The assay buffer and the diluted extract were incubated in Synergy H4 Hybrid Multi - Mode Microplate Reader (BioTek Instruments. Inc. USA) at 40°C for 30 min. The excitation wavelength was 340 nm, and the emission was monitored at 460 nm.

Heme pigment /hemin analysis: The method was based on the procedure described by *Lombardi-Boccia et al. (2002)*, adapted to Eppendorf tubes.

Total peroxide value using the ferric-xyleneol orange method: The frozen and aged samples were prepared according to the procedure described by *Yi et al. (2013)*.

TBARS: Lipid oxidation was assessed by the TBARS (thiobarbituric acid reactive substances) assay on the aged samples. Two g frozen meat was pulverized (IKA 11 basic Analytical mill, Germany) and mixed with 10 ml stock solution (0.375 % TBA and 15% TCA in 0.25 N HCl). All samples were treated in a water bath at 98 °C for 10 min and cooled on ice for the next 30 min. Solutions under the upper fat layer (1.5 ml) were carefully removed and centrifuged for 25 min at

25 186 x g and 4°C. The absorption (at 532 nm) of the supernatant was measured immediately after centrifugation using Shimadzu UV-1800 (Shimadzu corp. Kyoto, Japan).

Statistical analysis: All statistical analyses were performed using one way ANOVA or a general linear model (Minitab version 16 or 17, Minitab Ltd., Coventry) in combination with Tukey's test for individual comparisons. Significant differences were reported for $P \leq 0.05$.

Results and Discussion

Physical characteristics of sheep/lamb LTL

Carcass characteristics: Carcass weight, fat and conformation grading, tenderness, cooking loss and pH₂₄ for the six different age and breed categories are shown in Table 1. NO carcasses had nominally higher slaughter weights when compared to carcasses from WB. The carcasses from NO and B&H lambs had similar slaughter weights. The B&H sheep were small, had more fat, but good conformation score (Table 1), while the B&H lamb had the lowest fat and conformation score. The conformation score was highest for NO lambs. Due to unusual WB weather conditions in 2012 with pasture in surplus, the WB sheep and lamb were slaughtered one month later than usual; consequently the animals were also fatter (Bjelanovic et al., 2013). A significant difference ($P < 0.001$) in fatness and conformation score was found between groups.

Table 1. Carcass and meat physical quality assessments (mean and standard error square).

	Norwegian white sheep			WB Pramenka sheep		
	NO old	NO young	NO lamb	MN sheep	B&H sheep	B&H lamb
Age (years)	4-5	2	0.5	4-5	4-5	0.5-0.6
Carcass w. (kg)	30.4(±5.2) ^{ab}	33.1(±3.2) ^a	17.1(±2.6) ^d	27.3(±3.6) ^{bc}	25.0(±3.1) ^c	16.0(±1.7) ^d
EU fatness s.*	8.0(±1.4) ^b	7.4(±0.8) ^b	5.6(±1.3) ^c	7.7(±1.3) ^b	9.8(±1.0) ^a	5.1(±1.2) ^c
EU conformation s.**	5.0(±0.0) ^b	7.6(±0.6) ^a	8.0(±0.0) ^a	5.3(±1.5) ^b	7.9(±1.6) ^a	3.4(±0.9) ^c
pH	5.55(±0.12) ^b	5.61(±0.07) ^{ab}	5.64(±0.07) ^{ab}	5.75(±0.08) ^a	5.75(±0.25) ^a	5.75(±0.15) ^a
>pH 5.8	0/14	0/15	0/18	4/15	2/15	0/15
SF (N/cm ²)***	52.4(±10.4) ^a	54.6(±12.3) ^a	40.1(11.06) ^{bc}	47.4(±7.9) ^{ab}	38.9(±6.1) ^{bc}	31.8(±5.9) ^c
Range	38-70	37-77	25-60	28-83	25-66	25-42
>50 (N/cm ²)	4/14	8/15	4/18	3/15	1/15	0/15
Cooking loss (%)	20.5(±5.1) ^{ab}	19.3(±4.2) ^b	21.8(±5.1) ^{ab}	25.4(±4.9) ^a	18.1(±1.7) ^b	21.5(±5.2) ^{ab}

*Scale 1-15 points: 1=P-; 2=P (poor); 3=P+; 4=O-; 5=O(normal); 6=O+; 7=R-; 8=R (good); 9=R+; 10=U-; 11=U(very good); 12=U+, 13=E-; 14=E (excellent), and 15=E+

**Scale 1-15 points: 1=1-; 2=1(very scarce); 3=1+; 4=2-; 5=2 (scarce); 6=2+; 7=3-; 8=3 (medium); 9=3+; 10=4-; 11=4 (important), 12=4+; 13=5-; 14=5 (excellent), and 15=5+

***8 days p.m.

^{abcd} Row means within factors with different letters indicate statistically significant differences at ($P < 0.001$).

Sheep and lamb meat quality related characteristics:

Mean pH_{24} ranged from 5.55 to 5.75 (Table 1). A significant difference between groups in pH_{24} ($P < 0.001$) was found. pH was higher in WB than in NO samples. This may indicate less stress in NO animals when slaughtered (Martínez-Cerezo et al., 2005), or less type I fibers (Park et al., 1987). PS is an indigenous breed, and may uphold its natural instincts (i.e. fear) and sensitivity to stress. Stress results in excretion of adrenaline causing a series of biochemical changes that indirectly catalyze the breakdown of glycogen ante mortem (*am*), leading to an elevated muscle pH_{24} (Voisinet et al., 1997). Priolo et al. (2002), also connected higher ultimate pH to physical activity of animals and extensive production system.

Generally, the samples from WB sheep and lamb were significantly tenderer when compared to NO sheep and lamb, and this may depend both on breed and production system in agreement with Guerrero et al., (2013). Meat samples from B&H sheep and lamb were tenderer compared to the other groups. The samples from young NO were the toughest, while the MN sheep varied the most (Table 1). Meat with shear force scores above 50 N/cm² is regarded as tough (Davey, Gilbert, and Carse, 1976) and will be discounted by consumers. The breeding aim for higher muscular mass is often at the expense of lower tenderness and lower IMF content (Więcek et al., 2008). Cooking losses were highest in the MN samples (Table 1). This may reflect these samples lower protein content (Table 2).

The average changes in surface meat color parameters ($L^*a^*b^*$) during the aerobic storage were significantly different among groups (Figure 1 a,b). The first measurement (4 h) would reflect a bloomed sample with dominantly oxy-myoglobin (OMb) in the surface. A decline in L^* and a^* with time would be interpreted as conversion to meat-myoglobin (MMb). Surface L^* may increase due to microbial growth after prolonged storage in air.

L^* (lightness) was always higher in WB animals (Figure 1a) with B&H lamb having the highest initial L^* value. L^* increased/remained the same for 72 h, except for the young NO and B&H sheep. L^* may dependent on production system. Some authors have reported darker meat from extensive production systems (Mancini and Hunt, 2005; Priolo et al., 2002), but Lorenzo et al. (2014), reported a higher L^* value in meat from a free extensive production system. This phenomenon may be explained by a higher IMF level in meat from extensive production systems (Priolo et al., 2002).

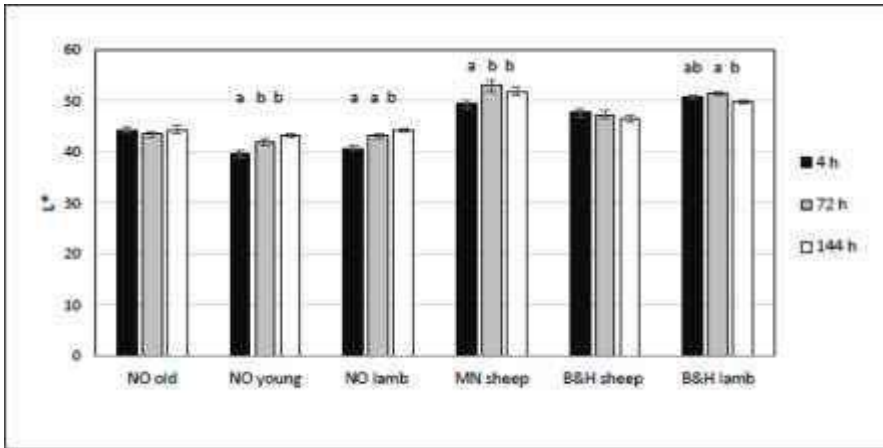


Figure 1a. The average changes in L* during aerobic incubation for different sheep/lamb groups and times. Different letters indicate significant ($P<0.05$) differences.

The variable a^* was not dependent on production system. Four h post mortem, only the NO lamb and B&H sheep had low a^* values. This could be due to low color stability for the NO lamb or the higher fat level in B&H sheep (Table 1). The variable a^* of MN sheep declined after 72 h, but still retained a higher level than in the other groups. a^* of the B&H sheep declined only moderately from 4 to 72 h. The color stability of NO sheep, using a^* as an indicator, was lower than in MN sheep and B&H sheep (Figure 1b). For lamb, a^* declined the least for the NO lamb.

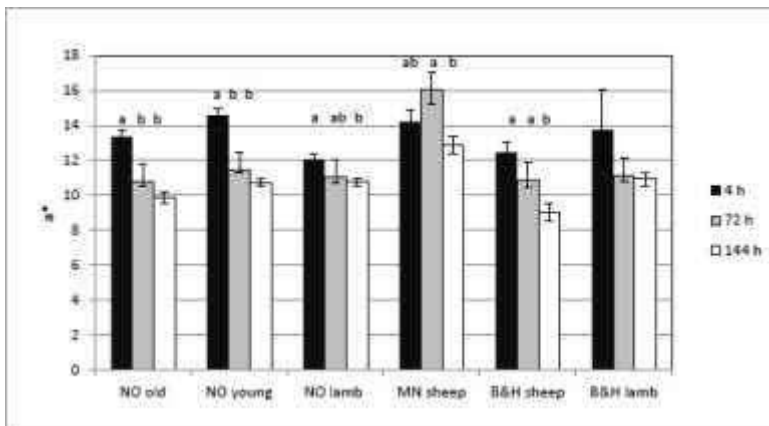


Figure 1b. The average changes in a^* during aerobic incubation for different sheep/lamb groups and times. Different letters indicate significant ($P<0.05$) differences.

NO young sheep and NO lamb had the lowest b^* and a much lower b^* than NO old (not presented). Interestingly, b^* was also high in B&H meat. Differences in muscle lightness and yellowness can be attributed to dietary effects on pre-slaughter glycogen and on marbling levels (*Mancini and Hunt, 2005*) while differences in a^* depend largely on heme amount, myoglobin states plus marbling.

Composition of sheep/lamb LTL

The iron concentration in meat is highly dependent on breeding, age, sex and muscle type of the animal (*Lombardi-Boccia et al., 2002*). As expected, heme was highest in older sheep and lowest in lambs (Table 2). There was no difference in heme between NO and B&H lambs, but NO lambs had the nominally lowest heme concentration (0.15 mg/ml).

Water content depended on age and was higher in younger compared to older and more fatty animals. The low water content in B&H sheep meat was related to its higher fat content (supported by Table 1 and 2). Breed combined with production system had no significant impact on dry matter.

Table 2. Meat chemical quality assessments (mean and standard error square).

	Norwegian white sheep			WB Pramenka sheep		
	NO old	NO young	NO lamb	MN sheep	B&H sheep	B&H lamb
Heme (mg/ml)	0.23(±0.05) ^a	0.21(±0.04) ^{ab}	0.15(±0.03) ^c	0.24(±0.04) ^a	0.21(±0.05) ^{ab}	0.18(±0.03) ^{bc}
Water content*	73.13(±0.6) ^b	73.42(±1.0) ^b	75.30(±0.9) ^b	73.15(±0.4) ^b	70.93(±0.6) ^c	75.83(±0.4) ^a
Dry matter*	26.87(±0.6) ^b	26.58(±0.9) ^b	24.69(±0.6) ^b	26.85(±0.4) ^b	29.07(±0.6) ^a	24.17(±0.4) ^c
Protein content*	21.38(±0.9) ^a	21.61(±0.9) ^a	20.56(±0.6) ^b	17.12(±0.4) ^c	20.49(±0.6) ^b	20.63(±0.4) ^b
Fat content*	3.88(±0.5) ^b	3.38(±0.2) ^b	2.58(±0.6) ^c	7.46(±0.8) ^a	7.39(±0.4) ^a	2.35(±0.1) ^c
Vitamin E (mg/100g)	0.23(±0.04) ^{ab}	0.12(±0.05) ^c	0.09(±0.07) ^c	0.29(±0.15) ^a	0.22(±0.08) ^{ab}	0.16(±0.06) ^{bc}
Vitamin E/Fat (mg/100g)	0.07(±0.05) ^{ab}	0.03(±0.03) ^b	0.05(±0.04) ^b	0.04 (±0.02) ^b	0.04(±0.02) ^b	0.11(±0.05) ^a
DPPH (total antioxidant)*	66.2(±5.2) ^b	66.5(±3.3) ^b	66.3(±4.8) ^b	70.9(±2.6) ^a	68.7(±3.7) ^{ab}	72.7(±3.7) ^a
Cathepsin B**	0.33(±0.07) ^{ns}	0.33(±0.04) ^{ns}	0.30(±0.03) ^{ns}	0.32(±0.05) ^{ns}	0.31(±0.04) ^{ns}	0.32(±0.03) ^{ns}
TBARS***	0.33(±0.13) ^{ab}	0.33(±0.21) ^{ab}	0.22(±0.05) ^b	0.47(±0.25) ^a	0.23(±0.23) ^b	0.43(±0.03) ^a

* expressed in %

** μM MCA/min/g meat

*** 8 days p.m. / mg malondialdehyde/kg

^{abcd}Row means within factors with different letters indicate statistically significant differences at ($P < 0.001$) except TBARS ($P < 0.005$).

Protein content was significantly different among all animal groups (Table 2). Both old and young NO had higher protein content than B&H and MN sheep. MN sheep had the lowest protein content, but with no difference for lamb groups. *Hofman et al. (2003)* reported that the muscles with the highest protein content were characterized by lower fat content. NO sheep had a more favourable fat/protein ratio (Table 2) in agreement with general breeding goals. The results also indicated that old and young NO sheep, with the highest protein content, were

less tender (Table 1). This can again relate to types of muscular fibers. *Wood et al. (1999)* suggested that genetic selection for modern breeds with increased meat yield and lean content increases the proportion of white glycolytic fibers (type IIB), and consequently less tender meat (*Karlsson et al., 1993*).

Vitamin E (α -Tocopherol) is a fat-soluble vitamin. Its content was significantly different among all six animal groups (Table 2). Green pasture or supplementation in feeds increase vitamin E in meat (*Jose et al., 2008*). Vitamin E can delay OMB oxidation via inhibition of lipid oxidation (*Faustman et al., 1998*). Color and lipid stability of fresh beef *longissimus muscle* can be improved if α -tocopherol concentrations of tissues is between 3.0 to 3.3 μg α -tocopherol/g meat (*Faustman et al., 1989*). MN sheep had a high concentration of vitamin E (0.29 mg/100g), close to this threshold. This can be a possible explanation of the delayed OMB conversion to MMB in MN sheep. Older sheep groups had a higher vitamin E concentration than younger groups. Unexpectedly, vitamin E/fat (mg/100g fat) was nominally highest in B&H lamb, and significantly different from the other groups (Table 2).

α -Tocopherol level is interesting from a nutritional perspective, assuming that its antioxidative power protects cells against the effects of free radicals which can contribute to the development of chronic diseases like cancer and cardiovascular diseases. This vitamin can enhance the immune function and block the formation of cancerogenous nitrosamines in the stomach from nitrates used as additive in food products. Vitamin E also prevents against cataracts (*Daley et al., 2010*).

Cathepsin B is a relevant enzyme for dry cured sheep production since its level is closely related to textural defects during the ripening phase of pig hams (*Priolo et al., 2002*). The activity of cathepsin B in *LTL* (Table 2) did not differentiate between groups, only within groups; the highest variation was for old NO and MN sheep. The variation was lowest for NO lamb and B&H lamb.

Table 3 shows average values and standard errors (SE) of intramuscular fatty acid composition (mg/100 g meat). The concentrations of total fatty acids were age dependent. Sheep had more total fatty acids than lambs, and WB sheep more than NO in agreement with their amount of total fat (Table 2). The concentration of the polyunsaturated fatty acids C18:2 (*n*-6) and C18:3 (*n*-3) showed the greatest variation, as indicated by their SE, while the concentration of C20:4 (*n*-6), C20:5 (*n*-3), C22:5 (*n*-3) and C22:6 (*n*-3) showed the lowest SE. The total amount of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA was also age dependent, and significantly higher in older animals. The percentage of PUFA dropped with age, but was also significantly dependent on production systems, as described by *Enser et al. (1998)*. The nominally highest % SFA was found in MN sheep.

Table 3. Fatty acid composition (mean and standard error square).

	Norwegian white sheep			WB Pramenka sheep		
	NO old	NO young	NO lamb	MN sheep	B&H sheep	B&H lamb
C18:2 n-6 Linoleic acid*	1.81(±1.58) ^{ab}	1.93(±0.72) ^{ab}	0.98(±0.27) ^c	2.34(0.84) ^a	2.21(±0.69) ^a	1.17(±0.24) ^{bc}
C18:3 n-3 α - Linolenic acid*	1.15(±1.33) ^a	1.49(±0.73) ^a	0.49(±0.17) ^b	1.42(±0.76) ^a	1.06(±0.45) ^{ab}	0.43(±0.10) ^b
C20:4 n-6 Arachidonic acid*	0.32(±0.03) ^b	0.39(±0.08) ^{ab}	0.33(±0.06) ^b	0.33(±0.04) ^b	0.38(±0.04) ^{ab}	0.43(±0.07) ^a
C20:5 n-3 Eicosapentaenoic acid*	0.20(±0.02) ^b	0.24(±0.05) ^a	0.20(±0.04) ^b	0.17(±0.03) ^{bc}	0.17(±0.03) ^c	0.16(±0.03) ^c
C22:5 n-3 Docosapentaenoic acid*	0.26(±0.11) ^b	0.36(±0.10) ^a	0.21(±0.04) ^b	0.27(±0.06) ^b	0.25(±0.05) ^b	0.22(±0.04) ^b
C22:6 n-3 Docosahexaenoic acid*	0.07(±0.02) ^{bc}	0.10(±0.04) ^a	0.06(±0.02) ^c	0.09(±0.03) ^{ab}	0.09(±0.01) ^{ab}	0.09(±0.02) ^{ab}
n-6/n-3*	1.37(±0.17) ^b	1.12(±0.15) ^c	1.44(±0.15) ^b	1.46(±0.15) ^b	1.73(±0.20) ^a	1.84(±0.14) ^a
SFA*	30.87(±42.48) ^a	27.66(±14.77) ^{ab}	8.67(±1.87) ^b	47.41(±23.20) ^a	29.51(±10.61) ^a	7.51(±2.69) ^b
MUFA*	29.12(±41.93) ^a	22.11(±12.69) ^{ab}	6.78(±1.68) ^c	36.77(±16.54) ^a	24.97(±10.41) ^{ab}	6.5(±2.57) ^{bc}
PUFA*	3.88(±3.09) ^{ab}	4.58(±1.65) ^a	2.33(±0.55) ^c	4.71(±1.70) ^a	4.23(±1.21) ^a	2.55(±0.46) ^{bc}

* mg/100g meat

^{abcd}Row means within factors with different letters indicate statistically significant differences at ($P < 0.001$).

Total amounts of C18:2 (*n*-6) was higher in sheep compared to lamb. Total amounts of α -Linolenic acid C18:3 (*n*-3) tended to follow this pattern. Sañudo et al. (2006) reported similar results for Spanish and British lambs. Old NWS had the greatest amount of *n*-3 LC-PUFA.

The ratio *n*-6/*n*-3 was still favorable for lamb/sheep (Russo, 2009). Interestingly, this ratio showed no variation with age in both NO and B&H systems. But the *n*-6/*n*-3 ratio was significantly higher for B&H sheep and lamb (Table 3) than other systems. The ratio *n*-6/*n*-3 was the lowest in young NO sheep. C18:3 (*n*-3) is regarded as the preferred fatty acids leading to C20:5 (*n*-3), docosapentaenoic acid C22:5 (*n*-3), and docosahexaenoic acid C22:6 (*n*-3) (Brenna et al., 2009). Additionally, it inhibits the conversion of C18:2 into the others *n*-6 LC-PUFA (Smink et al., 2012).

A favorable *n*-6/*n*-3 ratio is important for the regulation of SFA in human body. The dietary SFA can raise unfavorable blood lipids, but sufficient intake of *n*-3 PUFA can neutralize this effect (Dias et al., 2014), and prevent coronary heart diseases, diabetes 2, obesity and cancer. The SFA intake is a major contributor to calcium, vitamin D, vitamin B12 and the other essential nutrients absorption; a reducing of SFA without substituting lower-fat versions may result in serious unintended nutritional consequences (Huth et al., 2013).

Oxidative stability measurements

The total antioxidant activity method detects the ability of a matrix to eliminate an unpaired valence electron in DPPH (Dawidowicz, Wianowska, and Olszowy, 2011). Low DPPH values are therefore favorable. The total antioxidant activity was highest in NO meat (Table 2). Antioxidant activity was not affected by the age of the animals.

TBARS values above 0.5 are considered as critical and indicate a lipid oxidation level which produces a rancid odor and taste that can be recognized by consumers (Wood *et al.*, 2008). TBARS was significantly different among the groups (Table 2). After 7 days of aging at 4°C, TBARS accumulation in NO old and young was equal. NO lamb had the lowest TBARS value, while MN sheep and B&H lamb had the highest. B&H sheep had the lowest TBARS among sheep groups. The TBARS value of 0.47 in MN sheep was near the threshold of 0.5 suggesting that the high fat content and poor ratio vitamin E/fat content may have some impact on its low oxidative stability (Table 2). All together factors such as concentration of the fat, heme pigment and antioxidant status in the muscle tissue can influence color stability and FA oxidation, and are tightly related to the diet (Ponnampalam *et al.*, 2012). Lourenço *et al.* (2007) suggested that different grazing regions can induce changes in the rumen microbial population, and therefore differences in the biohydrogenation of PUFA. Dietary effects in form of different grass types might have an impact on the FA composition in ruminants. Lee *et al.* (2003) suggested that white clovers (*Trifolium repens*) can limit biohydrogenation of *n-3* PUFA. It seems that vitamin E had a positive impact on color stability in MN sheep, but not on FA oxidation stability.

Polar peroxides (0.12-0.39 mmol/kg meat) originating from lipids (Volden *et al.*, 2011) were highest in VP lamb followed by NO old and young. Proteins bound peroxides (Yi *et al.*, 2013) also varied significantly among groups from 0.09 in MN sheep to 0.191 mmol/kg in NO old. No significant difference was found for unpolar (chloroform soluble) peroxides. These data are partly in agreement with TBARS (Table 2).

Conclusion

The different production systems influenced meat color, pH, tenderness and fatty acid composition. Pramenka sheep, collected from their natural grazing areas, were smaller animals with more fatty carcasses relative to NWS from Hardangerevidda and Jotunheimen regions. WB meat (*LTL*) had higher pH₂₄, and a low protein to *IMF* ratio. Its total antioxidant capacity was lower, and the *n-6/n-3* ratio tended to be higher. The marketing potential of PS meat seems to be related to its higher color stability and good tenderness. This quality can be used to

encourage the production of B&H sheep and lamb in future. The marketing advantages of NO carcasses seemed related to their high protein/fat ratio, low $n-6/n-3$ ratio and good antioxidant capacity.

B&H sheep were muscular but with more fat, lower water content and lower cooking losses, lower $L^*a^*b^*$ with higher $n-6/n-3$ and became more rancid than MN sheep. The B&H lambs were smaller than NO lambs, with a higher level of vitamin E, but lower antioxidant capacity, more TBARS and less EPA and higher $n-6/n-3$ ratio. Its marketing potential seemed only related to its high vitamin E content while the marketing potential of NO lamb seems related to its good oxidative stability with a favorable $n-6/n-3$ ratio.

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Uticaj različitih proizvodnih sistema na kvalitet mesa trupova ovaca i jagnjadi Zapadnog Balkana i Norveške

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Rezime

Definisanje kvaliteta mesa odabranih rasa ovaca i jagnjadi koje su bile na ispaši u posebnim regijama je od velike važnosti u postizanju tržišne konkurentnosti. U ovom eksperimentu korišten je mišić *Longissimus thoracis at lumborum* (LTL) autohtonih zapadno-balkanskih(WB) ovaca i jagnjadi vlašičke pramenke (VP) koje su bile na ispaši na planini Vlašić u Bosni i Hercegovini. Također je korišten LTL od ovaca pivske pramenke (PP) koje su bile na ispaši na planini Ljubišnja u Crnoj Gori. Kvalitet mesa trupova i LTL-a autohtonih balkanskih ovaca upoređivani su sa trupovima norveških belih ovaca i jagnjadi (NWS), koje su bile na ispaši u regionu hardangerske visoravni i Jotunheimen regiona. Jagnjeće meso iz ovih regiona smatra se gurmanskim proizvodom.

U poređenju sa NWS ovcama rase pramenka ovaca imale su nižu prosečnu težinu, manji oksidativni kapacitet, veću konačnu pH vrednost, intramuskularnu masnoću kao i viši odnos n-6/n-3, bolju mekoću mesa i stabilnost boje. Jagnjad zapadno-balkanske pramenke su imala nešto manju masu, viši odnos n-6/n-3, slabiji oksidativni kapacitet, njihovo meso je veoma brzo užeglo, bez obzira na viši sadržaj α -tocopherola. Tržišna prednost mesa zapadno-balkanskih rasa je u njihovoj mekoći, dok NWS jaganjci imaju bolji nutritivni profil.

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EFFECT OF GENETIC AND ENVIRONMENTAL FACTORS ON THE PHENOTYPE CHARACTERISTICS OF LAMBS

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Abstract: The aim of this study was to determine the influence of environmental factors affecting body weight variability of lambs in two crossbreed groups: Pirot x Württemberg and Sjenica x Württemberg. Both populations were managed under the same farm conditions. The data were analyzed to determine the effect of age of the dam, weight of dam, birth type, sex, year and season, on the birth weight and weaning weight of crossbreed lambs. Statistical analysis was performed by GLM procedure using the SPSS statistical package program. The average birth weight of Pirot x Württemberg lambs was 3.56 kg while Sjenica x Württemberg lambs was slightly higher at 3.69 kg. The difference on birth weight between the two crosses was not statistically significant ($P > 0.05$). The average weaning weight of Pirot x Württemberg lambs was 23.54 kg while Sjenica x Württemberg lambs had higher weight at 24.37 kg. The difference of 0.83 kg on weaning weight was statistically significant ($P < 0.05$). Body weight, depending on the environmental factors, ranged from 3.17 to 3.96 kg at birth and from 22.12 to 24.18 kg at weaning in Pirot x Württemberg lambs. Body weight of Sjenica x Württemberg lambs ranged from 3.39 to 3.99 kg at birth and from 22.69 to 25.44 kg at weaning. Statistical analysis showed that the differences were statistically significant ($P < 0.05$) and highly significant ($P < 0.01$).

Key words: sheep, crossbreeding, genotype, environment, lamb body growth

Introduction

For a successful sheep management we need to have a breeding program and know which factors that affect production (Ugarte, 2007). Body weight of lambs has a major role in achieving profitable results. Initial body weight affects not only growth, but also vitality and mortality of lambs (Morris et al, 2000; Cloete

et al., 2001; Zapasnikiene, 2002; Berhan and Arendonk, 2006; Petrovic *et al.*, 2009). Not all breeds of sheep have potential for high daily weight gains. Therefore, the crossbreeding is the most effective way to improve the production of lamb meat, because; it directly affects the increase in body weight of lambs (Leymaster, 2002, Petrovic *et al.*, 2011). The efficiency of meat production has maximized in terminal crossbreeding systems by using specialized sire breeds to complement the characteristics of crossbred ewes (Petrovic, 2000; Cloete *et al.*, 2003; Hoffman *et al.*, 2003). Body weight at birth and weaning depends on many environmental factors. Among them are year and season, which is primarily reflected through nutrition, housing and care of animals during the production cycle, especially during pregnancy. Other important factors on the growth of lambs are: maternal age, maternal body weight, type of birth and sex of lambs (Hansen and Shrestha, 2002; Fisher, 2004; Rosa and Bryant, 2003; Barbar *et al.*, 2004, Notter *et al.*, 2005; Susic *et al.*, 2005).

The objective of this study was to determine the effect of some important factors affecting body weight variability in two groups of crossbred lambs.

Materials and Methods

This three-year study was conducted at a sheep farm located in South-East Region of Serbia. Sheep included in this research were representatives of two genotypes: R₁ Pirot x Württemberg (¼ Pirot breed; ¾ Württemberg breed) and R₁ Sjenica x Württemberg (¼ Sjenica breed; ¾ Württemberg breed). Dams R₁ generations were mated with rams R₁ generation to produce the experimental lambs which are R₂ generation of the above crosses. Both genotypes managed under same farm conditions. From November to May, the herd fed with hay and concentrate. After this period, the sheep grazed in the mountain's natural pastures. Dams were divided into three groups based on their age at lambing: young (< 4 years), mature (from 4.1 to 6 years) and old (> 6.1 years), and two groups regarding their body weight: light (< 55kg) and heavy (> 55kg). After lambing, the body weights of lambs at birth (1. day) and at weaning (90.days) were obtained and recorded. All determinations of weight were rounded to the nearest 0.1 kilogram. The data were (200 lambs per class of effects) analyzed to determine the effect of age of the dam, weight of dam, birth type, sex, year and season, on birth weight and weaning weight of lambs.

Statistical analysis was performed by GLM procedure of SPSS v.20 (2012) statistical package program using the following model:

$$Y_{ijklmnop} = \mu + G_i + J_j + S_k + A_l + W_m + T_n + L_o + \epsilon_{ijklmnop},$$

where: Y_{ijklmnop} = birth weight of pth lamb of oth sex, nth birth type, mth weight of dam, lth age of dam, born during kth season in jth year and ith genotype

μ = overall population mean

G_i = effect of genotype (fixed effect –2 classes)

J_j = effect of year (fixed effect –3 classes)

S_k = effect of season (fixed effect –2 classes)

A_l = effect of age of the dam (fixed effect –3 classes)

W_m = effect of weight of the dam (fixed effect –2 classes)

T_n = effect of type of birth (fixed effect –2 classes)

L_o = effect of sex of lamb (fixed effect –2 classes)

$e_{ijklmnop}$ = residual error

Results and Discussion

Results on the effect of genotype and environmental factors on body weight of lambs at birth and weaning are shown in Tables 1 and 2.

Effect of genotype. Average birth weight of Pirot x Württemberg lambs was 3.56 kg while Sjenica x Württemberg lambs was slightly higher at 3.69 kg. The difference of 0.13 kg was not statistically significant ($P>0.05$). Average weaning weight of Pirot x Württemberg lambs was 23.54 kg. The Sjenica x Württemberg lambs had higher weight, which was 24.37 kg. The difference of 0.83 kg between the two crosses was statistically significant ($P<0.05$).

Results of the present study on the effect of genotype and environmental factors on the birth weight and weaning weights in lambs were similar to other studies. *Momani et al. (2010)* stated that genotype of lambs significantly affected average daily gain, birth weight and body weight of lambs at 15, 30, 45 and 60 days. A significant effect of genotype was also reported by *Dawson and Carson (2002)*.

Table 1. Effect of genotype and environmental factors on body weight (kg) of lambs at birth

Effect		Genotype			
		Pirot x Württemberg (A)		Sjenica x Württemberg (B)	
		\bar{X}	\pm SE	\bar{X}	\pm SE
Overall population mean		3.56	\pm 0.10	3.69	\pm 0.09
Age of dam	Young (A)	3.38 ^B	\pm 0.10	3.43 ^B	\pm 0.12
	Mature (B)	3.82 ^A	\pm 0.07	3.95 ^A	\pm 0.09
	Old (C)	3.48 ^{aB}	\pm 0.09	3.69 ^{AB}	\pm 0.10
Weight of dam	Light (A)	3.49 ^b	\pm 0.10	3.56 ^b	\pm 0.11
	Heavy (B)	3.63 ^a	\pm 0.08	3.82 ^a	\pm 0.07

Effect of dam age. Variations of body weight as seen on Tables 1 and 2, depending on the mother's age, ranged from 3.38 to 3.82 kg at birth and from 22.48 to 24.18 kg at weaning in Pirot x Württemberg lambs. Variations in Sjenica x Württemberg lambs ranged from 3.43 to 3.95 kg at birth and from 22.69 to 25.44 kg at weaning. Young and old ewes had lighter lambs, while mature sheep had the heaviest lambs. Statistical analysis showed that there were a significant differences ($P < 0.01$ and $P < 0.05$) for both ages of lambs.

Petrović et al. (2011) reported the influence of dam age on birth weight variability on local Pramenka breeds. *Said et al. (2000)* found that the age of dams significantly affected body weight from birth until weaning in Awassi lambs. Other researchers (*Shahroudi et al. 2003; Kalantar 2003; Dixit et al. 2001; Matika et al. 2003; Rashidi et al. 2008*) observed similar results. However, *El Fadilli et al. (2000)* and *Abegaz et al. (2005)* showed different results.

Effect of dam weight. Lambs in both genotypes were heavier if their mother were also heavier. The differences were 0.14 kg for Pirot x Württemberg lambs ($P < 0.05$) and 0.26 kg for Sjenica x Württemberg lambs ($P < 0.05$). Relative to weaning weight, the difference in weight of lambs were 0.84 kg in Pirot x Württemberg lambs ($P < 0.05$) and 1.02 kg in Sjenica x Württemberg lambs ($P < 0.05$).

Momani Shaker et al. (2002) said that dam weight did not affect the growth of lambs at 30, 45 days until weaning, but effect on lamb growth from birth until 15 days was significant. *Krizek et al. (1983)* declared that body weight of dams significantly affected live weight of lambs at birth and at the age of 30 and 60 days.

Effect of type of birth. Birth type had also effect on the weight in both genotypes. Single Pirot x Württemberg lambs were heavier by 0.79 kg at birth ($P < 0.01$) and by 0.94 kg at weaning ($P < 0.05$) than twins. Similar result was found in Sjenica x Württemberg lambs. Weight of single lambs was 0.60 kg higher than in twins at birth, and 1.62 kg at weaning. The existing differences on the average body weight of lambs at birth and at weaning were statistically very significant ($P < 0.01$).

Baneh and Hafezian (2009) reported that type of birth was significant on weight traits of lambs to weaning. Single lamb's body weight in all ages and their average daily gain were more than twins because of competition between twins to fed on their mother's milk resulting in suckling less milk compared to the singles. Other authors (*Kalantar 2003; Dixit et al. 2001*) also observed higher weaning weight in singles. However, *Shahroudi et al. (2003)* and *Matika et al. (2003)* reported that type of birth had no effect on body weight of lambs.

Effect of sex. Sex of lambs at birth had also an effect on body weight but only the difference between Pirot x Württemberg lambs was significant ($P < 0.05$).

At weaning, sex of lambs significantly affected the body weight of both genotypes ($P < 0.05$). Male Pirot x Württemberg lambs were heavier by 1.13 kg and male Sjenica x Württemberg lambs were heavier by 1.01 kg than the females.

Notter et al. (1991) noted that birth weight of lambs is greatly influenced by lamb sex. Similar to our results, many authors stated that sex has an important effect on growth (*Said et al. 2000, Dawson et al. 2002; Momani Shaker et al. 2002*). Various authors (*Matika et al. 2003; Nourian 2000; Shahroudi et al. 2003; Rashidi et al. 2008*) reported the differences between male and female lambs bodyweight. Type and measure of hormone secretion especially sexual hormones, lead to difference in animal growth. Estrogen hormone has a limited effect on the growth of long bones in females. That could be one of the reasons for which females have smaller body and lighter weight compared to males (*Baneh and Hafezian 2009; Rashidi et al. 2008; Shahroudi et al. 2002*).

Effect of year. The birth weight observed for three years ranged from 3.35 to 3.87 kg in Pirot x Württemberg lambs and from 3.40 to 3.93 kg in Sjenica x Württemberg lambs. Statistical analysis showed significant differences ($P < 0.05$ and $P < 0.01$) between year 3 and years 1 and 2. Differences in body weight at weaning were highest on the third year in both genotypes which was significantly higher ($P < 0.05$) than years 1 and 2.

The results obtained in our study regarding the effect of year are in accordance with other authors (*Said et al. 2000; Momani Shaker et al. 2010; Petrovic et al. 2011*). Whereas, *Staikova and Stancheva (2009)* found out in their study that the year of birth significantly influenced the live weight at all ages.

Effect of season. Lambs born in spring-summer had a heavier body weight at birth (0.08 kg in Pirot x Württemberg lambs and 0.12 kg in Sjenica x Württemberg lambs) than those born in autumn-winter. However, differences between seasons were not significant ($P > 0.05$). The lambs weaning weight born in spring-summer season were also heavier (0.92 kg for Pirot x Württemberg and 1.06 kg for the Sjenica x Württemberg). The differences on weaning weight between seasons were significant ($P < 0.05$).

Petrovic et al. (2011) noted that difference depending on the lambing season can be interpreted as the factor of food, in other words, the effect of pasture grass and natural environment. *Dixit et al. (2001)* showed that year, season, sex, birth type and dam's age significantly affect the weight at first year of age of lambs.

Laes-Fettback and Peters (1995) observed that birth weight of lambs are affected by dam size, dam body condition and litter size that influences the survival rate and pre-weaning growth performance of the offspring. Effect of seasons on sheep production has been studied by several authors (*Demiroren et al. 1995; Sormunen and Suvela 1999; Hansen and Shrestha 2002; Fisher 2004; Rosa and Bryant 2003*).

Table 2. Effect of genotype and environmental factors on body weight (kg) of lambs at weaning

Effect		Genotype			
		Piroć x Württemberg (A)		Sjenica x Württemberg (B)	
		\bar{X}	\pm SE	\bar{X}	\pm SE
Overall population mean		23.54 ^b	\pm 0.80	24.37 ^a	\pm 0.85
Age of dam	Young (A)	22.48 ^{BC}	\pm 0.71	22.69 ^{BC}	\pm 0.68
	Mature (B)	24.18 ^A	\pm 0.82	25.44 ^{Ac}	\pm 0.91
	Old (C)	23.96 ^A	\pm 0.79	24.98 ^{Ab}	\pm 0.80
Weight of dam	Light (A)	23.12 ^b	\pm 0.78	23.86 ^b	\pm 0.80
	Heavy (B)	23.96 ^a	\pm 0.79	24.88 ^a	\pm 0.84
Birth type	Single (A)	24.01 ^b	\pm 0.86	25.18 ^B	\pm 0.92
	Multiple (B)	23.07 ^a	\pm 0.76	23.56 ^A	\pm 0.86
Sex of lamb	Male (A)	24.11 ^b	\pm 0.82	24.87 ^b	\pm 0.81
	Female (B)	22.98 ^a	\pm 0.77	23.86 ^a	\pm 0.78
Year	1(A)	23.14 ^{bc}	\pm 0.80	23.43 ^{bc}	\pm 0.80
	2(B)	23.57 ^{ac}	\pm 0.79	24.27 ^{ac}	\pm 0.84
	3(C)	23.91 ^{ab}	\pm 0.74	25.41 ^{ab}	\pm 0.85
Season	Autumn-winter (A)	23.08 ^b	\pm 0.81	23.84 ^b	\pm 0.80
	Spring-summer (B)	24.00 ^a	\pm 0.86	24.90 ^a	\pm 0.84

A,B,C P \leq 0.01; a,b,c P \leq 0.05

Results of this study are compatible with those of *Mendel et al. (1989)* who stated that Merinolandschaf lambs born in spring and summer are heavier than those born in autumn and winter. The present results confirmed the results of other reports (*Kalantar 2003; Matika et al. 2003; Ozcan et al. 2005*).

In the majority of cases, the results have shown that season has a significant influence on body growth and economic features. A particular connection with the

food source, temperature and day length depend on seasonal and certain climate characteristics for different geographical regions. The lambs born in different seasons of the year tend to have different birth weights.

Conclusion

Results of this study showed that genotype and environmental factors have important effect on lambs' growth from birth to weaning. Effect of genotype of lambs was not statistically significant on birth weight, but was statistically significant at weaning weight. Effect of dam age shows that young and old mothers gave birth to lighter lambs, while mature sheep have heavier lambs at birth. It also observed that lambs in both genotypes were heavier at birth if born from heavier ewes. Maternal weight also influenced the weight of lambs at weaning. The birth type had an effect on the body weight of lambs in both genotypes. Single lamb crosses had significantly higher weight than in twins. Sex of lambs had effect on body weight at birth, but significant differences had observed only in Pirot x Württemberg lambs. At weaning, sex of lambs had a significant effect on the body weight of both genotypes. Effect of the year on the birth and weaning weights was statistically significant. Lambing season shows that lambs born in spring-summer had a higher body weight at birth and at weaning.

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Uticaj genetskih i faktora životne sredine na fenotipske karakteristike jagnjadi

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Rezime

Cilj ovog istraživanja je bio da se utvrdi uticaj faktora životne sredine na varijabilnost telesne težine jagnjadi u dve grupe meleza: pirotska x virtemberg i

sjenička x virtemberg. Obe populacije su držane pod istim uslovima na farmi. Podaci su analizirani da se utvrdi uticaj starosti majke, njene težine, tipa rođenja, pola, godine i sezone, na težinu na rođenju i odbijanju jagnjadi meleza. Statistička analiza je izvedena pomoću GLM procedure, koristeći SPSS statistički program paket. Prosečna telesna masa meleza pirotska x virtemberg je 3,56 kg, dok sjenička x virtemberg jagnjad bila nešto veća – 3,69 kg. Razlika u težini na rođenju između dve grupe meleza nije bila statistički značajna ($P > 0,05$). Prosečna težina na odbijanju jagnjadi meleza pirotska x virtemberg je bila 23,54 kg, dok su jagnjad melezi sjenička x virtemberg imala veću težinu – 24,37 kg. Razlika težine na zalučenju od 0,83 kg je statistički značajna ($P < 0,05$). Telesna masa, u zavisnosti od faktora sredine, kretala se u rasponu od 3,17 do 3,96 kg na rođenju i od 22,12 do 24,18 kg na odbijanju u jagnjadi pirotska x virtemberg. Telesna masa jagnjadi sjenička x virtemberg kretala se u rasponu od 3,39 do 3,99 kg na rođenju i od 22,69 do 25,44 kg na odbijanju. Statistička analiza pokazala je da su razlike statistički značajne ($P < 0,05$) i visoko značajne ($P < 0,01$).

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EFFECT OF BODY CONDITION SCORE AND LIVE WEIGHT OF FERTILITY OF MERINO SHEEP AFTER INDUCTION OF OESTRUS IN THE OUT-OF-BREEDING SEASON

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Abstract: Object of the study were merino sheep raised in the farm of the Agricultural institute – Stara Zagora. The experiment was conducted with a group of 68 animals of different ages, lambing after treated with hormonal preparation according to adopted scheme during the out-of-breeding season – in May. In the experimental group were included ewes which lambed earlier without making a selection in respect to their productivity.

Animals were kept under the same conditions (stall-pasture) and fed the same rations with the concentrate mixture, rough, succulent feed and grazing in quantity and composition according to their physiological status and season from the fertilization until lambing. Hormonal pattern: setting pads for sheep type Sincro-part (30mg), removing pads after 12 days and giving ewes a PMSG injection at a dose of 500 UI, applying artificial insemination at the 50-55th hour. Body condition score and live weight of the animals were determined in 4 separate periods: 1st period (after mating), 2nd period (during pregnancy), 3rd period (after lambing), 4th period (before next mating service). Improving fertility in merino sheep is significantly influenced by the preparation of ewes for the mating by reaching the respective physiological status which is expressed by score over 2.5 according to the Body condition score method and live weight over 60 kg. Animals scored 2.75-3.50 before mating have a share of 91.18% from all the sheep in the flock and have the biggest number of lambs.

Key words: sheep, body condition score, hormonal preparation

Introduction

Determination of body condition score of sheep is widely used in the countries where sheep farming is well developed. Applied as an express assessment of the physiological status of the animals and opportunity for monitoring and

providing for their complete nutrition, this method has significant influence over the management of the flocks. In this respect, to achieve a better production efficiency it is necessary to study the existing dependence between the Body condition score and productive traits.

An important factor for improving fertility is the intensification of propagation process. Using the method for inducing oestrus in the out-of-breeding season and application of different hormonal patterns for superovulation (*Boscos et al., 2002; Jafar Yadi et al., 2011; Osama et al., 2010; Ralchev et al., 2008*) would provide more lambs per ewe.

According to *Torre et al. (1991), Attia et al. (2001) and Hatcher et al. (2007)* live weight and determination of Body condition score in the beginning of the mating have significant influence over fertility. *Sejian et al. (2009)* established that ewes with Body condition score 3-3,5 have the best reproduction rate.

Due to experiments conducted, a number of researchers report that fertility is influenced by the Body condition score (*Doney et al, 1982; Guerra et al., 1972; Koyuncu, 2005; Madani et al., 2009*).

Davoud et al. (2012) in their study found that Body condition score before mating has significant effect over the number of the newborn lambs and ewes scored 3 have a higher fertility. In our previous studies we established dependence between some selection traits and Body condition score in sheep from different productive range (*Ivanova et al., 2008; Dimova et al., 2008; Slavova et al., 2009, 2010*).

The object of the present study is to establish the existence of relation between the Body condition score and fertility of merino sheep after application of hormonal pattern for inducing oestrus during the out-of-breeding season.

Materials and Methods

Object of the study were merino sheep raised on the farm of the Agricultural institute – Stara Zagora. The experiment was conducted with a group of 68 animals of different ages, lambing after treated with hormonal preparation according to adopted scheme during the out-of-breeding season – in May. In the experimental group were included ewes which lambed earlier without making a selection in respect to their productivity.

Animals were kept under the same conditions (stall-pasture) and fed the same rations with the concentrate mixture, rough, succulent feed and grazing in quantity and composition according to their physiological status and season from the fertilization until lambing.

Hormonal pattern: setting pads for sheep type Sincro-part (30mg), removing pads after 12 days and giving ewes a PMSG injection at a dose of 500 UI, applying artificial insemination at the 50-55th hour. Body condition score and live weight of

the animals were determined in 4 separate periods: 1st period (after mating), 2nd period (during pregnancy), 3rd period (after lambing), 4th period (before next mating service). In order to define the Body condition score we used the adopted 5 point system – from 1 (very thin) to 5 (fattened), (Todorov, 2008; Todorov et al., 1994; Thompson and Meyer, 1994).

Data was processed statistically by using software product STATISTICA for Windows. We use Descriptive statistical analyze with levels of significance: high $p < 0.001$; average $p < 0.01$; low $p < 0.05$. Data were presented on figures and tables.

Results and Discussion

Type of lambing is presented in figure 1. From the total number of 68 ewes (of different ages, lambing after treated with hormonal preparation), 32 ewes (47.06 %) lambed 1 lamb, 30 ewes (44.12 %) lambed twins and 6 ewes (8.82 %) lambed triplets.

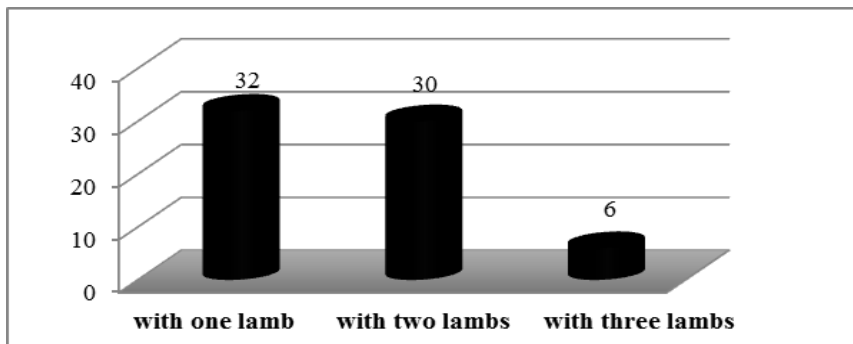


Figure 1. Distribution of sheep type of lambing, number

Table 1. Assessment of body condition of sheep, treated with hormonal preparations

Body condition score										
I assessment (after mating)		II assessment (during pregnancy)			III assessment (after lambing)			IV assessment (before the next mating)		
score	n	n	$\bar{X} \pm S\bar{x}$	VC	n	$\bar{X} \pm S\bar{x}$	VC	n	$\bar{X} \pm S\bar{x}$	VC
With one lamb										
2.50	4	4	3.125 ± 0.125	8.00	4	2.937 ± 0.062	4.25	4	2.937 ± 0.119	8.14
2.75	7	7	3.214 ± 0.085	7.00	7	2.929 ± 0.105	9.49	6	2.875 ± 0.191	16.28
3.00	7	7	3.321 ± 0.071	5.69	7	3.173 ± 0.033	3.78	5	3.200 ± 0.050	3.50
3.25	7	7	3.429 ± 0.071	5.51	7	3.214 ± 0.036	2.92	7	3.179 ± 0.046	3.84
3.50	7	7	3.536 ± 0.085	6.36	7	3.179 ± 0.118	9.81	6	3.292 ± 0.077	5.71
Total	32	32	3.344 ±	7.30	32	3.101 ± 0.	7.61	28	3.107 ±	9.40
3,047b			0.043c			042			0,055b	
With two lambs										
2.75	10	10	3.100 ± 0.076	7.77	10	3.100 ± 0.076	7.77	8	3.281 ± 0.031	2.68
3.00	13	13	3.096 ± 0.053	6.20	13	3.096 ± 0.053	6.20	13	3.212 ± 0.055	6.23
3.25	6	6	3.083 ± 0.083	6.62	6	3.083 ± 0.083	6.62	5	3.300 ± 0.093	6.33
3.50	1	1	3.000 ± 0.000	0.00	1	3.000 ± 0.000	0.00	1	3.250 ± 0.000	0.00
Total	30	30	3.092 ± 0.037	6.53	30	3.092 ± 0.037	6.53	27	3.250 ±	5.23
2.983a									0.033b	
With three lambs										
3.00	1	1	3.250 ± 0.000	0.00	1	3.000 ± 0.000	0.00	1	3.000 ± 0.000	0.00
3.25	1	1	3.500 ± 0.000	0.00						
3.50	2	2	3.625 ± 0.125	4.88	2	3.250 ± 0.000	0.00	2	3.125 ± 0.125	5.66
3.75	2	2	3.750 ± 0.000	0.00	2	3.125 ± 0.125	5.66	2	3.250 ± 0.000	0.00
Total	6	6	3.583 ±		5	3.150 ± 0.061	4.35	5	3.150 ±	4.35
3.458ab			0.083bc	5.69					0.061	

Significance: a – p<0.001; b - p<0.01; c - p<0.05

Body condition score of sheep is shown in table 1. The point system applied after mating was as follows: score from 2.75 to 3.5 was evaluated in 87,50% of the ewes which had 1 lamb; in 100% of the ewes which had twins and in 67,78 % of the ewes that had triplets, while 32.22% of the ewes with triplets were scored 3.75. The highest average score were given the ewes that lambed three lambs – 3,458 and the lowest - those that lambed twins – 2,983. Established differences were significant at p<0.001.

The biggest increase in points of Body condition score during the second period can be observed in ewes that yeanned 2 lambs – with 0,350. Differences between the groups of lambed ewes were statistically significant at p<0.01.

During the third period (after lambing) the most significant decrease in point of Body condition score is observed in ewes that have triplets - with 0,433. Differences between groups in respect to the trait which was analyzed not statistically significant at p<0.05.

The observed tendency in the Body condition score to vary according to the physiological status of the animals is unidirectional although it is different in magnitude – increasing from the first to second evaluation as the pregnancy increases, followed by decrease after lambing and after that again increasing,

connected to stabilization of physiological status and preparation of ewes for the next mating procedure.

Live weight in certain periods follows the tendency of variation of Body condition score (table 2). During the first evaluation live weight is lowest in ewes which have twins - 61,567 kg, followed by the ewes that have singles with the small difference of 0,277 and the highest in ewes that have triplets - 65,333 kg. During the second evaluation (friendly pregnancy), the increase of live weight in ewes with 1 lamb is 7.531 kg and in ewes with 2 lambs - 9.400 kg. The most significant is variation of live weight during the certain periods in the ewes with triplets - increase of 9.834 kg (from first to second evaluation) and decrease of 15.767 kg (from second to third evaluation). During the period of preparation for next mating live weight increases in all three groups. The obtained results match the physiological status of the animals but the established variances in respect to the analyzed trait are not statistically significant ($p > 0.05$).

Table 2. Live weight of sheep, treated with hormonal preparations

BCS on I assess- ment (after mating)	Live weight											
	I assessment (after mating)			II assessment (during pregnancy)			III assessment (after lambing)			IV assessment (before the next mating)		
	n	$\bar{X} \pm S\bar{x}$	VC	n	$x \pm Sx$	VC	n	$\bar{X} \pm S\bar{x}$	VC	n	$\bar{X} \pm S\bar{x}$	VC
With one lamb												
2,50	4	58,250 ± 2,529	8,68	4	65,750 ± 2,529	7,69	4	61,500 ± 4,444	14,45	4	65,000 ± 1,779	5,48
2,75	7	59,000 ± 1,603	7,19	7	67,571 ± 2,653	10,39	7	59,571 ± 2,836	12,59	6	65,833 ± 4,658	17,33
3,00	7	61,000 ± 1,464	6,35	7	68,286 ± 1,426	5,53	7	60,923 ± 1,238	7,32	5	69,000 ± 4,050	13,12
3,25	7	62,286 ± 2,179	9,26	7	69,714 ± 2,504	9,50	7	59,714 ± 2,551	11,31	7	69,429 ± 2,983	11,37
3,50	7	67,143 ± 3,188	12,56	7	74,000 ± 3,024	10,81	7	66,000 ± 4,065	16,29	6	71,333 ± 3,040	10,44
Total	32	61,844 ± 1,105	10,11	32	69,375 ± 1,162	9,47	32	61,562 ± 1,367	12,86	28	68,357 ± 1,549	11,99
With two lambs												
2,75	10	59,800 ± 1,597	8,45	10	72,800 ± 2,004	8,71	10	59,300 ± 1,469	7,83	8	71,500 ± 2,383	9,43
3,00	13	62,385 ± 1,328	7,67	13	70,692 ± 1,666	8,49	13	58,769 ± 1,455	8,93	13	68,692 ± 2,073	10,88
3,25	6	62,500 ± 2,997	11,75	6	69,167 ± 2,301	8,15	6	58,500 ± 3,106	13,01	5	68,000 ± 4,990	16,41
3,50	1	63,000 ± 0,000	0,00	1	67,000 ± 0,000	0,00	1	56,000 ± 0,000	0,00	1	60,000 ± 0,000	0,00
Total	30	61,567 ± 0,972	8,64	30	70,967 ± 1,084	8,37	30	58,800 ± 0,974	9,07	27	69,074 ± 1,521	11,44
With three lambs												
3,00	1	54,000 ± 0,000	0,00	1	59,000 ± 0,000	0,00	1	54,000 ± 0,000	0,00	1	60,000 ± 0,000	0,00
3,25	1	64,000 ± 0,000	0,00	1	78,000 ± 0,000	0,00	-	-	-	-	-	-
3,50	2	63,000 ± 3,000	6,73	2	72,000 ± 1,000	1,96	2	57,500 ± 7,500	18,45	2	63,000 ± 11,000	24,69
3,75	2	74,000 ± 0,000	0,00	2	85,000 ± 0,000	0,00	2	64,000 ± 5,000	11,05	2	77,000 ± 3,000	5,51
Total	6	65,333 ± 3,211	12,04	6	75,167 ± 4,020	13,10	5	59,400 ± 3,473	13,07	5	68,000 ± 5,177	17,02

The yearned ewes were separated according to their Body condition score during the first evaluation (after mating) which is shown in table 3. Within ewes that had singles there was a higher share of animals scored 2.75-3.50 (87.50 %), than of those scored up to 2.50 (12.50%). Within ewes lambing twins, a higher share had the animals with score 2.75-3.00 (76.67 %), than animals with score 3.25-3.50 (23.33 %), but all of them had score from 2.75 to 3.50. Ewes having triplets were few and 83.33 % of them are scored from 3.25 to 3.50. Totally, ewes scored from 2.75 to 3.50 after mating, which is 91.18%.

Table 3. Distribution of sheep with lambs depending on body condition score at I assessment /after mating/

Fertility	Body condition score									
	to 2,50		2,75 – 3,00		3,25 – 3,50		over 3,50		total	
	n	%	n	%	n	%	n	%	n	%
With one lamb	4	12,50	14	43,75	14	43,75	0	0	32	100
With two lambs	0	0	23	76,67	7	23,33	0	0	30	100
With three lambs	0	0	1	16,67	3	50,00	2	33,33	6	100
Total	4	5,88	38	55,88	24	35,30	2	2,94	68	100

Animals scored 2.75-3.00 have the highest share – 55.88%, followed by those scored 3.25-3.50 – 35.30%. Results correspond to those shown by *Sejian et al.* (2009) in their study conducted with sheep.

Conclusion

Improving fertility in merino sheep is significantly influenced by the preparation of ewes for the mating by reaching the respective physiological status which is expressed by score over 2.75, according to the Body condition score method and live weight over 60 kg. Animals scored 2.75-3.50 before or after mating have a share of 91.18% from all the sheep in the flock and have the biggest number of lambs.

Uticaj telesne kondicije i telesne mase na plodnost merino ovaca posle vansezonske indukcije estrusa

P. Slavova, S. Laleva, Y. Popova

Rezime

Objekat istraživanja su bile merino ovce gajene na farmi Poljoprivrednog instituta - Stara Zagora. Eksperiment je izveden sa grupom od 68 životinja različitog uzrasta, koje su se jagnjile nakon tretmana sa hormonskim preparatima u skladu sa usvojenom šemom za period van sezone - u maju. U eksperimentalnoj grupi su bile uključene ovce koja su se već jagnjile bez pravljenja odabira u odnosu na njihovu produktivnost.

Životinje su držane pod istim uslovima (zatvoreni objekat-ispasa) i hranjene istim obrokom sa mešavinom koncentrata, krmivom, sočnom hranom i ispašom u količini i sastavu u skladu sa njihovim fiziološkim statusom i sezonom od oplodnje do jagnjenja.

Hormonski obrazac: postavljanje jastučića za ovce - tip Sincro-part (30 mg), uklanjanje jastučića nakon 12 dana i davanje ovcama PMSG injekcije u dozi od 500 UI, primena veštačke oplodnje u 50.-55. satu.

Telesna kondicija i telesne mase životinja su utvrđeni u 4 odvojena perioda: 1. period (posle parenja), 2. period (tokom trudnoće) 3. period (posle jagnjenja), 4. period (pre sledećeg parenja).

Na poboljšanje plodnosti merino ovaca značajno utiče priprema ovaca za parenje, i to dostizanjem odgovarajućeg fiziološkog statusa koji se izražava rezultatom preko 2,5 prema metodi ocene telesne kondicije i telesne mase preko 60 kg. Životinje koje su osenjene sa 2,75-3,50 pre parenja imaju udeo od 91,18% od svih ovaca u stadu i imaju najveći broj jagnjadi.

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PRODUCTIVE CHARACTERISTICS AND BODY MEASUREMENTS OF ALPINE GOATS RAISED UNDER SMALLHOLDER PRODUCTION SYSTEMS IN CENTRAL SERBIA

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Abstract: The purpose of this study was to evaluate present level of productivity and determine linear body traits of Alpine goats raised in Serbia on smallholder farms. Data were collected from 22 smallholder farms located in Belgrade district, with total of 330 purebred Alpine does 2-9 years of age, 145 yearling does and 476 kids. Traits measured were: body weight of does, body weight of kids at birth, 30 days of age and at weaning (90-120 days), prolificacy of mature and yearling (primiparous) does, six linear body traits of does (withers height, body length, hearth girth, chest depth, chest width, pelvic width) and milk production (milk yield, milk fat and milk protein content). The analysis showed the average body weight of does to be 54.96 kg, while the average body weight of kids at birth, 30 days of age and weaning was 2.73 kg, 8.7 kg and 18.3 kg., respectively. Prolificacy was 144% in mature and 125% in yearling does. Measurements of linear body traits were: withers height 67.87 cm, body length 71.92 cm, hearth girth 81.79 cm, chest depth 32.93, chest width 21.49 cm and pelvic width 17.63 cm. Among dairy production traits, following results were obtained: lactation length 220.73 days, total milk yield 531.66 kg, milk fat content 3.33% and milk protein content 3.16%. It was concluded that the overall productivity of Alpine goats raised under smallholder production systems in Serbia is satisfying. Giving the fact that these animals are usually kept under poor conditions, many of these productive traits are very good.

Key words: goats, body weight, body traits, prolificacy, milk

Introduction

Within the sector of farm animals in Serbia, the goat industry is the least developed. Despite the fact that Serbia has very favourable natural conditions for goat breeding, this production is not attractive to farmers, primarily due to the bad economy situation in the country. The main reason for this negative situation, in which the goat breeding has been for a long period, is the lack of organized and guaranteed purchase of goat milk, which would provide some security for farmers. Production of our goats is directed towards milk-meat, but priority is milk. This relates especially on households where the production of goat milk is more acceptable than rearing of cows (Žujović *et al.*, 2011). Dairy goat is considered the cow of the poor. The goat eats little, occupies a small area and produces enough milk for the average unitary family, whereas maintaining a cow at home cannot be afforded by the homeowner, hence the growing popularity of goat as the poor person's cow (Aziz, 2010).

In Central Serbia goats are mainly raised extensively in very small herds on individual family farms. Produced milk is mainly used for making of cheese, which is sold at local green markets.

A very small number of goats are under the control of productive and reproductive traits, approximately 1800 of them, which is about 1% of the total number of goats that are bred on this territory. From total number of goats registered in central heard book, Alpine breed is the most dominant with 87%, followed by local breeds, Balkan goat and Serbian white goat. Alpine goats can be found all over Central Serbia, in different regions, from lowlands to hilly-mountainous areas.

The Alpine is the most common breed of goats in France with 60% of the females being registered at the Official Milk Control organization. It is an animal of average size and the females are the good milk yielders. It is a goat with close-cropped hair, often towed colour. Rustic, the Alpine breed is well adapted to both off-grazing production system and pasture.

In developing countries, the performance of high-yielding breeds imported from countries with highly advanced production systems is often negatively affected due to genotype-environment interactions (Smith *et al.*, 1988; Bondoc *et al.*, 1989).

In goat breeding the easiest and fastest way to describe the breed is the description of external markings, measurement of body characteristics and production traits (Nemeth, 2010). Thanks to the good adapting abilities Alpine goat breed is quite spread around the world, and in Serbia it is raised very successfully in various regions of the country. However, there is little information available regarding body measurements and productivity of Alpine goats raised in Serbia.

Accurate data are required to determine the future outlook of the goat populations and their productivity. Therefore, the purpose of this study was to

determine present level of productivity of Alpine goats raised in Serbia on smallholder farms.

Materials and methods

Data were collected from 22 smallholder farms located in Belgrade district, with total of 330 purebred Alpine does 2-9 years of age, 145 yearling does and 476 kids. The average flock size was 15 goats, with a range of 2 to 76. Animals were kept extensively, mostly at pasture, except for the winter, when they were kept indoors. Nutrition was based primarily on pasture (during warm part of the year) and quality alfalfa hay (during cold part of the year). Supplementary diet was consisted of grain and mineral-vitamin mix. Goats were bred through natural service.

Body weight at mating and prolificacy was measured in 330 mature does. Prolificacy was also measured in 145 yearling does. Body weight of kids was measured at birth, at the age of 30 days and at weaning (the age of 90 – 120 days). Prolificacy was calculated as the percentage of number of kids born on total number of does delivered according to the following equation.

$$\text{Prolificacy (\%)} = (\text{No. of kids born} / \text{No. of does kidding}) \times 100$$

Six linear body traits were measured in 330 mature does as follows: body weight (BW), wither height (WH), body length (BL), hearth girth (HG), chest depth (CD), chest width (CW) and pelvic width (PW). The length, width and depth data were measured by stick and hearth girth was measured by tape. Body length was measured as the distance between the shoulder and pin bone (tuber ischii). Wither height was measured as the distance from the surface of a platform to the withers. Heart girth represented the circumference of the chest. Chest depth was measured as vertical distance from sternum to withers. Chest width was measured as width of the rib cage between the fore legs. Pelvic width was taken as the distance between the two pelvic bones (Tubercxae), across the dorsum.

Among dairy production traits, following traits have been analyzed: the milk yield in full lactation, milk fat content, milk protein content and lactation duration (in days).

The milk recording was conducted by AT method, which was done in the time interval of 28-34 days, once in the morning and next time at evening, by official recorder (International agreement, 2009). First recording was done 40 days after kidding. Milk components (fat and protein) were analyzed using the ultrasonic milk analyzer Ekomilk.

The collected data were analyzed by the statistical package Statistica for Windows 7 (stat. Soft. Inc.). Obtained results were presented using descriptive statistics.

Results and Discussion

Descriptive statistics for live weight of does, birth weight of kids, body weight of kids at the age of 30 days, body weight of kids at weaning and prolificacy of mature and yearling does are set out in Table 1.

Table 1. Descriptive statistics for productive and fecundity traits in does and kids

Traits	N	Mean	SD	CV%
BW of does (kg)	330	54.96	2.11	3.84
BW of kids at birth (kg)	476	2.73	0.46	16.85
BW of kids at 30 days of age (kg)	476	8.7	1.05	12.07
BW of kids at weaning (kg)	476	18.3	1.13	6.17
Prolificacy (mature does), %	330	144		
Prolificacy (yearling does), %	145	125		

BW – body weight

Body weight is an important economic trait in the selection of animals and the main purpose of animal breeding practices is to improve traits of economic value.

Live weight of adult goats, as presented in Table 1, was 54.96 kg and prolificacy rate was 144%. *Nemeth et al. (2005)* reported average live weight of 54.08 in Alpine does raised in Hungary, which is in accordance with present study. In the study of *Memiši and Stanišić (2014)* it was reported that Alpine does raised in Serbia had live weights of 46.47 to 52.77 kg, which was lower than in present study. According to *Kume et al. (2012)* Alpine does in the country of origin have live weight of 80 kg, which is far more than observed in the present study.

Average body weights of kids were 2.73 kg at birth, 8.7 kg at the age of 30 days and 18.3 kg at weaning (90 -120 days of age). *De Menezes et al. (2007)* found body weights of Alpine kids to be: 3.61 kg at birth, 7.35 kg at 30 days of age and 19.11 kg at 90 days of age. *Kume and Hajno (2010)* also reported higher birth weights (3.11-3.15 kg), but lower weights at 30 days of age (6.71 kg), compared to the present study.

The prolificacy obtained in the present study (144%) is somewhat lower than in research of *Drobnic et al. (1998)* who determined litter size of 1.64 in controlled Slovenian herds of Alpine goats. *Crepaldy et al. (1999)* also reported bigger litter size of 1.6 kids/doe of Alpine breed raised in Italy. However, *Kasap et al. (2012)* obtained almost exact value for litter size of 1.46 kids/doe when investigating reproductive parameters of Alpine goats in Croatia. In primiparous does, prolificacy of 125% was lower than in mature does which is consistent with numerous statements that litter size is influenced by parity (*Amoah and Gelaye, 1990; Awemu et al., 1999; Kasap et al., 2012*).

The prolificacy has influence on the economy and milk production. It is expected for twin kidding mothers to have more of the milk yield compared to

those having one kid. More kids also mean more meat and more incomes from selling them as quality breeding animals.

The mean values along with standard deviation and coefficient of variation for wither height, body length, chest width, chest depth, heart girth and pelvic width in Alpine goats are presented in Table 2.

Table 2. Descriptive statistics of analyzed body measurements in Alpine does

Traits	N	Mean	SD	CV%
WH (cm)	330	67.87	1.72	2.53
BL (cm)	330	71.92	1.97	2.73
HG (cm)	330	81.79	8.55	10.45
CD (cm)	330	32.93	3.77	11.45
CW (cm)	330	21.49	1.57	7.30
PW (cm)	330	17.63	3.38	19.17

WH – wither height, BL – body length, HG – heart girth, CD – chest depth, CW – chest width, PW – pelvic width

Morphological measurements have been traditionally used for characterisation of different breeds of animals by many researchers. Furthermore, external body measurements have been studied to predict body weight, as well as to predict carcass characteristics (*Khan et al., 2006; Pesmen and Yardimci, 2008; Abd-Alla 2014*). Body size and shape measured objectively could improve selection for growth by enabling the breeder to recognize early maturing and late maturing animals of different sizes (*Akpa et al., 2013*). Where genetic evaluation has still limited use, identification of some descriptive linear traits may be useful and farmers' friendly tools for selecting goats with desirable characters (*Haldar et al., 2014*).

The body measurements obtained in this study were as follows: wither height 67.87 cm, body length 71.92 cm, hearth girth 81.79 cm, chest depth 32.93, chest width 21.49 cm and pelvic width 17.63. *Nemeth et al. (2005)* reported very similar values for almost all studied body traits of Alpine goats raised in Hungary: 67.9 cm for wither height, 74.3 cm for body length, 32.5 cm for chest depth an 17.2 cm for pelvic width, except for chest width which was somewhat lower (19.8 cm) than in the present study. There is a lack of information on body traits of Alpine goats in the literature and therefore these results are hard to compare.

Table 3. Mean \pm se milk traits depending on the order of lactation

Order of lactation	N	Lactation length, days	Total milk yield, kg	Milk fat, %	Milk protein, %
I	136	219.75 \pm 13.48	486.63 \pm 6.11	3.4 \pm 0.00	3.16 \pm 0.00
II	36	222 \pm 3.59	595.16 \pm 12.69	3.3 \pm 0.01	3.15 \pm 0.01
III	39	208.08 \pm 3.31	537.28 \pm 10.52	3.31 \pm 0.01	3.19 \pm 0.01
IV	34	215.29 \pm 3.73	568.44 \pm 12.09	3.32 \pm 0.02	3.16 \pm 0.02
V	22	205.95 \pm 4.17	556.05 \pm 18.11	3.31 \pm 0.02	3.15 \pm 0.01
VI+	57	240.47 \pm 31.79	564.68 \pm 11.03	3.32 \pm 0.01	3.14 \pm 0.01
Overall	325	220.73 \pm 7.95	531.66 \pm 4.67	3.33 \pm 0.00	3.16 \pm 0.00

Milk traits of Alpine goats, such as milk yield, lactation length, milk fat and milk protein content are set out in Table 3.

Overall milk production in studied population was 531.66 kg for the lactation of 221 days, with 3.33% of milk fat and 3.16% of milk protein content, on average. These results are in accordance with findings of *Crepaldy et al. (1999)* who found milk yield in Alpine goats to be 567 kg in the lactation of 231 days. *Mioč et al. (2008)* also obtained similar results for milk yield (557 kg) and somewhat higher milk fat content (3.47%) in Alpine goats in Croatia. However, values from the present study are higher than those of *Memiši et al. (2011)* who found that the milk yield of Alpine does in Serbia was 362.83 kg for the lactation of 252 days. These authors also determined somewhat lower content of milk protein (2.93%), but milk fat content was at the same level as in present study (3.32%). *Pavliček et al. (2006)* also established lower milk yield of Alpine goats in Croatia, being from 288.26 kg at first lactation to 382.96 kg at third lactation, with lactation lasting for 201-203 days. According to *Kume et al. (2012)* milk yield of Alpine goat breed in the country of origin is 950 kg for the lactation of 256 days.

When observed by order of lactation, milk yield was lowest in first lactation (486.63 kg) which is in agreement with findings of other authors (*Mourad et al., 2001; Pavliček et al., 2006; Memiši et al., 2011*) who also reported lower milk yield in primiparous does. Highest milk yield was observed in second lactation (595.16 kg) and then varied in subsequent lactations. It is expected to have linear increase in milk yield from first to third lactation (*Bogdanović et al., 2010*), however, milk production is highly sensitive production, affected by many different factors and therefore some deviations can be expected. Milk performance is polygenic property caused by numerous genes which directly or indirectly have impact on its expression. Production of milk is closely associated with environment factors, such as: nutrition of mothers/dams before and after partus, number of kids, climatic and soil conditions, housing and care, and many other factors (*Memiši et al., 2011*).

Lactation length also differed, being shortest in V lactation (206 days) and longest in VI+ lactation (240 days), which also influenced variations in milk yield. Milk fat content was highest in first lactation, while proteins remained almost constant.

Conclusion

Based on the results obtained in the present study and compared to results of other authors it can be concluded that the overall productivity of Alpine goats raised under smallholder production systems in Serbia is satisfying. Giving the fact that these animals are usually kept under poor conditions, many of these productive traits are very good.

There is always room for improvement, but it must be kept in mind that high outputs need high inputs. Better housing, better diet, better health care and stricter selection could lead to productivity improvement, but with higher production cost. With the current trend in goat production in Serbia, with a lack of organized purchase of goat milk and unstable market for goat products, all major investments in the production would not be justified.

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Proizvodne karakteristike i telesne mere koza alpske rase gajenih na malim porodičnim gazdinstvima u Centalnoj Sbjiji

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Summary

Cilj ovog istraživanja bio je da se proceni postojeći nivo produktivnosti i da se utvrde vrednosti linearnih telesnih mera koza alpske rase koje se gaje u Srbiji kod individualnih poljoprivrednih proizvođača. U ispitivanje su uključena 22 poljoprivredna gazdinstva locirana u beogradskom okrugu, sa ukupno 330 koza alpske rase uzrasta 2-9 godina, 145 prvojarenica i 476 jaradi. Analizirane su sledeće osobine: telesna masa koza, telesna masa jaradi na rođenju, sa 30 dana uzrasta i pri odlučanju (90-120 dana), plodnost odraslih koza i prvojarenica, linearne telesne mere (visina grebena, dužina trupa, obim grudi, dubina grudi, širina grudi, širina karlice) i osobine mlečnosti (dužina laktacije, količina mleka za laktaciju, sadržaj mlečne masti i proteina). Prosečne vrednosti telesne mase i plodnosti ispitivanih kategorija bile su: telesna masa koza 54,96 kg, telesna masa jaradi na rođenju 2,73 kg, telena masa jaradi sa 30 dana 8,7 kg i telesna masa jaradi pri odlučanju 18,3 kg, plodnost odraslih koza 144%, plodnost prvojarenica 125%. Utvrđene su sledeće vrednosti telesnih mera: visina grebena 67,87 cm, dužina trupa 71,92 cm, obim grudi 81,79, dubina grudi 32,93 cm, širina grudi 21,49 cm i širina karlice 17,63 cm. Prosečna laktacijska mlečnost je iznosila 531,66 kg mleka u laktaciji od 221 dan, sa 3,33% mlečne masti i 3,16% proteina. Na osnovu utvrđenih rezultata i poređenjem sa rezultatima drugih autora zaključeno je da je produktivnost koza alpske rase gajenih na malim poljoprivrednim gazdinstvima zadovoljavajući. Plodnost koza je na nešto nižem nivou, kao i porođajne mase jaradi. Međutim, ako se ima u vidu da su uslovi gajenja ovih životinja često veoma

skromni, mnoge od ovih proizvodnih osobina su veoma dobre. Prostora za poboljšanje ima, ali uz veća ulaganja koja uslovljavaju i veću cenu proizvodnje. Ipak, uz nepostojanje organizovanog i zagarantovanog otkupa mleka koza, kao ni zaštitnih cena mleka, što bi proizvođačima pružilo neku sigurnost u proizvodnji, sva veća ulaganja u ovom trenutku ne bi bila isplativa.

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THE IMPORTANCE OF BUFFALO IN MILK PRODUCTION AND BUFFALO POPULATION IN SERBIA

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Original research paper

Abstract: This research paper gives an analysis on the size of world's domestic buffalo populations, their milk production and the size of buffalo population in Serbia. Population of domestic buffalo in the world is constantly increasing so that in 2013 there were 199 783 549 individuals, out of which in India in the same year they raised 57.77% of buffalo world population, in Pakistan 18.87%, and in China 11.64%. The share of total world production of buffalo milk in total world milk production in 2012 was 12.92 % or 97 417 135 t out of which 67.76% was produced in India. In Serbia buffalo is raised in the regions of Raška (about 1000 individuals) and Kosovo. Populations of buffalo in central Serbia show a tendency of decreasing in size what was the reason to start a programme of *in situ* conservation 10 years ago. On the sample of buffalo population encompassed by the programme of conservation the body measures were analysed indicating that the population of buffalo is quite unequalised and that average values obtained for exterior measures are similar to the results obtained by the authors of earlier period for the population of buffalo in the area of former Yugoslavia.

Key words: domestic buffalo, exterior, state in population

Introduction

Buffalo is the most distant relative of domestic beef cattle. They are at the lowest degree of evolutionary development. As wild animals they live in Asia and Africa while as domestic animals they live in Asia, Africa, Europe and South America, and as half domestic in Australia. According to most researchers and criteria for systematisation of this species, the division of buffaloes on Asian and African seems to be acceptable.

Based on: conventional morphological analysis (Bohlken, 1958), as well as craniological morphology (Groves, 1981), cited by Gates et al. (2010), subfamily of *Bovinae* se is divided into several genera, among which the two independent genera are: *Bubalus* (Asian buffalo) and *Syncerus* (African buffalo). There are also different divisions or sorting of buffaloes into separate genera or subgenera. Thus, according to Antoniusu (1922), cited by Mitić et al. (1987), *Bubalus* is divided into 3 subgenera: *Bubalus depressicornis*, *Bubalus mindorensis*, *Bubalus Bubalus* with two species: *Bubalus bubalis* (Asian buffalo) and *Bubalus caffer* (African buffalo). **Asian wild buffalo** (*Bubalus*) has three subgenera: Anoa buffalo with two varieties (*Bubalus depressicornis* and *Bubalus quarlesi*); Tamaru buffalo (*Bubalus mindorensis*); Asian buffalo – Indian buffalo- Arni (*Bubalus bubalis*).

African wild buffalo (*Syncerus caffer*) belongs to the genus *Syncerus*, within which there are two very different subgenera: 1) Black buffalo- savannah buffalo (*Syncerus caffer*, Cape buffalo) which has three varieties and 2) forest – red buffalo (*Syncerus caffer nanus*).

Domestic buffalo has an important place in agricultural production of Asia, Mediterranean countries and some African countries, such as for example in Egypt, as reported by Barakat and Alhimaidi (2012). Domestic buffaloes are raised in the regions with hot and humid climate, where cattle originating from *tura* cannot be raised. Buffaloes are resistant to many diseases being even genetically resistant to some ailments (Borriello et al., 2006).

Number of domestic buffaloes in the world is constantly increasing. According to data reported by Sambraus (2006) in 1998 in the world there was about 162 million domestic buffalo animals altogether. The countries with largest population of buffalo were India (91.8 million), China (27.8 million) and Pakistan (21.2 million). In Europe, in the same year, for example, in Italy, there was 162 000 animals, and in the republics of former Yugoslavia 16000 individuals.

Table 1. Number of cattle and buffalo in the world in 2013 (FAOSTAT, 2015)

Country	number cattle +buffalo	number of cattle		number of buffalo	
	Population (million)	Population (million)	% of population in the world	Population (million)	% of population in the world
World in total	1694132318	1494348769	100.00	199783549	100.00
Europe	122503662	122078279	8.17	425383	0.21
Asia	713844389	519972278	34.79	193872111	97.04
Africa	305277527	301077502	20.15	4200025	2.10
North America	101515311	101515311	6.79	-	-
South America	355325873	354046153	23.69	1279720	0.64
Oceania	40221756	40221546	2.69	210	0.0001
India	329770000	214350000	14.34	115420000	57.77
Pakistan	72000000	38300000	2.56	33700000	16.87
China	136890500	113636600	7.60	23253900	11.64

Since 1961 the number of buffalo in Asia has been constantly increasing, as well as a yield of milk quantities produced by buffalo cows in total world's milk production. According to FAO data (2015) in 2013 in India was raised 57.77% of world buffalo population, in Pakistan 18.87%, and in China 11.64%. In these three countries the number of buffalo is the highest. In India and Pakistan a constant rise of buffalo population since 1961 has been present, and it is predicted that due to rise in human population in these two countries the number of buffaloes will continue to increase and will in the highest degree determine the size of overall world population of buffalo.

Domestic buffalo, in many regions in which they are raised, are triple-purpose (milk, work, meat) animals. As draught animals, they are most useful in the countries where rice is grown since thanks to their wide and strong hooves, they can work well in swampy terrain covered with water and mud.

In total world milk production, according to FAO data, buffalo milk participates with about 12%, and according to data reported by *Pasha and Hayat* (2012), milk produced by buffalo cows yielded 12.75% of total world milk production. In South Asia, as reported by *Khan et al.* (2011), 85.4 million tons of milk were produced, of which 66.7% in India and 25.2% in Pakistan.

There are buffalo species with various production purposes (dairy type, draught type, meat type, combined type). The greatest variety of the types of species can be found in India (jaffarabadi, kundhi, mehsana, magpuri, nili, ravaja, deli and others). Buffalo cow milk yield is in the range of 1000 kg to 1300 kg, with about 18 % milk dry matter. Buffalo milk has a similar composition to sheep milk. Also there are selected buffalo populations and breeds with pronounced milk production traits (in India, Transcaucasia, Asia Minor) which can produce 4000 kg milk with 6 % to 8 % milk fat. *Khan et al.* (2011) reported that in dairy buffalo in Pakistan average daily milk yield was 5.5 liters. Mean content of fat in milk was 7.47 ± 0.87 %, lactose 5.24 ± 0.15 %, protein 3.31 ± 0.13 % and mineral matters 0.77 ± 0.02 %.

As a raw material milk is important for making dairy products in households. A production of homemade butter is particularly important, which in certain nations is used as a substitute for a lard in cooking. Buffalo milk can be successfully used for making various kinds of cheeses, while one of a well-known cheese, originally made from buffalo milk is "mozzarella". Because of the production of dairy products made by buffalo milk, which are thought to be delicacy, in Italy there is a stable population of buffaloes of a more pronounced milk production traits compared to other populations of buffalo in Mediterranean. For the same reasons (production of dairy products from buffalo milk), the buffalo farms are being established in the countries where they were not bred in the past (for example Great Britain). In European countries, buffaloes are raised in Greece, Albania, Italy, Bulgaria, Romania, former Yugoslav republics (Serbia, Montenegro, Macedonia) and in lesser degree in Hungary.

Buffalo meat is characterised by a rough and tough muscle fibres so it is of poorer quality than beef meat. Meat is dry, tough, of dark red colour and a specific taste because of which it is used more as processed meat. The meat of young buffaloes is similar to those of calves. The buffalo hide is the strongest of the hides of other large ruminants and much appreciated by a leather industry.

Buffalo in Serbia

Buffalo in Serbia, alike buffalo in Europe, originate from the Asian wild buffalo. Body mass of buffalo depends on the conditions of rearing, especially in early juvenile period when scarce nutrition can be negatively reflected on the size and body mass of adult animals. Body mass of buffalo cows whose growth is finished most often ranges between 450 kg and 600 kg although female buffalo of body mass of 700 kg can be found on the terrain. According to the records of previous period, reported by *Ogrizek* (1940/41), and cited by *Mitić et al.* (1987), the mass of buffalo was between 274-507 kg. The mass of young buffalo at birth was 25-40 kg, depending on the size of a dam and conditions of nutrition. Milk yield trait of buffalo cows, according to the results cited by *Mitić et al.* (1987) ranged from 700 kg to 1300 kg (in better conditions) with 7 % to 8 % milk fat.

In Serbia buffaloes are raised in the region of Raška (municipalities of the towns of Novi Pazar, Sjenica and Tutin) and in the region of Kosovo. In central Serbia (in aforementioned three municipalities) there are about 1000 individuals.

Materials and Methods

We have analysed data regarding the size of populations of buffalo in the world. On the basis of data available we have shown the share of buffalo milk in overall produced quantities of milk in the world and a share of production of the most important countries in overall world production of buffalo milk.

For the purposes of analysis of the state of domestic buffalo in Serbia the measures of body dimensions were taken on total of 37 females in the region of the municipalities of Novi Pazar and Sjenica. Buffalo population in these municipalities is included in the part of buffalo population involved in *in situ* programme for buffalo conservation.

Linear measures were taken in breeding females which had already had a calf. The values for total of 10 measures were determined (body mass, ridge height, body length, chest depth, chest width, chest girth, hip width, thigh width, buttocks width, loins height). For these linear measures we have calculated main parameters of descriptive statistics.

Results and Discussion

Population of buffalo raised in the region of Raška has a relatively uniformed appearance. Their head is narrow and long, horns are strong and they grow outward, go backwards then curve upward. The height of withers in females in the measured population was 125.97 cm ranging from 115 to 138 cm. The height of loins was 126.98 cm. Body length of examined population was on average 142.19 cm, so the trunk was of a quadratic appearance. Chest depth is well expressed, while the chest girth and trunk width are poorly expressed. A front part of the body is more developed, the line of the backs goes down to the buttocks. Buttocks are wide in the region of thigh bone knobs and narrow in the region of buttocks bones. At the same time, buttocks are short, and the root of the tail is placed low in the trunk. Chest depth on average was 68.75 cm, chest width 44.48cm, and chest girth 184.25 cm.

Table 2. Exterior measures of female buffalo

Traits	Average	Stand.dev.	Cv(%)	Max	Min
Age, months	71.48	38.79	54.26	180	24
Body mass, kg	464.13	63.13	13.60	600	350
Ridge height, cm	125.97	5.96	4.73	138	115
Loins height, cm	126.98	5.17	4.07	138	117
Body length, cm	142.19	9.83	6.91	167	122
Chest depth, cm	68.75	5.67	8.25	81	55
Chest width, cm	44.48	5.68	12.76	56	33
Chest girth, cm	184.25	41.42	22.48	208	165
Hips width, cm	50.5	4.90	9.69	58	38
Thigh knobs distance , cm	51.6	8.00	15.50	66	38
Buttocks knobs distance, cm	20.10	3.46	17.19	27	13

An average female buffalo body weight was 464.13kg, with great variations existing (13.16%). Such a large discrepancy in buffalo body weight could be understood as a consequence of inadequate and diverse conditions of nutrition and care. The results obtained for exterior measures are similar to the results established by the authors in a much earlier period for buffalo population in the region of former Yugoslavia, cited by *Mitić et al.* (1987). This can all lead to a conclusion that in a previous period a breeding and selection work aimed to improve productive and therefore also exterior characteristics of buffalo which are now being raised in our country were not conducted.

Buffalo is mostly raised on farms in combination with beef cattle. There is an effort to produce sufficient quantity of butter from buffalo milk to last throughout a whole year. Because of that there is a practice among farmers not to let a baby buffalo suckle their dams but to feed them with bovine milk (by suckling

or bottle feeding) while the milk of female buffalo is taken for making dairy products immediately after the colostrum period.

Population of buffalo in central Serbia shows a decreasing tendency and due to this fact in a previous 10 year period the programme to subsidise the raising of buffalo was conducted in order to preserve the population. In most cases the programme of *in situ* conservation of buffalo was conducted (by selection and control of breeding and reproduction of buffalo in native region of breeding). There are breeders in other regions outside Raška who raise buffalo in a lesser number and they are included in the *ex situ* programme of conservation. There are some real threats that native buffalo, according to FAO criteria for preserving genetic resources, will become an endangered species, and then a critical population in Serbia.

Breeding of buffalo in Serbia in previous decades was unorganised and unsystematic. There was almost no control of productivity so that a production traits and production potential of buffalo are quite unknown. Breeding and selection work in buffalo has never been conducted except for natural selection on the resistance to ultimately extensive breeding conditions. All these resulted in very low production of milk of native females (1000-1300kg), which in Serbia is several times lower in relation to milk production realised by selected populations of buffalo in some Asian countries.

Table 3. Production of milk in the world in 2012 (ton), Faostat (2015)

Milk	World total, t	% of total world milk production	Production in Europe, t	% of total world milk production	Production in Asia, t	% of total world milk production	Production in India, t	% of total world milk production
Milk total	753925418	100	216089387	28.66	279666027	37.09	124850000	16.56
Milk, whole fresh cow	625754261	82.99	210336776	33.61	169765010	27.13	54000000	8.63
Milk, whole fresh buffalo	97417135	12.92	200706	0.21	94566429	97.07	66000000	67.75
Milk, whole fresh goat	17846118	2.37	2536773	14.22	10410137	58.33	4850000	27.18
Milk, whole fresh sheep	10122522	1.34	3015062	29.79	4729861	46.73	-	-
Milk, whole fresh camel	2785382	0.37	70	0.003	194590	6.99	-	-

Population of buffalo in the world is increasing therefore the production of buffalo milk is increasing as well. According to FAO (2015) participation of overall world production of buffalo milk in total world production of milk in 2012 was 12.92 % (Table 3). Of total world production of buffalo milk (97 417 135 t) 67.76% was produced in India in 2012.

Conclusion

The population of domestic buffalo in the world is on a constant rise and in 2013 there were 199 783 549 individuals, out of which number in India in 2013 was raised 57.77% of world buffalo population. The participation of total world production of buffalo milk in overall world production of milk in 2012 was 12.92 % or 97 417 135 t, out of which 67.76% was produced in India.

In Serbia buffalo is raised in the regions of Raška (about 1000 animals) and Kosovo. Primary importance of raising buffalos in the world and in our country is production of milk and butter as major products.

On the sample of the population included in the programme of *in situ* conservation body measures were analysed which indicate that population of buffalo is rather unequalised and that average values obtained for exterior measures are similar to the results established by the authors in a much earlier period in populations of buffalo in the area of former Yugoslavia. This can lead to a conclusion that breeding and selection work in buffalo in Serbia in earlier decades was not conducted except for a natural selection on resistance to extremely extensive breeding conditions.

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Značaj bivola u proizvodnji mleka i stanje populacije u Srbiji

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Rezime

U radu je analizirano brojno stanje domaćih bivola u svetu, proizvodnja mleka bivola, kao i stanje populacije bivola u Srbiji. Populacija domaćih bivola u svetu stalno raste i 2013. godine bilo je 199.783.549 grla, a od tog broja u Indiji je u 2013. godini gajeno 57,77% svetske populacije bivola, u Pakistanu 18,87%, a u Kini 11,64%. Učešće ukupne svetske proizvodnje bivoljg mleka u ukupnoj svetskoj proizvodnji mleka 2012. godine bilo je 12,92 % ili 97.417.135 t, a od toga je 67,76% proizvedeno u Indiji.

U Srbiji bivoli se gaje u području Raške oblasti (oko 1000 bivola) i na području Kosova. Populacija bivola u centralnoj Srbiji ima tendenciju smanjenja veličine, zbog čega je pre 10 godina počeo da se sprovodi program konzervacije. Na uzorku populacije (37 bivolica) obuhvaćene programom *in situ* konzervacije utvrđene su prosečne vrednosti za telesne mere. Visina grebena bivolica u populaciji koja je merena bila je 125,97 cm, visina krsta 126,98cm, dužina trupa 142,19cm, dubina grudi 68,75 cm, širina grudi 44,48cm, a obim grudi 184,25 cm. Populacija bivolica je bila dosta neujednačena što ukazuje na odsustvo odgajivačko-selekcijskog rada kod bivola u Srbiji u prethodnim decenijama.

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COMPARATIVE STUDY OF FATTENING AND SLAUGHTER TRAITS OF MALE SIMMENTAL BREED AND CROSSES WITH CHAROLAIS BREED

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Original scientific paper

Abstract: The objective of this study was to determine the slaughter traits, conformation score and fat covering of carcass and composition of carcasses of young cattle of two genotype groups: domestic Simmental breed (A) and its crosses with Charolais breed (B). The sample included a total of 30 animals, 15 in each group. Both groups were slaughtered at final weight of about 660 kg. After the slaughtering, warm carcass sides with and without kidney fat were weighed individually. After cooling, the left carcass sides were cut into main parts according to the Rulebook ("Off. Gazette of SFRY", No. 34/74, 26/75, 13/78 - Rulebook, 1/81 - Rulebook and 2/85 - Rulebook). The results of research show that the young cattle of group (B) achieved a statistically significant ($p < 0.05$) higher yield of warm carcass compared to group A, and statistically highly significant ($p < 0.01$) higher yield of warm carcass without tallow/fat. A statistically significant difference was found in the share of tongues ($p < 0.01$), which was higher in young cattle of group (B) and a statistically significant difference in the share of offal ($p < 0.01$), which was higher in group (A). Shares of tenderloin and the shoulder of young cattle of group (B) were statistically significantly ($p < 0.05$) higher than in young cattle of group (A). A statistically significant difference was determined in carcass conformation scores between groups of young cattle.

Key words: slaughter traits, carcass side composition, carcass conformation score

Introduction

The importance of meat in human nutrition is well known and meat is considered the indispensable and the best-quality component of proper and well balanced diet (*Biesalski, 2005*). Beef is characterized by exceptional nutritional

value, which sets it apart from other types of meat and makes highly respected food (Petrović et al., 2002).

Fattening traits and carcass/slaughter traits, are the basic characteristics of every breed which influence directly the quantity and quality of the final product. Based on data on body development, the amount of bone in the body, the meat : bone ratio, as well as the total amount of meat can be more or less predicted (Ostojić-Andrić et al., 2007).

The use of domestic Simmental breed as the basis for crossbreeding with specialized beef cattle breeds is the fastest and most economical way to improve the fattening and slaughter traits of cattle (Mišćević et al., 2003; Bogdanović et al., 2005). French beef breeds like Charolais are characterized by favorable fattening and slaughter traits, as well as good quality meat with a low fat content in carcasses, which is why they can be fattened to higher final weights ((Ostojić-Andrić et al., 2007).

Number of slaughtered cattle in the Republic of Serbia has been steadily declining (Aleksić et al., 2007). The continuing decline in the number of cattle leads to reduction in the number of calves for fattening (Aleksić et al., 2005 and Aleksić et al., 2012). In European Union member states, a deficit of beef is established with the estimate that the deficit will amount to 600,000 tons. Serbia today exports only 1,000 tons, even though the permitted export preferential quota in the EU is 8,870 tons. For comparison it should be noted that the largest beef export was achieved in 1985 and amounted to 20,000 tons, which is about 20 times more than today. All this points to the need that it is necessary to take advantage of cattle population of lower production characteristics and apply new methods in breeding, nutrition and reproduction in order to obtain a larger number of quality calves for fattening. One quick and efficient way to produce quality calves for fattening is by application of the method of industrial crossing with French beef cattle breeds, as well as through increase of pre-slaughter body weight to provide more meat per animal.

In this trial, final pre-slaughter weight of beef cattle amounted to approximately 660 kg. In the current production practices cattle are mainly fattened to final weight of 450 kg. In this way, we can provide a greater amount of meat per animal without compromising the quality of beef. In particular, given the reduction of breeding animals in Serbia, it is essential that future technologies are based on the crossing and increase of pre-slaughter body weight, in order to compensate for the reducing number of cows and heifers and thus the number of calves for fattening (Aleksić et al., 2005).

Materials and Methods

The trial was conducted at the experimental farm of the Institute for Animal Husbandry (Belgrade, Serbia). Two groups of male calves group were formed: A (n = 15) Simmental breed and group B (n = 15) of the F1 generation with Charolais breed. Both groups of cattle were fed a combined meal which consisted of a mixture of corn silage *ad libitum* according to the table of nutrition depending on the weight group. Final weight before slaughter was about 660. One day prior to slaughter young cattle did not receive food, but had free access to water. Slaughtering and primary processing were carried out in the experimental slaughterhouse of the Institute for Animal Husbandry. Animals were weighed immediately before slaughter, and then slaughtered according to standard commercial procedures. After primary treatment, carcasses were placed in cold storage at 4°C for another 24 hours. Warm carcass weight, weight of offal (heart, lungs, liver, kidneys, spleen and tongue), head, tail and kidney fat were measured one hour after slaughtering and processing. After chilling, the carcasses were measured and split along the vertebral column in two halves, and the left side was used for all measurements. The left side of each carcass was divided into twelve anatomical regions: round/leg, tenderloin, loin part, shoulder, back, neck, brisket, undershoulder, ribs, flank (belly), forshank and hindshank, using a standard technique. Carcass scores were determined based on two systems: JUS (carcass conformation and covering of carcass with fat tissue - measured using a scale of 1 to 5, where 1-very low; 2-low; 3-average; 4-high and 5-very high) and Europe (beef conformation and covering of carcass and round/leg with fat tissue measured using a scale from 1 to 5, where 5 (E) - Great, 4- (U) Very good, 3 - (R) Good, 2 - (O) - Moderate or 1- (P) -Poor).

The data obtained for certain parts were processed using the variance analysis of the single factorial experiment (One-way ANOVA) by SPSS Statistics 20. The statistical significance of differences between mean values was determined by t-test.

Results and Discussion

Average values of the slaughter results of the studied beef cattle group (A) and group (B) are shown in Table 1. The results show that the cattle of group (B) achieved higher carcass yield by 2.11% in relation to young cattle of group (A). *Kamieniecki et al. (2009)* state that the carcass yield of crosses between domestic Simmental and Charolais breed is 58.8%. *Oprządek et al. (2001)* come to similar results in their research.

Table 1. Average values of slaughter traits of young cattle

Indicator	A	B	t-test
Pre-slaughter mass (kg)	672.00 ± 16.67	644.12 ± 54.03	ns
Daily gain (g)	1436.82 ± 199.17	1699.20 ± 423.36	ns
Warm carcass mass (kg)	390.05 ± 9.27	385.72 ± 37.92	ns
Warm carcass yield (%)	58.07 ± 1.36	60.18 ± 2.20	*
Warm carcass mass without fat/tallow (kg)	408.55 ± 95.15	379.71 ± 37.86	ns
Warm carcass yield without fat/tallow (%)	56.93 ± 1.37	59.24 ± 2.04	**

ns – not significant

* significant at the level of ($p < 0.05$)

** significant at the level of ($p < 0.01$)

Share offal was not significantly different between groups of young cattle (Table 2). A statistically significant difference was found in the share of tongues ($P < 0.01$), which was 0.30% higher compared to young cattle of group B.

Aleksić et al. (2002) come to similar results. Data obtained are consistent with the study of *Aleksić et al. (2009)* for young cattle of domestic Simmental breed.

Table 2. Share of slaughter by-products

Share of carcass (%)	A	B	t-test
Kidney fat/tallow	1.12 ± 0.45	0.79 ± 0.43	ns
Liver	1.17 ± 0.11	1.10 ± 0.09	ns
Lungs	0.53 ± 0.08	0.58 ± 0.09	ns
Spleen	0.19 ± 0.03	0.17 ± 0.03	ns
Kidneys	0.18 ± 0.02	0.17 ± 0.02	ns
Tongue	0.22 ± 0.03	0.30 ± 0.06	**
Heart	0.37 ± 0.04	0.36 ± 0.06	ns
Head	2.37 ± 0.15	2.39 ± 0.20	ns
Tail	0.18 ± 0.04	0.20 ± 0.04	ns
Skin	8.35 ± 0.77	9.17 ± 0.66	ns

ns – not significant

* significant at the level of ($p < 0.05$)

Carcass conformation scores showed no statistical significance between the groups (Table 3). Scoring of conformation and fat covering of beef carcasses has great significance in the contemporary systems of carcass quality assessment. According to research of *Ostojić-Andrić et al. (2011)* Charolais crosses with domestic Simmental breed had carcass conformation score 3.94 and covering of

round/leg 3.77. In the research by *Chambaz et al. (2003)*, carcasses obtained from Simmental cattle demonstrated the poorest conformation compared to Charolais and Limousin carcasses which were significantly heavier. *Karolyi et al. (2006)* suggest that the young cattle of domestic Simmental breed have shown favorable carcass conformation with the highest scores.

Table 3. Evaluation of beef carcass conformation using two systems (jus and eu)

Trait	A	B	t-test
Conformation (jus)	4.95 ± 0.15	4.90 ± 0.20	ns
Covering (jus)	4.23 ± 0.41	4.20 ± 0.24	ns
Meat colour (jus)	4.59 ± 0.49	4.50 ± 0.45	ns
Conformation (eu)	4.95 ± 0.15	4.90 ± 0.20	ns
Covering of carcass (eu)	4.18 ± 0.40	4.00 ± 0.32	ns
Covering of leg/round (eu)	4.32 ± 0.40	4.10 ± 0.20	ns
Colour of fat/tallow (eu)	4.09 ± 0.20	4.10 ± 0.20	ns

ns – not significant

The share of parts of extra category (tenderloin) and category II (loin, back, shoulder) were significantly different between groups of young cattle (Table 4). A statistically significant difference ($P < 0.05$) was found in the share of steak that was higher in young cattle of group B (1.27%) compared to young cattle of group A (1.00%). Share of shoulder was lower in young cattle of group A (12.20%) compared to young cattle of group B (13.82%). Similar results are obtained by *Oprządek et al. (2001)*. *Oprządek et al. (2001)* suggest that the share of the shoulder in young cattle of domestic Simmental crosses with Charolais breed is 15.40%.

Table 4. Share of main beef carcass parts

Carcass parts (%)	A	B	t-test
Round/leg	28.36 ± 1.55	28.82 ± 1.20	ns
Hind shank	3.59 ± 0.40	3.72 ± 0.51	ns
Tenderloin	1.00 ± 0.17	1.27 ± 0.29	*
Back-loin part	9.71 ± 1.44	10.33 ± 1.30	ns
Shoulder	12.20 ± 1.25	13.82 ± 1.85	*
Fore shank	2.73 ± 0.28	3.07 ± 0.59	ns

ns – not significant

* significant at the level of ($p < 0.05$)

Conclusion

Based on the research results, statistically significant effect of genotype on slaughter traits and certain parts of the carcass and slaughtering by-products can be concluded. Based on the results presented in this paper it can be stated that young cattle of group (A) and young cattle of group (B) do not differ significantly in their carcass conformation scores. A statistically significant difference was found in a higher share of yield in young cattle of group (B), which amounted to 59.24%, in the share of by-products: tongue, which was higher in young cattle of group (B) – 0.30%; and statistically significant difference was established in the share of offal-cuts that was higher in young cattle of group (A) – 0.54%. The share of basic carcass parts differed between groups. Share of tenderloin and shoulder was higher in young cattle of group (B) and amounted to 1.27% and 13.82% respectively.

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Usporedno ispitivanje tovnih i klaničnih osobina muške junadi simentalske rase i meleza šarolea sa simentalskom rasom

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Rezime

Cilj ovog istraživanja je bio da se utvrde klanične osobine, ocena konformacije i prekrivenosti trupova lojem i sastav polutki junadi dve genotipske grupe: domaće simentalske rase (A) i njenih melaza sa šarole rasom (B). Uzorkom je obuhvaćeno ukupno 30 grla, po 15 u svakoj grupi. Obe grupe su zaklane pri dostizanju težine oko 660 kg. Nakon klanja izvršeno je pojedinačno merenje toplih polutki sa i bez bubrežnog loja. Posle hlađenja leva polutka je rasecana u osnovne delove prema pravilniku ("Sl. list SFRJ", br. 34/74, 26/75, 13/78 – dr. pravilnik, 1/81 – dr. pravilnik i 2/85 – dr. Pravilnik). Dobijeni rezultati istraživanja pokazuju da su junad grupe (B) ostvarila statistički značajno ($p < 0.05$) veći randman toplog trupa u poređenju sa grupom A, kao i statistički vrlo značajno ($p < 0.01$) veći randman toplog trupa bez loja. Statistički značajna razlika je pronađena u udelu

jezika ($p < 0.01$), koji je bio veći kod junadi grupe (B) i statistički značajna razlika u udelu obrezaka ($p < 0.01$), koji je bio veći kod grupe (A). Udeo bifteka i udeo plečke kod junadi grupe (B) su statistički značajno ($p < 0.05$) bili veći nego kod junadi grupe (A). Statistički značajna razlika nije utvrđena kod ocene konformacije trupova između grupa junadi.

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THE EFFECT OF INTENSE LIGHT PULSES ON THE SENSORY QUALITY AND INSTRUMENTAL COLOR OF MEAT FROM DIFFERENT ANIMAL BREEDS

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Abstract: Intense light pulses (ILP) are an emerging processing technology, which has a potential to decontaminate food products. The light generated by ILP lamps consists of a continuum broadband spectrum from deep UV to the infrared, especially rich in UV range below 400 nm, which is germicidal. Evaluation of the effect of intense light pulses (ILP) on sensory quality of meat, game and poultry was performed using two kinds of red meat (beef and pork), two kinds of poultry (chicken and turkey) and three game meat samples (deer, rabbit and kangaroo). All the samples were treated with 1 and 5 light pulses (pulse duration of 300 μ s and pulse intensity of 3.4 J/cm²) at a rate of one pulse per 2 seconds. Sensory quality changes induced by intense light pulses were different and depended on animal species, type of meat and ILP dose applied. Only the odour of all the meat, poultry and game samples suffered significant changes after the pulsed light treatment. Of all kinds of meat investigated only turkey received scores below the good quality grade after the treatment. Instrumental colour values remained unaffected in chicken and rabbit meat samples while higher doses of ILP significantly compromised both redness and yellowness only in pork and turkey meat.

Keywords: intense light pulses, meat, game, poultry, sensory quality, colour

Introduction

Intense light pulses (ILP), also known as pulsed light (*Oms-Oliu et al., 2010*), high intensity broad spectrum pulsed light (*Roberts and Hope, 2003*), pulsed white light (*Kaack and Lyager, 2007; Marquenie et al., 2003*) and pulsed UV light (*Bialka and Demirci, 2007, 2008; Keklik et al., 2009*) are included among the emerging technologies that are intensely investigated as an alternative to thermal treatment for killing pathogenic and spoilage microorganisms (*Barbosa-Canovas et*

al., 2000; *Elmnasser et al.*, 2007; *Gomez-Lopez et al.*, 2007; *Palmieri and Cacace*, 2005; *Woodling and Moraru*, 2005).

The inactivation mechanism of ILP is similar to that of continuous UV-C light; it causes the formation of thymine dimers which renders microbial cells unable to replicate; this is called the photochemical effect (*Gómez-López*, 2012). Additionally, photophysical and photothermal effects have been identified (*Krishnamurthy et al.*, 2010). Its big advantage is that it can inactivate microorganisms very fast. Different studies have demonstrated the sensitivity of bacteria to ILP on meat (*Hierro et al.*, 2012), poultry (*Keklik et al.*, 2010; *Paskeviciute et al.*, 2011) meat products (*Ganan et al.*, 2013; *Hierro et al.*, 2011), meat contact surfaces (*Rajkovic et al.*, 2010) and seafood (*Cheigh et al.*, 2013; *Ozer and Demirci*, 2006). However, if microbial inactivation is a critical requirement, it is also essential to keep the nutritional and sensory properties of the product, minimizing the possible loss of quality caused by the treatment (*Hierro et al.*, 2012).

The aim of this study was to systematically evaluate the effect of intense light pulses (ILP) on sensory quality and color of 7 different varieties of meat, game and poultry.

Materials and Methods

Samples preparation

Two kinds of red meat (beef and pork), two kinds of poultry (chicken and turkey) and three game meat samples (deer, rabbit and kangaroo) were used in this study. All of the samples used were purchased from a local retailer and kept refrigerated at $2\pm 2^{\circ}\text{C}$ until treated. All the fresh meat, poultry and game was cut into 10 cm chunks before the ILP treatment.

ILP equipment and treatment

The ILP treatments were performed using a laboratory-scale batch-fed pulsed-light system unit: Tecum - Mobile Decontamination Unit (Claranor, Manosque - France). Light pulses with duration of 300 μs and pulse intensity of 3.4 J/cm^2 , measured with SOLO 2 - Power and Energy Meter (Gentec Electro-Optics, Inc., Quebec, Canada), were generated by four 20 cm cylindrical Xenon flash lamps (Flashlamps Verre & Quartz, Bondy, France), with an input voltage of 3000 V.

The samples were ILP-treated with 1 pulse (1P) and 5 pulses (5P) at a rate of one pulse per 2 seconds, respectively. During treatments, samples were placed in the system unit at a distance of 6 cm from the top and bottom lamps, and 10 cm from the left-hand and right-hand lamps. No treatment was applied to the control groups of samples.

Sensory Analyses

Sensory evaluation was performed by a professional panel of eight panelists, members of the Department of Food Safety and Food Quality-University of Ghent, Belgium and of the Meat Science and Technology Department-University of Belgrade, Serbia. The panel was trained according to international standards (*ISO, 1993*) and additionally trained for three days in the sensory assessment of meat and meat products by a panel leader with over 2,000 h of sensory testing experience of meat and meat products.

Sensory tests were performed in a controlled sensory analysis laboratory (Food Safety and Food Quality Department/University of Ghent - Belgium) built in accordance to the general guidance for the design of test rooms intended for the sensory analysis of products (*ISO, 2007*) with individual booths equipped with computer terminals and provided with red light to mask any differences in color when needed.

Five-Point-Scale Scoring Method

The test was carried out as described by *Tomic et al. (2008)* with slight modifications. Selected sensory attributes (Table 1) were assessed using the 5-point scale with the following descriptions: 5=(excellent, typical quality, without visible defects); 4=(good quality, with minimal visible defects); 3=(neither good nor poor quality, still can be used for its intended purpose); 2=(poor quality, reworked could be used for its intended purpose); and 1=(unacceptable, extremely poor quality, cannot be used for its intended purpose), with ability of giving semi scores (4.5, 3.5, 2.5 and 1.5). Scores given to each of assessed attributes were corrected by corresponding coefficients of importance (Table 1).

Table 1. Selected sensory attributes of the samples assessed using the 5-point scale, with corresponding coefficients of importance (CI)

Meat, poultry & game: Beef, Pork, Chicken, Turkey, Deer, Rabbit, Kangaroo		Meat products:		
			Cooked ham, Parisian sausage, Bacon	Parma ham, Fermented sausage
Attribute	CI	Attribute	CI	
Appearance	7	Appearance	4	4
Color	8	Color	5	4
Odor	5	Odor and Taste	7	8
		Texture and Juiciness	4	4

Coefficients of importance (CI) show the relative importance of a single sensory attribute to the total sensory quality. Sum of all CIs is arranged to be 20, and in that way the sum of corrected scores gives the "percentage of total sensory

quality" in a given situation. Dividing the total value by the sum of CI gives the "pondered average value of total sensory quality". A section in the score card was included for panelists to leave their comments.

Instrumental color measurement

Instrumental color readings of samples were measured using a Konica Minolta spectrophotometer CM-2500d (Konica Minolta, Osaka, Japan), operating in the CIE $L^*a^*b^*$ color space. The L^* (lightness), a^* (redness) and b^* (yellowness) values (a single repetition) were determined from the mean of 10 random readings on the surface of each sample, using D_{65} illuminant and 10° standard observer. The measurement was repeated in triplicate ($n=3$) and the values averaged. The instrument was calibrated with a white calibration tile and black calibration box. Data acquisition was performed using the Spectramagic NX color data software, version 1.52 (Osaka, Japan).

Statistical analysis

Data entry and decoding were 100% verified. A one-way ANOVA was conducted to compare the results of the different assays, using SPSS Statistics 17.0 (Chicago, Illinois, USA) data analysis software. An alpha level of $p<0.05$ was used to determine significance.

Results and Discussion

Five-Point-Scale Scoring Method

ILP treatment did not significantly change ($p<0.05$) appearance and total score values of the beef samples (Table 2). The color score also remained unchanged regardless of the level of fluence applied which is in contrast of the findings of *Hierro et al. (2012)* where the color of beef was assessed by panel members as slightly lighter after the treatment of 11.9 J/cm^2 . The application of 1 pulse (3.4 J/cm^2) in our investigation significantly decreased score for odor of beef while the same happened only after 8.4 J/cm^2 when applied to beef *carpaccio* in the experiments of *Hierro et al. (2012)*. The similar in both investigations was the fact that the beef odor was assessed as acceptable in both cases even after the highest fluency rate applied.

Table 2. Sensory evaluation scores (mean±SD) for 5-Point-Scale Scoring test of the ILP treated meat, poultry and game

		Beef	Pork	Chicken	Turkey	Deer	Rabbit	Kangaroo
Control	Appearance	4.9 ±0.2	4.9 ±0.2	4.9 ±0.2	4.4 ±0.2	4.9 ±0.2	4.9 ±0.2	4.9 ±0.2
	Color	4.9 ±0.2	4.9 ±0.2	4.9 ±0.2	4.4 ±0.2	4.9 ±0.2	4.9 ±0.2	4.9 ±0.2
	Odor	4.9 ±0.2 a	4.9 ±0.2 a	4.9 ±0.2 a	4.4 ±0.2 a	4.9 ±0.2 a	4.9 ±0.2 a	4.9 ±0.2 a
	Total score	4.9 ±0.2	4.9 ±0.2	4.9 ±0.2	4.4 ±0.2	4.9 ±0.2 a	4.9 ±0.2	4.9 ±0.2
1 pulse	Appearance	4.9 ±0.2	4.9 ±0.2	4.9 ±0.2	4.2 ±0.3	4.9 ±0.2	4.9 ±0.2	4.9 ±0.2
	Color	4.9 ±0.2	4.9 ±0.2	4.9 ±0.2	4.3 ±0.3	4.9 ±0.2	4.9 ±0.2	4.9 ±0.2
	Odor	4.4 ±0.2 b	4.6 ±0.4 a,b	4.4 ±0.4 b	3.8 ±0.3 b	4.4 ±0.2 b	4.4 ±0.2 b	4.6 ±0.4 a,b
	Total score	4.8 ±0.2	4.8 ±0.3	4.8 ±0.2	4.1 ±0.2	4.8 ±0.1 a,b	4.8 ±0.2	4.8 ±0.3
5 pulses	Appearance	4.9 ±0.2	4.9 ±0.2	4.7 ±0.4	4.1 ±0.7	4.8 ±0.3	4.9 ±0.2	4.9 ±0.2
	Color	4.9 ±0.2	4.6 ±0.4	4.7 ±0.4	4.1 ±0.7	4.7 ±0.3	4.9 ±0.2	4.7 ±0.3
	Odor	4.4 ±0.2 b	4.3 ±0.3 b	4.2 ±0.4 b	3.4 ±0.2 b	4.1 ±0.2 b	3.9 ±0.2 c	4.4 ±0.2 b
	Total score	4.8 ±0.2	4.6 ±0.3	4.6 ±0.4	3.9 ±0.5	4.6 ±0.1 b	4.7 ±0.2	4.7 ±0.0

a,b,c Values in the same column with different letter are significantly different ($p < 0.05$)

According to our results the odor of beef meat is a bit more sensitive to the ILP than the odor of pork meat, because the odor scores for pork meat have significantly decreased only after the 5-pulses treatment. For the poultry, the only sensory attribute affected by the ILP treatment was odor but not to such extent that could also affect the pondered average values of the total sensory quality for the chicken and turkey meat (Table 2). Similar was found by *Paskeviciute et al. (2011)* where UV light dose higher than 6 J/cm^2 had only some moderate effect on odor of chicken. The odor scores significantly decreased in all game meat samples after the 5-pulses treatment but they were most easily observable in deer meat and essentially contributed to the significant change of its pondered average value of total sensory quality. The effect of the treatment on odor was least pronounced in kangaroo meat. The panelist's comments were unanimous that the effect of ILP on game meat was reflected only by subtle changes in its naturally sour odor.

Instrumental color measurement

The instrumental color values of beef meat were not affected by 1-pulse treatment, since no significant differences ($p>0.05$) were observed (Table 3). Treatment of 5 pulses significantly decreased redness in beef, while no significant differences were observed for lightness and yellowness. In beef *carpaccio* subjected to ILP, Eva *Hierro et al.* (2012) also observed decrease in a^* values but they were followed with the significant differences in b^* value when the samples were treated with fluences equal to or higher than 8.4 J/cm^2 .

Table 3. Instrumental color values (mean \pm SD) of the ILP treated meat, poultry and game

		Beef	Pork	Chicken	Turkey	Deer	Rabbit	Kangaroo
Cont rol	L^*	42.0 \pm 1.0	54.7 \pm 0.5	58.1 \pm 1.1	53.0 \pm 0.5	33.4 \pm 0.9	57.3 \pm 1.0	35.4 \pm 0.1a
	a^*	16.2 \pm 1.1a	11.1 \pm 0.3a	0.2 \pm 0.3	3.8 \pm 0.1a	9.2 \pm 0.1a	0.5 \pm 0.0	13.1 \pm 0.4
	b^*	14.6 \pm 0.1	16.6 \pm 0.1a	8.8 \pm 1.0	10.3 \pm 0.2a	9.2 \pm 0.1	7.1 \pm 0.2	9.3 \pm 0.1a
1 pulse	L^*	42.3 \pm 1.2	54.2 \pm 0.5	56.8 \pm 0.8	53.0 \pm 0.5	33.0 \pm 1.0	58.1 \pm 1.1	34.6 \pm 0.1b
	a^*	15.8 \pm 0.7a,b	11.0 \pm 0.3a	0.2 \pm 0.3	3.2 \pm 0.1b	9.3 \pm 0.2a	0.5 \pm 0.8	13.2 \pm 0.5
	b^*	13.9 \pm 1.1	16.4 \pm 0.3a	8.7 \pm 0.6	10.2 \pm 0.3a	9.1 \pm 0.3	7.5 \pm 0.3	9.3 \pm 0.1a
5 pulses	L^*	42.5 \pm 1.0	53.8 \pm 0.2	56.5 \pm 1.3	53.0 \pm 0.4	32.9 \pm 0.1	59.3 \pm 1.1	34.1 \pm 0.1b
	a^*	14.1 \pm 0.4b	9.9 \pm 0.1b	0.1 \pm 0.0	2.7 \pm 0.2c	8.6 \pm 0.3b	0.1 \pm 0.0	12.4 \pm 0.3
	b^*	13.4 \pm 0.6	15.3 \pm 0.1b	8.6 \pm 0.1	9.5 \pm 0.3b	8.7 \pm 0.3	7.4 \pm 0.1	8.7 \pm 0.1b

^{a,b,c} Values in the same column with different letter are significantly different ($p<0.05$)

The same was the case in our investigation with the pork meat treated with 17 J/cm^2 when both values, a^* and b^* , significantly decreased after the treatment. Chicken color values were not significantly changed ($p>0.05$) irrespective of the level of treatment. This is in agreement with the results of *Keklik et al.* (2010) indicating that mild and moderate pulsed light treatments also did not affect the color of chicken samples ($p>0.05$), although extreme ILP treatment did increase the lightness (L^*), redness (a^*), and yellowness (b^*) of samples significantly ($p<0.05$). The a^* value of treated turkey samples were significantly lower than that of the untreated samples with the significant difference observed among the fluences assayed. The redness gradually decreased as fluence increased. The yellowness was found significantly lower to control samples only after the treatment of 5 pulses. Similar ILP color resistance to the one of chicken meat, in our experiment, was observed only in rabbit meat samples (Table 5). Dear meat suffered significant decrease in redness value after the 5-pulses treatment while the kangaroo meat was significantly lower in L^* (after 1 pulse) and in b^* (after 5 pulses).

Conclusion

Our study indicated that the sensory quality changes induced by intense light pulses are different and depend on animal species, type of meat and ILP dose applied. Only the odor of all the meat, poultry and game samples suffered significant changes after the pulsed light treatment. Of all kinds of meat investigated only turkey received scores below the good quality grade after the treatment. Instrumental color values remained unaffected in chicken and rabbit meat samples while higher doses of ILP significantly compromised both redness and yellowness only in pork and turkey meat.

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Efekat intenzivnih svetlosnih pulseva na senzorni kvalitet mesa, divljači i živine

I. Tomašević

Rezime

Ispitivanje efekata dekontaminacione tehnike intenzivnih svetlosnih pulseva na senzorni kvalitet i boju mesa obavljeno je na dve vrste crvenih (govedina i svinjetina), na dve vrste mesa (piletine i ćuretina) i na tri vrste mesa divljači (jelen, zec i kengur). Sve vrste uzoraka tretirane su sa 1 i 5 svetlosnih pulseva (dužina trajanja pulsa 300 μ s uz intenzitet pojedinačnog pulsa od 3.4 J/cm²) učestalošću od 1 pulsa svake dve sekunde. Senzorni kvalitet mesa varirao je u odnosu na vrstu mesa i jačinu primenjenog tretmana. Miris je jedini senzorni atribut koji je kod svih vrsta ispitivanog mesa pretrpeo značajne promene nakon primenjenog tretmana. Samo je ćureće meso ocenjeno kao "ispod prosečnog kvaliteta" nakon promena pretrpljenih dejstvom svetlosnih pulseva. Instrumentalne vrednosti boje ostale su

nepromenjene kod pileline i zečijeg mesa dok je jači primenjeni tretman značajno izmenio vrednosti udela crvene i žute boje samo kod svinjskog i ćurećeg mesa.

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FATTY ACID COMPOSITION OF MEAT FROM THE HIND LEG CUT OF RABBITS (*ORYCTOLAGUS CUNNICULUS*) FED DIETS CONTAINING GRADED LEVELS OF PROCESSED TALLOW (*DETARIUM MICROCARPUM*) SEED MEAL

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Abstract: Eighty one (81) weaned rabbits of mixed breeds and sexes (male and female) were randomly allotted to nine treatment groups with nine rabbits per treatment. Each treatment had three replicates with three rabbits per replicate. Processed tallow was included in the diets as a source of protein which was set at 16 % CP. The control diet had 100 % palm kernel cake (PKC) and 0 % tallow seed meal (TSM). Diets 1 – 4 contained cooked tallow seed meal (CTSM) included at 75 % PKC: 25 % CTSM, 50 % PKC: 50% CTSM, 25% PKC:75 % CTSM and 0 % PKC: 100 % CTSM, while groups 5 – 8 had fermented tallow seed meal diets (FTSM) and included at the same levels as in the cooked diets. Fifty four rabbits were randomly selected for slaughtering from the nine groups with six rabbits (male and females) per group. Fatty acid content of the hind leg of rabbits were determined. All the fatty acids measured were significantly ($P<0.05$) influenced by the processing methods except decosenoic acid methyl ester and pentadecanoic acid methyl esters. The levels of inclusion of tallow also significantly ($P<0.05$) affected all the fatty acids composition measured. It was therefore concluded that irrespective of the processing methods the use of tallow in the diets of rabbits has no negative effect on the fatty acid composition of rabbit meat.

Key words: fatty acid, hind leg, rabbits, tallow seed meal

Introduction

The information available on chemical composition of rabbit meat is extremely variable, especially regarding fat content (*Pla et al., 2004*). These compositions depend on the part of the carcass studied (*Pla et al., 2004*) and also on the different productive factors (*Dalle-Zotte, 2002*). The author also

corroborated that feeding factors have a strong influence on the chemical composition of rabbit meat, in particular, on its lipid composition. Rabbit meat is characterized by its lower energy value compared with red meats (Combes, 2004). This might be due to its low fat content. Fat content varies widely depending on the carcass portion from 0.6 to 14.4 % (fat from edible meat with intramuscular and intermuscular fat content) with an average value of 6.8 % (Hernández and Gondret, 2006) with the loin being the leanest part of the carcass (1.2 % of lipids). There is an increasing interest in the lipid composition of edible meat and fat of domestic animals because of its relationship with human health, particularly with cardiovascular illnesses (Hu and Willett, 2002). The quantity and composition of fatty acids found in monogastrics is directly influenced by the composition of the diet (Bernardini et al., 1999) The aim of this study was to determine the fatty acid composition of meat from the hind leg cut of rabbits (*Oryctolagus cuniculus*) fed diets containing graded levels of processed tallow (*Detarium microcarpum*) seed meal.

Materials and Methods

Eighty one (81) weaned rabbits of mixed breed and sexes were randomly allotted to nine treatment groups with nine rabbits per treatment. Each treatment had three replicates with three rabbits per replicate. The crude protein level was set at 16 %. The control diet (0 % TSM) had 100 % Palm kernel cake (PKC) as the main source of protein. Groups 1-4 contained cooked tallow (*Detarium microcarpum*) seed meal (CTSM) diets and included as 75 % PKC: 25 % CTSM, 50 % PKC: 50 % CTSM, 25 % PKC: 75 % CTSM and 0 % PKC: 100 % CTSM, respectively. While groups 5-8 had fermented tallow (*Detarium microcarpum*) seed meal diets (FTSM) and included as 75 % PKC: 25 % FTSM, 50 % PKC: 50 % FTSM, 25 % PKC: 75 % FTSM and 0 % PKC: 100 % FTSM. Table 1 shows the composition of experimental diets. All diets were supplemented with equal amounts of bone meals, oyster shell, salt, vitamin-mineral premix, methionine and lysine. The rabbits were treated against endo parasites. In addition, medications were administered where was necessary. The diets were supplemented with some quantities of *Tridax procumbens* as source of forage in the evenings. The cages were equipped with feeders and drinkers. Prior to the start of the experiment, the animals were fed common diets and allowed an adjustment period of 5 days to enable the animals get accustomed to their cages and diets. The diets and fresh water were provided *ad-libitum* throughout the duration of the experimental period. The experiment lasted for 12 weeks. The design of the experiment was 2/4 factorial experiment and arranged as a completely randomized design (CRD). At the end of the growth studies, a total of 54 rabbits were randomly selected from the nine (9) dietary groups, with six (6) rabbits per group. The fatty acid content of the meat

from the hind leg of rabbits was analyzed using the method described by *Schafer (1998)*. This includes the extraction of total lipids using chloroforms methanol (2:1, v/v). The lipids were methylated with Trimethylsulfonium hydroxide (TMSH). Fatty acids (FA) methylesters were thereafter separated by a gas chromatograph (HP 5890, Hewlett Packard GmbH, Germany) equipped with a polar capillary column (30cm FFAP, 8.53 mm I.D.Macherey and Nagel, Duren, Germany), a flame ionization detector and an automatic en-column injector. Helium was used as the carrier gas with a flow of 5.4ml min⁻¹. The data collected from this study were subjected to analysis of variance (ANOVA) using statistical package (*SAS, 1998*). The variations in means were separated using the Duncan Multiple Range Test (*Duncan, 1955*).

Table 1. Composition of experimental diets (%)

Ingredients	Control					FTSM			
	0	25	50	75	100	25	50	75	100
Maize	17.69	23.48	27.13	32.20	35.18	24.76	30.31	33.95	37.75
PKC	58.06	39.20	24.31	10.89	0.00	38.24	22.72	10.45	0.00
TSM	0.00	13.07	24.31	32.66	40.57	12.75	22.72	31.35	38.00
Maize offal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vita premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100	100
Determined analysis									
Dry matter	88.47	87.50	89.05	87.95	87.88	88.89	88.69	89.13	87.85
Crude protein	16.85	16.48	16.20	16.65	16.00	16.55	16.20	16.85	16.00
Crude fibre	15.62	16.12	16.50	15.50	15.50	15.50	16.60	16.03	16.03
Ash	4.50	4.50	4.50	4.00	4.00	4.00	4.50	5.00	5.50
Ether extract	9.00	11.00	11.00	10.50	9.50	10.50	9.00	9.00	9.50
NFE	42.50	39.40	40.85	41.30	42.88	42.34	42.39	42.25	40.82
Energy (Kcal/kg/ME)	2756	2840	2864	2941	2891	2842	2846	2615	2749

TSM = Tallow seed meal, CTSM= Cooked tallow seed meal, FTSM = Fermented tallow seed meal
 Premix supplied per 2.5kg/tonne contains: Retinol acetate (10000000 iu), Vit. D₃ (2000000 iu), Vit E (15000 iu), Vit B (3000mg), Niacin (15000mg), Calcium pantothenate (800mg), Vit . B₆ (3000mg), Vit. B₁₂ (10mg) Vit. K₃ (2000mg), Biotin (20gm), Folic acid (500mg), Choline chloride (250,000mg), Manganese (75000mg), Iron (25000mg), Copper (5000mg), Zinc (70000mg), Selenium(150mg), Iodine(1300mg), Magnesium (100mg), 500g ethoxyquin and BHT (700g)
 NFE=Nitrogen free extract

Results and Discussion

The results of fatty acid content of hind legs of rabbits fed diets containing graded levels of processed tallow seed meal are shown in Table 2a. Content of all the fatty acids measured was significantly ($P < 0.05$) influence of the processing methods except decosenoic acid methyl ester and pentadecanoic acid methyl esters. Tallow share also significantly ($P < 0.05$) affected all the fatty acids composition measured. Meat from rabbits fed cooked diets had higher fatty acid content except for stearic acid methyl ester, arachidic acid, arachidonic acid methyl ester and pentadecanoic acid methyl ester. With regard to the effect of the tallow share in the diets of the rabbits, no particular trend was observed except in myristic acid methyl ester, palmitic acid methyl ester, linoleic acid methyl ester, arachidic acid methyl ester and saturated fatty acids (SFA) where a slightly decrease observed in the content of the fatty acids with increase processed tallow share in the diets of the rabbits.

Table 2a. Fatty acid content of the hind leg of rabbits fed diets containing graded levels of processed tallow seed meal TSM) (%)

Methods	Lauric ME	Myristic ME	Palmitic ME	Palmitic acid	Margaric ME	Oleic ME	Stearic ME	Oleic Acid	Linoleic ME	Arachidic Acid	Arachidonic ME	Decoic ME	Pentadecanoic ME	SFA	MUFA	PUFA	PUFA/SFA
Cooked	5.74 ^a	3.94 ^a	28.39 ^a	3.31 ^a	1.82 ^a	31.85 ^a	10.78 ^b	11.50 ^a	1.23 ^a	0.42 ^b	1.39 ^b	1.46	0.81	54.97 ^a	45.51 ^a	3.90 ^a	0.06 ^b
Fermented	5.44 ^b	3.62 ^b	26.45 ^b	2.90 ^b	1.32 ^b	28.98 ^b	11.04 ^a	11.09 ^b	0.87 ^b	0.54 ^a	2.45 ^a	1.46	0.85	51.51 ^b	42.93 ^b	3.03 ^b	0.08 ^b
SE \pm	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LOS	*	*	*	*	*	*	*	*	*	*	*	NS	NS	*	*	*	*
Levels																	
0	1.68 ^d	8.11 ^a	32.17 ^a	6.10 ^a	1.78 ^c	31.70 ^c	11.86 ^a	6.70 ^c	0.00 ^c	0.00 ^c	1.00 ^c	0.00 ^d	0.00 ^c	55.98 ^a	43.02 ^d	1.00 ^c	0.02 ^c
25	6.57 ^b	3.50 ^b	27.35 ^b	1.10 ^c	1.86 ^b	28.50 ^d	10.39 ^d	14.17 ^a	0.53 ^d	0.53 ^d	1.56 ^d	1.81 ^{bc}	1.30 ^b	51.70 ^c	44.50 ^c	3.86 ^c	0.10 ^a
50	7.06 ^a	3.25 ^c	27.31 ^c	3.87 ^b	0.61 ^d	28.24 ^e	10.23 ^e	12.44 ^b	0.54 ^c	0.54 ^c	1.58 ^c	1.82 ^b	0.73 ^c	52.60 ^b	43.00 ^e	4.43 ^a	0.09 ^{ab}
75	6.60 ^b	2.79 ^d	26.51 ^d	2.57 ^b	1.06 ^d	31.74 ^b	10.77 ^c	11.18 ^d	0.59 ^b	0.59 ^b	2.36 ^b	1.86 ^a	1.57 ^a	51.40 ^d	44.80 ^b	3.78 ^d	0.08 ^b
100	6.15 ^c	2.23 ^e	23.73 ^e	1.88 ^d	2.53 ^a	31.89 ^a	11.32 ^b	12.00 ^c	0.74 ^a	0.74 ^a	3.10 ^a	1.80 ^c	0.57 ^d	49.90 ^e	45.80 ^a	4.26 ^b	0.09 ^{ab}
SE \pm	0.05	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LOS	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
M x L	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

^{abc}Means with different superscripts in the same column are significantly ($P < 0.05$) different.

The interaction effect of processing methods and levels of inclusion of TSM on the fatty acid content of the hind leg of rabbits fed processed tallow seed meal diets (Table 2b) revealed that SFA were significantly ($P < 0.05$) higher in meat obtained from rabbits fed control (0 % TSM) diets. MUFA was higher in the meat of rabbits fed 25 % FTSM diets and the least in the meat of those fed 50 % CTSM diets. PUFA was higher in the meat of rabbits fed 75 % FTSM diets and least in the meat of rabbits fed 0 % TSM diets. SFA / PUFA ratio was least in meat obtained from rabbits fed control (0 % TSM) diets and highest in meat from rabbits fed 75 % FTSM diets.

Table 2b. The interaction effect of processing methods and levels of inclusion of TSM on the fatty acid content of the hind leg of rabbits fed processed fallow seed meal diets

Levels (%)	Lauric ME	Myristic ME	Palmitic ME	Stearic ME	Myristic ME	Palmitic ME	Stearic ME	Arachidonic ME	Arachidonic ME	Arachidonic ME	Docosahexaenoic ME	Docosahexaenoic ME	PUFA/SEA
Cooked													
0	1.58 ^a	7.11 ^a	32.17 ^a	6.10 ^a	1.78 ^a	31.70 ^a	11.86 ^a	0.00 ^a	0.00 ^a	1.00 ^a	0.90 ^a	0.00 ^a	45.02 ^a
25	6.14 ^a	7.50 ^a	28.83 ^a	2.20 ^a	1.53 ^a	24.43 ^a	9.61 ^a	1.80 ^a	0.43 ^a	1.10 ^a	1.80 ^a	1.05 ^a	56.32 ^a
50	7.62 ^a	2.24 ^a	29.40 ^a	3.78 ^a	1.21 ^a	25.31 ^a	9.41 ^a	12.87 ^a	0.50 ^a	1.15 ^a	1.81 ^a	1.46 ^a	39.99 ^a
75	7.10 ^a	0.00 ^a	28.08 ^a	2.41 ^a	2.11 ^a	31.93 ^a	11.44 ^a	11.20 ^a	0.52 ^a	1.11 ^a	1.89 ^a	1.53 ^a	45.02 ^a
100	7.00 ^a	3.35 ^a	23.39 ^a	2.06 ^a	2.45 ^a	31.53 ^a	11.59 ^a	12.65 ^a	0.50 ^a	2.58 ^a	1.79 ^a	0.00 ^a	50.04 ^a
Fermented													
25	6.25 ^a	0.00 ^a	25.87 ^a	0.00 ^a	2.10 ^a	32.58 ^a	11.16 ^a	14.23 ^a	0.60 ^a	2.01 ^a	1.82 ^a	1.54 ^a	48.63 ^a
50	6.50 ^a	3.33 ^a	25.12 ^a	3.95 ^a	0.00 ^a	31.16 ^a	11.05 ^a	12.00 ^a	0.58 ^a	2.01 ^a	1.83 ^a	0.00 ^a	49.95 ^a
75	6.10 ^a	4.46 ^a	25.01 ^a	2.73 ^a	0.00 ^a	31.55 ^a	10.10 ^a	11.15 ^a	0.63 ^a	3.60 ^a	1.83 ^a	1.60 ^a	50.22 ^a
100	6.04 ^a	3.18 ^a	24.06 ^a	1.70 ^a	2.61 ^a	32.24 ^a	11.05 ^a	11.35 ^a	0.30 ^a	3.62 ^a	1.81 ^a	1.13 ^a	49.77 ^a
SE	0.03 ^a	1.35x10 ^{-3a}	1.28x10 ^{-3a}	1.62x10 ^{-3a}	1.02x10 ^{-3a}	1.40x10 ^{-3a}	8.46x10 ^{-3a}	2.12x10 ^{-3a}	8.77x10 ^{-3a}	1.29x10 ^{-3a}	8.77x10 ^{-3a}	9.62x10 ^{-3a}	8.48x10 ^{-3a}
M													1.69x10 ^{-3a}

^a Means with different superscripts in the same column are significantly (P<0.05) different. TSM, 25 % TSM, 50 % TSM, 75 % TSM and 100 % TSM Abbreviation for Table 2a and 2b: SEM=Standard error of mean ME= methyl ester, Myristic= Myristic Pal= Palmitic Marg= Margarine Lino= Linoleic Arach= Arachidonic Arach= Arachidonic Deco= Decosanoic Pent= Pentadecanoic SFA= Saturated fatty acid MUFA= Mono unsaturated fatty acid, PUFA= Poly unsaturated fatty acid

Hernández and Gondret (2006) stated that rabbit meat fat comprises mostly saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFAs), with percentages of 36.9 %, and 34.60 %, respectively, total fatty acids in the hind leg. Mono unsaturated fatty acids (MUFAs) are less representing about 28.50 %. The most ubiquitous fatty acids are oleic (C18:1), palmitic (C16:0), and linoleic (C18:2) acids, showing percentages higher than 20 % of total fatty acids. Altogether, rabbit meat has a high ratio of PUFA to SAFs (0.85) for the meat of hind leg (*Alasnier et al., 1996* and *Ramírez et al., 2005*). From the results of this study when the fatty acids are grouped as SFAs, MUFAs and PUFAs were better in rabbits fed CTSM based diets except the ratio of SFA/PUFA that was better in rabbits fed FTSM based diets though quite the results were lower than those reported by *Alasnier et al. (1996)* and *Ramírez et al. (2005)*. The levels of inclusion and interaction effects were significant ($P < 0.05$) in all the fatty acids determined. The high significant ($P < 0.05$) difference observed in the results of SFA's might be due to the fatty acid content of the test ingredient which was directly deposited in the meat of rabbits. The high amount of palmitic acid methyl ester, palmitic acid and margaric acids in the meat of rabbits fed the control diets might be due to the high inclusion levels of PKC in the diet. This is in accordance with the report of *Hernández (2008)*, who observed that rabbits and other non-ruminants are able to incorporate dietary fatty acids into adipose and muscle tissue lipids. The mono unsaturated fatty acids values were also higher in the meat of the rabbits in all the treatments compared to about 28.50 % given by *Hernández and Gondret (2006)* making the meat a good source of nutrition for diabetic and hypertensive patients (*Aduku and Olukosi, 2000*). PUFA was generally low in all the treatments even though appreciable values were recorded in the FTSM based diets. *Xiccato, (1999)* and *Zsedely et al. (2008)* reported that high PUFA concentration in meat can have a negative effect on its storage stability due to higher susceptibility to peroxidation. It showed that the meat of rabbits from this study could store well for a longer period.

Conclusion

The present study showed that irrespective of the processing methods used in tallow meal diets preparation, they have no negative effect on the fatty acid composition of rabbit's meat.

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Sastav masnih kiselina mesa dela trupa (zadnje noge) kunića (*Oryctolagus cuniculus*) hranjenih sačmom prerađenog semena *Detarium microcarpum*

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Rezime

Osamdeset jedan (81) odbijeni kunić mešovitih rasa i polova (muškog i ženskog) su nasumično podeljeni u devet tretiranih grupa sa devet zečeva po tretmanu. Svaki tretman je imao tri ponavljanja sa tri kunića po ponavljanju. Prerađeno seme je uključeno u ishranu kao izvor proteina koji je postavljen na 16% CP. Kontrolni obrok je sadržavao 100% pogače palminog zrna (PKC) i 0% sačme *Detarium microcarpum* (TSM). Obroci 1 - 4 sadržavali su sačmu kivanog semena *Detarium microcarpum* (CTSM) koja je bila uključena u sledećim nivoima: 75% PKC: 25% CTSM, 50% PKC: 50% CTSM, 25% PKC: 75% CTSM i 0% PKC: 100% CTSM, dok su grupe 5 - 8 hranjene obrocima koji su sadržavali sačmu fermentisanog semena (FTSM) i uključena na istom nivou kao i kod kuvanih obroka. Pedeset četiri kunića su nasumice odabrani za klanje iz devet grupa sa šest kunića (mužjaka i ženki) po grupi. Određivan je sadržaj masnih kiselina mesa zadnjih nogu kunića. Sve izmerene masne kiseline su značajno ($P < 0.05$) bile pod uticajem načina obrade semena osim metil estra dokozenoinske i metil estra pentadekanske kiseline. Nivoi uključivanja semena je takođe značajno ($P < 0.05$) uticalo na sve masne kiseline. Stoga je zaključeno da, bez obzira na metode obrade, upotreba *Detarium microcarpum* u ishrani zečeva nema negativan efekat na sastav masnih kiselina zečeva mesa.

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MYCOBIOTA AND MYCOTOXINS IN FRESHLY HARVESTED AND STORED MAIZE

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Abstract: The incidence of mycobiota and mycotoxin levels were investigated in the freshly harvested maize kernel samples from October 2014 and in the samples of stored maize kernels from February 2015. Toxigenic fungal species (moulds) were isolated, cultivated and identified on agar plates according to standard mycological methods, while mycotoxins were detected by enzyme-linked immuno-sorbent assay (ELISA).

Mycological analyses of kernels showed the presence of toxigenic species from genera *Aspergillus*, *Fusarium* and *Penicillium*. Among the *Aspergillus* species, *Aspergillus flavus* was identified with higher incidence in the stored kernels (10.25%), than in freshly harvested kernels (3.67%) whereas *A. parasiticus* was the predominant species in the freshly harvested kernels (4.17%) compared to the stored kernels (0%). From the genus *Fusarium* three species were identified: *F. graminearum*, *F. subglutinans* and *F. verticillioides*, with the incidence of 1.08%, 8% and 25.75%, respectively in freshly harvested kernels and the incidence of 2.50%, 7.10% and 29.75%, respectively in the stored kernels. Species from genus *Penicillium* had higher incidence in freshly harvested kernels (14.25%) than in the stored kernels (9%).

In addition, tested samples of harvested and stored maize kernels were 100% positive with aflatoxin B₁ (AFB₁), deoxynivalenol (DON) and total fumonisins B₁, B₂ and B₃ (FBs). The mean levels of AFB₁, DON and FBs were 2.77 µg kg⁻¹, 117.83 µg kg⁻¹, and 3700.84 µg kg⁻¹, respectively in the freshly harvested kernels and a mean levels of 2.16 µg kg⁻¹, 2034.40 µg kg⁻¹, and 5976.50 µg kg⁻¹, respectively in the stored maize kernels.

In the freshly harvested maize kernel samples, statistically significant ($P \leq 0.05$) positive correlations of kernel moisture content with the incidence of *Penicillium* spp. ($r = 0.47$), and levels of AFB₁ ($r = 0.46$) and FBs ($r = 0.47$), and between the incidence of *Penicillium* spp. and level of AFB₁ ($r = 0.53$) were established. In the stored maize kernel samples, statistically significant ($P \leq 0.05$) positive correlations were found between the incidence of *F. subglutinans* and level of FBs ($r = 0.50$) and between levels AFB₁ and FBs ($r = 0.52$). A highly significant

($P \leq 0.01$) positive correlation was established between the incidence of *F. verticillioides* and level of FBs ($r = 0.64$) in freshly harvested maize kernel samples.

These results indicate that the incidence of toxigenic fungi and levels of mycotoxins, in particular DON and FBs, were higher in the stored maize kernel samples than in freshly harvested maize kernels. Therefore, to prevent the development of toxigenic fungi and mycotoxins accumulation in post-harvest period it is necessary to thoroughly dry maize and keep it in hygienic food storages.

Key words: mycobiota, mycotoxins, harvest, storage, maize

Introduction

Maize is cereal crop used for human and animal nutrition. According to data of *Statistical Yearbook of Serbia (2012)*, maize has been grown on about 1.2 million hectares with an average yield of 5.4 t ha^{-1} and with a production of 6.5 million tons in Serbia in 2011.

The contamination of maize with fungi (moulds) and their secondary metabolites (mycotoxins) represents a serious hazard to humans and animals. The most important mycotoxins in maize kernels are aflatoxins produced by *Aspergillus flavus* and *A. parasiticus*. Equally important mycotoxins in maize kernels are fumonisins produced mostly by *Fusarium verticillioides* and *F. proliferatum*, as well as deoxynivalenol produced by *F. graminearum* (Chulze, 2010; Pereyra et al., 2011).

Climatic conditions and maize growing on large areas in Serbia are suitable for development of numerous toxigenic species, resulting with frequent animal feed contamination by toxic products of fungi – mycotoxins. Development of toxigenic fungi and bio-synthesis of mycotoxins most often depend on ample precipitation and low temperatures at the end of summer or beginning of autumn during sensitive phenophase of maize growing (Krnjaja et al., 2009). Mould growth and mycotoxin contamination can occur in the field pre-harvest and later during storage condition in the post-harvest period. The higher moisture and temperature are abiotic factors which positively influence on fungal growth and mycotoxin accumulation (Niaz et al., 2011; Kocasari et al., 2013). Production of farm animals, poultry and swine, in particular, requires a large amount of cereal grain. For this reason, most of the grain is stored until utilized. Storage conditions are determined by abiotic and biotic factors including microorganisms, insects, mites, rodents and birds (Santin et al., 2005).

Consumption of mycotoxin contaminated diet may cause acute and chronic toxicity in humans and animals. Among farm animals, swine are considered to be

the most sensitive species to mycotoxins. Aflatoxin B₁ (AFB₁) is main hepatotoxin that causes various pathological effects on organs and tissues. Deoxynivalenol (DON) causes feed refusal, emesis, anaemia, haemorrhage, immunosuppression and neurotoxic effects. Fumonisin B₁, B₂ and B₃ (FBs) cause leukoencephalomalacia and porcine pulmonary edema (Biagi, 2009; Berardo, 2011; Pereyra et al., 2011).

Unfortunately, there are no direct measures for prevention of fungal infection and mycotoxin contamination. However, unfavourable conditions for development of toxigenic fungi could be provided by implementation of appropriate agricultural practices as preventive measures in the field. Pre-harvesting control strategies primarily consist of crop practices designed to reduce the fungal development and mycotoxin accumulation and the utilization of genetically resistant hybrids (Blandino et al., 2008). Post-harvest control strategy includes the practices directed at reducing mycotoxin levels, mycotoxin risk assessment in crop products and controlling its use through regulation. Monitoring of fungal and mycotoxin contamination in crops and products can be implemented in pre-harvest and post-harvest period (Dohlman, 2003). Reduction moisture in grains to moisture level of 13% or below is also very important post-harvest control measure (Lutfy et al., 2008).

The aim of this research was to determine the incidence of toxigenic fungal species, levels of mycotoxins (AFB₁, DON and FBs) and to establish the signification of correlation coefficients between investigated variables in freshly harvested and stored maize kernel samples, used for animal feed.

Material and Methods

During the harvest time in October of 2014 and storage time in February of 2015, 20 and 20 maize kernel samples intended for farm animals feeding (pigs, sheep and poultry) were randomly collected from parcels for agricultural production and warehouse with natural-air-drying conditions in the Institute for Animal Husbandry, Belgrade. The samples were collected according to the Commission Regulation (EC) No 401/2006 (European Commission, 2006). All samples were kept at 4°C in the refrigerator before further analysis. Moisture content of milled maize kernels was determined using a moisture analyzer (OHAUS MB35, USA).

For the mycological analysis, maize kernels were disinfected in 1% sodium hypochlorite (NaOCl) for 2-3 minutes, and rinsed twice in distilled water. A total of 50 maize kernels per each sample were distributed in Petri dishes (5 kernels per Petri dish) with water-1,8% salt agar (18g NaCl per 1 litre of agar medium) (Krnjaja et al., 2015). Plates were incubated for 14 days at 20°C with alternating light and darkness. Potential toxigenic fungi were identified according to the fungal

key of Singh *et al.* (1991). The incidence of potential toxigenic fungal species was calculated according to Lević *et al.* (2012).

For the mycotoxicological analysis, the maize kernel samples were ground to a fine powder with an analytical mill (IKA A11, Germany). The levels of AFB₁, DON and FBs were detected using the competitive ELISA method according to the manufacturer's instructions Celer Tecna® ELISA kits. Absorbance was determined at a wavelength of 450 nm on an ELISA plate reader spectrophotometer (Biotek EL x 800TM, USA). The limit of detection for AFB₁, DON and FBs were 1 µg kg⁻¹, 40 µg kg⁻¹ and 750 µg kg⁻¹, respectively.

The correlation between individual values for moisture content of maize kernels, the incidence of toxigenic fungal species and the levels of AFB₁, DON and FBs was determined using the Pearson correlation coefficient.

Results

The average moisture content for freshly harvested maize kernel samples was 15.20% (range 14.28 – 16.05%) and for stored maize kernel samples was 14.04% (range 13.41 – 14.92%).

In mycological analyses, toxigenic fungi from genera *Aspergillus*, *Fusarium* and *Penicillium* were identified. Fungal species identified in both, freshly harvested and stored maize kernels, were *A. flavus*, *F. graminearum*, *F. subglutinans*, *F. verticillioides* and *Penicillium* spp., with the exception of the species *A. parasiticus* which was identified only in the freshly harvested maize kernels. The most frequent fungal species was *F. verticillioides*, with an average incidence of 25.75% (range 10 - 43%) in freshly harvested and 29.75% (range 17 – 50%) in stored maize kernels. Considering the average values, the incidence of *A. flavus* was higher in stored (10.25%) than in freshly harvested maize kernels (3.67%). The species *A. parasiticus* was identified only in freshly harvested kernels with presence of 4.17% on average. The presence of *F. graminearum* was higher in stored (on average 2.50%) than in freshly harvested kernels (on average 1.08%), while the incidence of *F. subglutinans* and *Penicillium* spp. was higher in freshly harvested (on average 8% and 14.25% respectively) than in stored maize kernels (on average 7.10% and 9% respectively) (Table 1).

In the mycotoxicological analyses, freshly harvested and stored maize kernels samples were 100% positive for the presence of all tested mycotoxins. In the freshly harvested samples the mean levels of AFB₁, DON and FBs were 2.77 µg kg⁻¹ (range 2.31 – 3.34 µg kg⁻¹), 117.83 µg kg⁻¹ (range 42 – 238 µg kg⁻¹) and 3700.84 µg kg⁻¹ (range 1519 – 9780 µg kg⁻¹), respectively (Table 2). In the stored maize samples the mean levels of AFB₁, DON and FBs were 2.16 µg kg⁻¹ (range 1.03 – 4.11 µg kg⁻¹), 2034.40 µg kg⁻¹ (range 380 – 10684 µg kg⁻¹) and 5976.50 µg kg⁻¹ (range 760 – 35760 µg kg⁻¹), respectively (Table 3).

Table 1. Incidence of fungal species in freshly harvested and stored maize kernel samples

Fungal species	Freshly harvested kernel		Stored kernel	
	Incidence (%)			
	Average	Range	Average	Range
<i>Aspergillus flavus</i>	3.67	0 - 10	10.25	0 - 28
<i>Aspergillus parasiticus</i>	4.17	0 - 17	0	0
<i>Fusarium graminearum</i>	1.08	0 - 7	2.50	0 - 10
<i>F. subglutinans</i>	8	0 - 20	7.10	0 - 20
<i>F. verticillioides</i>	25.75	10 - 43	29.75	17 - 50
<i>Penicillium</i> spp.	14.25	0 - 27	9	0 - 27

Table 2. Levels of aflatoxin B₁ (AFB₁), deoxynivalenol (DON) and total fumonisins (FBs) in freshly harvested maize kernel samples

Item	Freshly harvested kernel		
	AFB ₁	DON	FBs
Sample size ^a	20/20	20/20	20/20
Incidence (%)	100	100	100
Range (µg kg ⁻¹)	2.31 - 3.34	42 - 238	1519 - 9780
Mean ^b (µg kg ⁻¹)	2.77	117.83	3700.84

^a Number of positive samples/Number of total samples

^b Mean level in positive samples

Table 3. Levels of aflatoxin B₁ (AFB₁), deoxynivalenol (DON) and total fumonisins (FBs) in stored maize kernel samples

Item	Stored kernel		
	AFB ₁	DON	FBs
Sample size ^a	20/20	20/20	20/20
Incidence (%)	100	100	100
Range (µg kg ⁻¹)	1.03 - 4.11	380 - 10684	760 - 35760
Mean ^b (µg kg ⁻¹)	2.16	2034.40	5976.50

^a Number of positive samples/Number of total samples

^b Mean level in positive samples

Statistical analyses and Pearson's correlation coefficient, established highly significant positive correlation ($P \leq 0.01$) between the incidence of *F. verticillioides* and level of FBs ($r = 0.64$) in the freshly harvested kernel maize samples. There was also significant positive correlation ($P \leq 0.05$) of kernel moisture content with levels of AFB₁ ($r = 0.46$) and FBs ($r = 0.47$) and between the incidence of *Penicillium* spp. and level of AFB₁ ($r = 0.53$). Likewise, the positive correlation, but not statistically significant, was found between kernel moisture content and level of DON ($r = 0.27$), then between levels of AFB₁ and FBs ($r = 0.31$), between the incidence of *F. subglutinans* and level of FBs ($r = 0.30$), and between the incidence of *A. flavus* and AFB₁ ($r = 0.20$). It was only established the

statistically insignificant negative correlation between the incidence of *A. parasiticus* and level of AFB₁ ($r = -0.06$) in the freshly harvested maize kernels. Considering correlation coefficients in the stored maize kernel samples, statistically significant positive correlation ($P \leq 0.05$) was also found between levels of AFB₁ and FBs ($r = 0.52$), then between the incidence of *F. subglutinans* and level of FBs ($r = 0.50$) and between the incidence of *A. flavus* and AFB₁ ($r = 0.34$). There were also positive correlations but not significant between the incidence of *F. verticillioides* and level of FBs ($r = 0.31$), then positive correlation of kernel moisture content with levels of FBs ($r = 0.26$), AFB₁ ($r = 0.17$) and DON ($r = 0.03$). The negative, but not significant, correlation was found only between the incidence of *F. graminearum* and level of DON ($r = -0.30$) in the stored maize kernel samples (data not presented).

Discussion

In this study the incidence of mycobiota and levels of mycotoxins in the samples of freshly harvested and stored maize kernels were researched. The contamination of cereals with toxigenic fungi under favourable climatic and storage conditions may lead to mycotoxin accumulation to injurious levels for farm animals and human health. The production of mycotoxins is consequence of increased presence of toxigenic fungi. Therefore, determination of fungal species at the right time is very important step to reduce the detrimental effects of mycotoxin problems.

All tested samples of freshly harvested and stored maize kernels were positive for the presence of mycotoxins AFB₁, DON and FBs. In samples of freshly harvested maize kernels, mean levels of mycotoxins did not exceed the maximum permitted limits for unprocessed maize prescribed by the regulations of the Republic of Serbia (*Sl. glasnik, 2014*), but in some samples FBs level was above the maximum permitted limit ($4000 \mu\text{g kg}^{-1}$), with a maximum level of $9780 \mu\text{g kg}^{-1}$ (Table 2). In samples of stored maize kernels, mean level of DON was above the maximum permitted limit ($1750 \mu\text{g kg}^{-1}$) with a maximum level of $10684 \mu\text{g kg}^{-1}$, as well as certain samples with FBs levels above the maximum permitted limit – maximum level of $35760 \mu\text{g kg}^{-1}$ (Table 3). The incidence of DON producer, *F. graminearum*, and FBs producers, *F. subglutinans* and *F. verticillioides*, was higher in the stored than in the freshly harvested maize kernels (Table 1). These results can be explained by the favourable climate conditions during the maize harvest in 2014 and during the winter period in 2015. Namely, according to data of the Republic Hydro-meteorological Service of Serbia for 2014, heavy total rainfall was recorded in September (126 mm) with a mean daily temperature of 18.3°C before maize harvest. For this reason the average moisture content of harvested maize kernels was high ($> 15\%$). After harvest, the maize was stored in a warehouse and naturally dried without conditions control (temperature and

humidity). Mild winter during 2015 and uncontrolled conditions of temperature and relative humidity in the warehouse caused the intensive development of moulds and increased mycotoxins content, especially of DON in samples of stored kernels.

Similarly to this, in earlier mycotoxicological studies in Serbia, *Krnjaja et al. (2013a)* have found in maize kernels harvested in 2012 the mean level of AFB₁ in 100% of tested samples exceeding the maximum permitted limit (5 µg kg⁻¹; *Sl. glasnik, 2014*) in unprocessed maize, with incidence of *A. flavus* of 36.69%. These authors also concluded that the mean levels of DON and FBs have not exceeded the maximum permitted limits. It has been assumed that the drought in 2012 had also been the reason for the occurrence of aflatoxigenic fungi and high level of AFB₁ up to >40 µg kg⁻¹. In addition, in the stored maize samples collected from October 2011 to October 2012 with an average kernel moisture content of 11.02% *Krnjaja et al. (2013b)* have detected AFB₁, DON and FBs with the mean levels of 1.39 µg kg⁻¹, 128.17 µg kg⁻¹ and 1610.83 µg kg⁻¹, respectively. Compared to year 2014, in 2011 less rainfall was recorded before the maize harvest in September (47.7 mm) with a mean daily temperature of 17.2°C. Such climatic conditions before harvesting and especially kernel moisture content during the period of maize storage were not suitable for intensive development of toxigenic fungi and thus for the increased production of mycotoxins.

In total 26 maize samples from Turkey (n=19) and USA (n=7) collected between April 2002 and 2003, the mean aflatoxin and fumonisin levels were higher in samples from Turkey (10.94 µg kg⁻¹ and 88240 µg kg⁻¹, respectively) than in samples from USA (0.78 µg kg⁻¹ and 74150 µg kg⁻¹, respectively) (*Oruc et al., 2006*). By examining a total of 82 consignments of French and Argentinean maize as raw, imported in the United Kingdom between 2004 and 2007, *Scudamore and Patel (2009)* have detected the maximum level of 444 µg kg⁻¹ for DON and 5002 µg kg⁻¹ for FBs. These authors have found clear differences in the levels of mycotoxins between harvests and geographic regions. Maize from Argentina contained lower levels of DON and higher levels of FBs than maize from France, although the level of FBs up to 2000 µg kg⁻¹ or more were present in samples taken from both regions. Likewise, levels of fumonisins were higher in 2004 in Argentina and in 2006 in France due to exceptionally hot and dry summer and dry period before harvest. In the USA, *Dowd and Johnson (2010)* over a 4-year period (2005-2008) conducted mycotoxicological studies of popcorn samples and have rarely detected AFB₁, but FBs and DON were present in all years, with mean levels in fields up to 1700 µg kg⁻¹ (sample max. 2770 µg kg⁻¹) and 1900 µg kg⁻¹ (sample max. 2660 µg kg⁻¹). These authors have concluded that the damage from insects is the main cause of higher levels of FBs in relation to the DON levels, while higher levels of DON are caused by higher rainfall and lower temperatures during the maize ripening. In mycotoxicological analysis of 2258 maize samples collected over a 3-year period (2006-2008) from 93 storage centres in Italy, *Berardo et al.*

(2011) have found a high level of FBs, with the highest mean level in 2006 (10900 $\mu\text{g kg}^{-1}$) and lowest in 2008 (4800 $\mu\text{g kg}^{-1}$). These authors have assumed that climatic factors in conjunction with the specific growing area, played an important role in the accumulation of FBs in maize. In maize samples collected from July through August 2011, from 15 swine farms in the Beijing region in China, *Li et al.* (2014) have reported the natural occurrence of AFB₁ and DON with the highest levels of 58.9 $\mu\text{g kg}^{-1}$ (on average 6 $\mu\text{g kg}^{-1}$) and 2130 $\mu\text{g kg}^{-1}$ (on average 1091 $\mu\text{g kg}^{-1}$), respectively. Similar to the above mentioned data our results confirm high maximum levels of FBs in both harvested and stored maize kernels and high maximum level of DON in stored maize kernels.

After examining the correlation coefficients, in most variables from both tested groups in this research, harvested and stored maize samples, positive correlations were found, particularly emphasizing the positive correlation between the incidence of *F. verticillioides* and *F. subglutinans* with FBs level, and the incidence of *A. flavus* and AFB₁ level, then positive correlation between AFB₁ and FBs levels and between kernel moisture content and DON level. These results are similar to studies of *Kimanya et al.* (2008), *Sun et al.* (2011), *Berardo et al.* (2011) and *Krnjaja et al.* (2013a).

In this study, in tested harvested and stored maize kernel samples, the presence of potentially toxigenic fungi and mycotoxins AFB₁, DON and FBs was recorded. Because of favourable conditions for the growth and development of toxigenic fungi and mycotoxin contamination of maize kernels, it is of outmost importance to implement the preventive measures to reduce the risk of these contaminants in Serbia, especially in years when weather conditions are suitable for their development. Preventive measures, such as fast drying of maize for the medium- and long-term storage in hygiene maintained warehouses, without the presence of insects and microorganisms, and proper regulation of the moisture content of kernels, could significantly reduce the mycotoxins contamination of maize grains (*Bruns, 2003*). Good ventilation of warehouse has also been one of the most important preventive measures for the reduction of mycotoxins production (*Jakic-Dimic et al., 2011*).

Conclusion

Fungal and mycotoxin contamination of maize has been increasing worldwide, as a result of climate change. Some mycotoxins could be synthesized in maize before harvest but their level may increase after harvest during the storage period and further in the food chain.

Based on the obtained results it can be concluded that the potentially toxigenic species of fungi from the genera *Aspergillus*, *Fusarium* and *Penicillium* were present in the harvested and stored maize kernel samples. Species of the genera *Aspergillus* and *Fusarium* had higher incidence in stored samples compared

to freshly harvested maize. Also, in both groups of maize samples, AFB₁, DON and FBs were detected, but in the samples of stored maize kernels, the mean levels of DON and FBs exceeded the maximum permitted levels for unprocessed maize as stipulated by Serbian Regulation. For this reason, constant supervision and monitoring of mycotoxins occurrence in maize pre- and post-harvest and application of preventive measures are very important to reduce risks to human and animal health.

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Mikrobiota i mikotoksini u sveže požnjevenom i uskladištenom zrnu kukuruza

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Rezime

U radu je proučavana učestalost mikrobiota i sadržaj mikotoksina u uzorcima zrna kukuruza sakupljenih tokom berbe u oktobru 2014. godine i u uzorcima uskladištenog zrna sakupljenih u februaru 2015. godine. Toksigene vrste gljiva (plesni) su izolovane, odgajene i identifikovane na hranljivoj podlozi prema standardnim mikološkim metodama, dok je sadržaj mikotoksina detektovan primenom imunoadsorpcione enzimske metode (ELISA).

Mikološkim analizama zrna kukuruza ustanovljeno je prisustvo toksigenih vrsta iz rodova *Aspergillus*, *Fusarium* and *Penicillium*. Među *Aspergillus* vrstama, *Aspergillus flavus* je identifikovana u većem procentu u uzorcima uskladištenog zrna (10,25%) nego u uzorcima sveže požnjevenog zrna (3,67%), a *A. parasiticus* bila je dominantna vrsta (4,17%) u uzorcima sveže požnjevenog u odnosu na uskladišteno zrno kukuruza (0%). Tri vrste roda *Fusarium* su identifikovane sa učestalošću od 1,08% (*F. graminearum*), 8% (*F. subglutinans*) i 25,75% (*F. verticillioides*) u požnjevenom zrnu, i sa učestalošću od 2,50% (*F. graminearum*), 7,10% (*F. subglutinans*) i 29,75% (*F. verticillioides*) u uskladištenom zrnu kukuruza. Vrste iz roda *Penicillium* imale su veću učestalost u uzorcima požnjevenog (14,25%) nego u uzorcima uskladištenog zrna (9%).

Ispitivani uzorci sveže požnjevenog i uskladištenog zrna bili su 100% pozitivni sa aflatoksinom B₁ (AFB₁), deoksinivalenolom (DON) i ukupnim

fumonizinima FB₁, FB₂ i FB₃ (FBs). Prosečne koncentracije ovih toksina su iznosile 2,77 µg kg⁻¹ (AFB₁), 117,83 µg kg⁻¹ (DON) i 3700,84 µg kg⁻¹ (FBs) u uzorcima sveže požnjevenog zrna i 2,16 µg kg⁻¹ (AFB₁), 2034,40 µg kg⁻¹ (DON), i 5976,50 µg kg⁻¹ (FBs) u uzorcima uskladištenog zrna.

Statistički značajne ($P \leq 0.05$) pozitivne korelacije ustanovljene su između sadržaja vlage zrna sa učestalošću *Penicillium* spp. ($r = 0,47$) i koncentracijama AFB₁ ($r = 0,46$) i FBs ($r = 0,47$), kao i između učestalosti *Penicillium* spp. i koncentracije AFB₁ ($r = 0,53$). U uzorcima uskladištenog zrna, statistički značajne ($P \leq 0.05$) pozitivne korelacije ustanovljene su između učestalosti *F. subglutinans* i koncentracije FBs ($r = 0,50$) i između koncentracija AFB₁ i FBs ($r = 0,52$). Statistički veoma značajna ($P \leq 0.01$) pozitivna korelacija ustanovljena je između učestalosti *F. verticillioides* i koncentracije FBs ($r = 0,64$) u uzorcima sveže požnjevenog zrna kukuruza.

Rezultati ovih istraživanja ukazuju da su učestalosti toksigenih vrsta gljiva i koncentracije mikotoksina, posebno DON i FBs, bile više u uzorcima uskladištenog zrna nego u uzorcima sveže požnjevenog zrna. Zbog toga, da bi se sprečio razvoj toksigenih gljiva i akumulacija mikotoksina u postžetvenom periodu neophodno je kukuruz dobro osušiti i čuvati u higijensko ispravnim skladištima.

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SILAGE FERMENTATION CHARACTERISTICS OF GRASS-LEGUME MIXTURES HARVESTED AT TWO DIFFERENT MATURITY STAGES

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Abstract: The objective of our study was to investigate the diversity of individual parameters of quality of grass-legume mixture silages harvested in two stages of crop utilization and the impact of the interaction of studied factors on the quality. Legumes as an important source of protein are very difficult to ensilage. However, in mixture with grasses their fermentable characteristics are improved. During the growth and development of plants, their chemical composition changes, hence their ability and suitability for ensiling also changes. In regard to the studied factors, the phase of exploitation had a highly significant impact on almost all quality parameters in both years. By delaying the harvest period, dry matter content in silage has significantly increased and the level of crude protein and $\text{NH}_3\text{-N}$ decreased. In regard to the content of lactic acid, the studied silages fall into category of good quality silages. Its content in the first year ranged from 24.3-31.5 in the early harvest stage and from 27.9-36.2 g kg^{-1} DM at the late harvest stage, and in the second year from 27.4 to 31.4 in the early harvest stage and from 28.2-31.9 g kg^{-1} DM at the stage of late harvest. According to the content of acetic and butyric acids, studied silages can also be considered as silages of good quality.

Key words: silage, grass-legume mixtures, maturity stage, quality

Introduction

Silage quality depends on many factors such as plant species, implemented agro-technical measures and practices, preparation time, applied inoculants, especially stages of plant development in which it is used for the preparation of silage. *McEniry et al. (2013)* state that the two most important pre-ensiling factors responsible for the quality of silage are plant species and stage of plant development at the time of harvest. At certain stages of development plants are

characterized by different chemical composition. Thus, in the younger stages of development plants contain more protein, less dry matter, higher buffer capacity, which results in great losses in the silage dry matter (King *et al.*, 2012) and the incidence of clostridial fermentation. However, plants in later stages of development are also characterized by reduced buffering capacity, reduced concentration of easily soluble sugars, higher content of dry matter and structural carbohydrates, lignin (Keady and O'Kiely, 1998) as well as the incidence of problems in compaction of forage, resulting in silage of poorer quality with a lower concentration of lactic acid, higher pH and butyric acid content, reduced digestibility (Vranić *et al.*, 2008). By delaying the harvest period in the study of Knežević *et al.* (2009) higher content of dry matter, and the ADF and NDF, NH₃-N and acetic acid, as well as pH value of the silage were achieved. Also, Dawson *et al.* (2002), by harvesting the plants in the later stages, obtained silages with substantially higher content of acetic acid, NDF and NH₃-N, but lower pH values and digestibility. In studies of Keady *et al.* (2000), delayed harvest period resulted in silage with high content of dry matter, NDF, ADF, ADL, cellulose and hemicellulose, and reduced content of crude protein and butyric acid.

Legumes as an important source of protein are very difficult to ensilage due to the high buffering capacity and low concentrations of easily soluble sugars. In mixture with grasses their fermentable characteristics are improved, so that silage of adequate quality can be obtained. Also different grass species have different ensiling abilities, depending on the content of WSC and buffering capacity. In the study of the mixture of red clover with perennial and Italian ryegrass and cocksfoot, Wyss (2004) concluded that the ryegrasses had positive, and cocksfoot negative impact on silage quality. According Knotek (1997), crude protein content in cocksfoot ranges from 142.4-130.7, soluble carbohydrates 52.9-52.3 g kg⁻¹, the buffer capacity from 1.34 to 1.59, in tall fescue CP content is 113.7, soluble carbohydrates 89.9 g kg⁻¹, the buffer capacity of 2.19, which means that tall fescue is more suitable for silage.

The aim of our study was to investigate the diversity of individual parameters of quality of grass-legume mixture silages harvested in two stages of crop utilization and the impact of the interaction of studied factors on the quality.

Materials and Methods

The trial was performed at the Institute for Animal Husbandry in Zemun, Belgrade. The experiment was set in a semi random block system in four replications. The main plot size was 10 m². The survey covers lucerne as a pure crop (A) and its mixtures with cocksfoot (50 : 50) - B1, cocksfoot and tall fescue (33.3 : 33.3 : 33.3) - B2 and cocksfoot, tall fescue and sainfoin (25 : 25 : 25 : 25) -

B3. One half of the basic plot was harvested in the bud stage (early harvest), the other half in the stage of 50% flowering of lucerne (late harvest).

The second cut was used for the preparation of silage. After cutting the biomass was weighed, wilted and used for the preparation of silage in experimental silos of 10 dm³ volume. For the purpose of good fermentation before filling the silo, the material was treated with micro-biological preparation - mixture of four homofermentative strains of lactic-acid fermentation (*Lactobacillus plantarum*, *Pediococcus acidilactici*, *Streptococcus faecium*, *Lactobacillus salivarius*) and four different enzymes (cellulases, hemicellulase, amylase, xylanase), in the amount of 10g + 2l H₂O t⁻¹ of green mass. Sampling of silage was performed 90 days after the silos were closed.

At the end of the fermentation process the silages were analysed. Dry matter was determined by oven-drying over the night at 105°C. Crude protein was determined according to Kjeldahl. The concentration of ammonia-N was analysed by steam distillation method with a Kjeltex 1026 analyser. Lactic, acetic and butyric acid were determined by Flieg method. pH value was measured with a Hanna Instruments HI 83141 pH meter.

Data were analysed as a completely randomized design using the generalized factorial ANOVA of the software package STATISTICA 8 (StatSoft, Inc. 2007), where mixture type and harvest time were included as fixed factors. Means with a significant F-value were tested with Fisher's LSD test.

Results and Discussion

The dry matter content of silage is one of the important parameters that determines the success of fermentation of the ensiling material. The low level of DM in the silage is a sign of poor fermentation and such silage has a high pH value, less lactic and acetic acids and often high values of butyric acid due to the development of butyric bacteria. Excess DM is also not desirable, because due to more difficult compaction of ensiling material the aerobic processes are extended, resulting in oxidative losses and the occurrence of mould.

In our research, in the first year, the level of DM was quite high. Significant differences were observed only in phases of exploitation. In the early harvest stage, the DM content was lower than in the later stage of harvesting. Also in the second study year, the level of dry matter of silage depended only on the crop utilization phase. DM values were lower than in the first year of the study and ranged from 311.3 to 416.4 g kg⁻¹. Also, in the phase of early harvest lower DM content was obtained compared to the late harvest stage. According *Knotek (1997)*, in order to make good quality silage it is necessary to produce silage from wilted material which contains DM of 320-380 g kg⁻¹, or according to *Đorđević et al. (2001)*, the dry matter content of plant material should be above 35% in order to

ensure successful fermentation. According to these findings, all silages obtained in the second year of research had a satisfactory level of DM, while the silages of the first year had a slightly higher content of DM than expected for successful fermentation.

Table 1. The quality of grass-legume silages, depending on the structure of the mixture and utilization phase in 2008

Quality paramet. (gkg ⁻¹ SM)	Early harvest				Late harvest				level of significance		
	A	B1	B2	B3	A	B1	B2	B3	mix. type	harv time	inter act.
Dry matter	415.4	416.9	429.3	360.8	403.4	459.3	430.6	433.1	ns	*	ns
Crude protein	179.1	170.0	173.0	165.1	146.2	148.9	152.1	149.7	ns	**	ns
NH ₃ -N†	112.8	109.9	120.6	123.5	103.9	105.6	96.1	100.4	ns	**	ns
soluble N	504.1	465.6	463.7	501.0	550.1	515.2	425.8	419.6	**	ns	**
pH	4.8	4.8	4.8	4.8	4.5	4.4	4.6	4.7	ns	**	ns
lactic acid	31.5	26.6	27.3	24.3	35.7	36.2	27.9	29.5	**	**	*
acetic acid	15.0	18.6	14.9	19.7	8.9	9.2	11.2	10.3	**	**	**
butyric acid	0.00	0.04	0.00	0.07	0.00	0.02	0.00	0.15	*	ns	ns

†- g kg UN; A-lucerne in monoculture; B1-mixture of lucerne and cocksfoot; B2- mixture of lucerne, cocksfoot and tall fescue; B3- mixture of lucerne, cocksfoot, tall fescue and sainfoin.

The crude protein content depended primarily on the phase of exploitation, and in the second year of research on the structure of the mixture and the mixture x phase interaction. The early harvest phase is characterized by a significantly higher content of CP compared to the phase of late harvest. This can be explained by the fact that in the early stages of exploitation share of leaves was equal to or greater than the share of stems, while in the later stages of the exploitation the share of stems was relatively higher than the share of leaves, and therefore at this stage the level of crude protein was lower and the crude fiber content higher (*Di Marco et al., 2002*). Similar to our research, *Keady and O'Kiely (1998)*, recorded in their research significantly higher (185 g kg⁻¹ DM) CP content of the grassland silage, prepared from the earlier harvested material, compared to the later harvest silage (143 g kg⁻¹ DM). *Dinić et al. (2008)* also confirmed the presence of significant differences between the stages of heading and flowering in tall oatgrass. Tall oatgrass silage prepared in the heading stage had 156.0 g kg⁻¹ DM of crude protein and prepared in the flowering stage, 138.2 g kg⁻¹ DM.

In the second year of research, the mixtures had a significant impact on the content of CP. Pure lucerne crop silage and mixture with lucerne and sainfoin had

higher CP content than the other two mixtures, which is in line with the findings of *Tekeli and Ates (2005)*, that the content of the CP in the mixture increases with increasing proportion of legumes. The differences are significantly more visible in the silage from the first harvest phase than from the second.

Table 2. The quality of grass-legume silages, depending on the structure of the mixture and utilization phase in 2009

Quality paramet. (gkg ⁻¹ SM)	Early harvest				Late harvest				level of significance		
	A	B1	B2	B3	A	B1	B2	B3	mix. type	harv time	inter act.
Dry matter	311.3	317.6	320.1	340.7	371.3	380.6	416.4	387.2	ns	**	ns
Crude protein	176.6	156.9	154.9	172.5	148.1	146.8	149.1	153.6	**	**	*
NH ₃ -N†	115.4	116.6	104.8	91.3	90.4	95.8	91.5	98.1	ns	**	*
soluble N	598.9	621.0	600.3	542.9	589.6	580.2	558.0	592.8	ns	ns	*
pH	4.6	4.6	4.6	4.6	4.5	4.6	4.5	4.6	ns	ns	ns
lactic acid	28.8	27.6	27.4	31.4	28.6	31.9	29.9	28.2	ns	ns	**
acetic acid	10.7	11.8	11.4	10.3	15.7	13.1	13.7	14.0	ns	**	ns
butyric acid	0.02	0.02	0.02	0.04	0.09	0.11	0.19	0.02	ns	**	ns

†- g kg UN; A-lucerne in monoculture; B1-mixture of lucerne and cocksfoot; B2- mixture of lucerne, cocksfoot and tall fescue; B3- mixture of lucerne, cocksfoot, tall fescue and sainfoin

One of the most important indicators of the quality of fermentation is the amount of NH₃-N, as an indicator of the degradation of proteins. Significant differences in the content of ammonia nitrogen were observed in different phases of exploitation. In the early harvest phase, NH₃-N content of the silage was significantly higher than in the silage of the late harvest phase. *Muck (1988)* found, by ensiling lucerne with different degrees of humidity, that the content of non-protein N and ammonia decreased with increase in dry matter, which explains the phenomenon that the higher content of NH₃-N in the first compared to the second phase of the research. Similar to our research, *Kuoppala et al. (2008)* and *King et al. (2012)* have established that the content of NH₃-N is significantly higher in silages from earlier phases of exploitation. However, *Knežević et al. (2009)*, regardless of the dry matter content of silage from different phases of exploitation, have established that the content of NH₃-N is higher in silages made from plants harvested in the later stages of development (maturity stage, growth stage).

In the course of fermentation, organic acids accumulate in the silage. The most valuable is lactic acid, which lowers the pH, has the bactericidal effect on harmful microorganisms and provides aerobic stability of silage. The content of

lactic acid was highly dependent on all examined parameters only in the first year of research. Its content ranged in the first year from 24.3-31.5 in the early stage of harvest and of 27.9-36.2 g kg⁻¹ DM at the late harvest stage, and in the second year from 27.4-31.4 in the early harvest stage and of 28.2-31.9 g kg⁻¹ DM at the stage of late harvest. According to *Dorđević and Dinić (2003)*, average 3-7% of lactic acid is contained in good quality silage. According to *Dorszewski (1997)*, these values are slightly lower from 2.28-3.90%. The studied silages in regard to the content of lactic acid fall into category of good quality silages.

In addition to lactic acid, during the fermentation process, also the acetic acid is produced. If the content of acetic acid is up to 5.5% of the dry matter, it is considered to be good quality silage (*Dorđević and Dinić, 2003*). The content of acetic acid in the investigated silages ranged from 8.9-18.6 in the first year and from 10.3-15.7 g kg⁻¹ DM in the second year. As in the case of the lactic acid content, in regard to the content of acetic acid, silages can be considered as good quality silages.

Butyric acid is undesirable fermentation product and an indication of the presence of clostridia in silage. It is usually not present or present in small concentrations. A large number of silages of the first year showed no butyric acid, as opposed to the second year silages, where the content ranged from 0.02 to 0.19 g kg⁻¹. According to *Huhtanen et al. (2012)*, wilting to 400 g kg⁻¹ of dry matter acts preventively in the production of butyric acid. Considering that in the first year dry matter content of the silage was higher, then this could explain this fact. Good quality silages should have less than 0.1% butyric acid (*Seglar, 2003*). All studied silages showed a lower content than the mentioned value, which is necessary for good quality silage.

Conclusion

Based on the obtained results of the study of two exploitation phases on the the quality of grass-legume silages, it can be concluded that the preparation of grass-legume mixture silages harvested in the later stages of growth and development are better from the standpoint of quality. Such silages had higher level of dry matter, higher content of lactic acid and less ammonia nitrogen. However, important deficiency in preparation of silages in the later stages (maturity stage) is significantly lower crude protein content. By increasing the degree of wilting good silages from earlier maturity stage could be produced, with high crude protein content, dry matter, lactic acid and a low content of NH₃-N.

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Fermentabilne karakteristike travno-leguminoznih silaža košenih u dve različite starosne faze

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Rezime

Cilj naših istraživanja je bio da ispitamo uticaj faze zrelosti useva u vreme kosidbe različitih travno-leguminoznih smeša, kao i interakciju faktora faza-vrsta smeše na pojedine parametre kvaliteta silaža od tih smeša. Leguminoze kao važni izvori proteina se vrlo teško siliraju. U smeši sa travama njihove fermentabilne karakteristike se popravljaju. U toku rasta i razvika biljaka menja se njihov hemijski sastav, pa i sposobnost za siliranje. Od ispitivanih faktora, faza iskorišćavanja imala je visoko značajnog uticaja na gotovo sve parametre kvaliteta u obe ispitivane godine. Odlaganjem vremena kosidbe značajno je povećan sadržaj suve materije u silaži i smanjen sadržaj sirovih proteina i $\text{NH}_3\text{-N}$. Ispitivane silaže po sadržaju mlečne kiseline spadaju u silaže dobrog kvaliteta. Njen sadržaj u prvoj godini kretao se od 24,3-31,5 u ranoj kosidbi i od 27,9-36,2 g kg^{-1} SM u kasnoj kosidbi i u drugoj godini od 27,4-31,4 u ranoj fazi i od 28,2-31,9 g kg^{-1} SM u kasnoj fazi košenja. Po sadržaju sirćetne i buterne kiseline, ispitivane silaže se takođe mogu okarakterisati kao dobrog kvaliteta.

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THE EFFECT OF PARAGENETIC FACTORS ON REPRODUCTIVE TRAITS OF SIMMENTAL COWS

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