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MANAGEMENT OF LAMB NUTRITION AS A WAY FOR MODELING FATTY ACID PROFILES IN MEAT

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Review paper

Abstract: In addition to nutritional value, a very important criterion for the selection of meat, for the modern consumer is the health aspect, i.e. the content of fat and the profile of fatty acids in meat. The content of fat and fatty acids, among other things, is conditioned by the feeding system and the rearing method. Lambs fed on pasture have a lower share of fat in the carcass than animals fed with a concentrated mixture, in a closed system. The recommended value for the ratio of polyunsaturated to saturated fatty acids is up to 0.45, and below 4.0 for the n-6 and n-3 fatty acids ratio. Taking into account that the influence of lamb nutrition on these relationships is significant, modelling of fatty acid composition should be directed to the lamb nutrition system which leads to a decrease in the content of saturated and an increase in the concentration of polyunsaturated (PUFA) fatty acids in meat. A feeding strategy involving a grazing feeding system of lambs results in a higher content of n-3 PUFA, CLA and a more favourable n-6/n-3 ratio of fatty acids, while the lamb meat originating from animals fed concentrated diets has a higher proportion of n-6 PUFA and a higher n-6 ratio/n-3 fatty acids, which exceeds the recommended value of 4.0. Conjugated linoleic acid (CLA) is of great importance since it has an anticancer, antidiabetic effect as well as an effect on the immune system, suggesting a direction for future research on lamb meat.

Keywords: lambs, fatty acids, grazing, CLA

Introduction

Meat, by its nutritional characteristics, primarily in terms of protein, fat, minerals and vitamin content, has a significant place in the human diet of modern times (*Ramos et al., 2020; Higgs, 2000; Celada et al., 2015)*. Demographic growth, urbanization and rising purchasing power are increasing the demand for meat. On

the other hand, the popular perception of fats contained in meat indicates the risk of a range of health problems such as heart disease, stroke, diabetes and some types of tumors (*Klir et al., 2012*). Consequently, food has lost its former primary role in providing nutrients for life, and has taken on many other roles, primarily related to human health status (*Kaić et al., 2013*). As a result, consumer interests are increasingly driven by the knowledge of the quantity and quality of fats and fatty acids in certain types of meat (*Saatchi et al., 2013; Yarali et al., 2014*). It should be taken into account that the fat and individual fatty acids content in lamb meat depends on: genotype, animal age, gender, diet, rearing method and anatomical position of the muscle (*Borys et al., 2012; Ružić-Muslić et al., 2014, Júarez et al., Ružić, 1999; Ružić-Muslić et al., 2013*). Numerous studies have confirmed that fatty acid composition in ruminants is significantly influenced by the diet (*Demirel et al., 2006; Díaz et al., 2005*).
The aim of this paper is to examine the influence of nutrition management on the fatty acid profile of lamb meat.

The effect of nutrition management on the fatty acid profile of lamb meat

Lamb nutrition, as well as the way they are reared/housed, affects the fat content of the carcasses. (*Ružić-Muslić et al., 2007; Ružić-Muslić 2011*). Fatty tissue is localized in the body cavities (renal, abdominal and pelvic fat), subcutaneous fat, fat between the muscles (intermuscular fat) as well as within the muscle, i.e. intramuscular fat. More than 90% of the animal body fat is triglycerides, while the remainder is mainly phospholipids and cholesterol (*Grebens, 2004*). *Mioč et al. (2007)* state that fat in sheep meat is in the form of triglycerides, phospholipids, stearides and sterin. Lambs fed on pasture have a lower share of fat in the carcass ($P < 0.001$) than lambs fed concentrated food indoors (*Carrasco et al., 2009; Cividini et al., 2014; Perlo et al., 2008*), which is certainly a consequence of the increased physical activity of grazing lambs and the use of energy for movement (*Ružić-Muslić et al., 2012*). Meat of lambs fed with concentrated nutrients or grazing and concentrated mixture, contains more fat tissue ($P < 0.001$), than meat of lambs fed exclusively with roughage (*Perlo et al., 2008*).
The qualitative properties of lamb meat, in addition to the amount of fat, are determined by the content and profile of fatty acids, which, in addition to being important energy source, play a significant role in the immune status of the organism (*Yaqoob and Calder, 2007*). Fatty acids can be: saturated (do not contain a double bond in the chemical structure) and unsaturated (contain at least one double bond). Microorganisms in rumen of ruminants hydrogenate unsaturated fatty acids into saturated fatty acids (*Klir et al., 2012*) The interest in fatty acid

composition mainly originates from the need to find ways to produce healthier meats, i.e. better ratio of polyunsaturated (PUFA) to saturated fatty acids, and balance between n-6 and n-3 PUFA (*Wood et al., 2003*). In recent years, there has been an increasing interest in the ways of manipulating the fatty acid composition of meat. This is because meat is considered to be the main source of fat in the human diet, and in particular of saturated fatty acids, which have a detrimental effect on human health.
Table 1 shows the fatty acid composition of beef, veal, lamb and pork.

Table 1. Fatty acid profile of beef, veal, lamb and pork meats, expressed in g*10-2 g edible portion (*Williams, 2007*)

Fatty acids	Beef	Veal	Lamb	Pork
C14:0	0.096	0.034	0.101	0.010
C15:0	0.012	0.006	0.016	0.000
C16:0	0.607	0.215	0.842	0.250
C17:0	0.028	0.009	0.051	0.000
C18:0	0.356	0.119	0.644	0.130
Total	1.149	0.409	**1.730**	0.400
C14:1	0.025	0.007	0.004	0.000
C16:1	0.082	0.033	0.066	0.030
C18:1	1.103	0.356	1.995	0.390
C20:1	0.015	0.048	0.010	0.010
Total	1.205	0.399	2.066	0.430
C18:2n-6	0.204	0.090	0.321	0.120
C18:3n-3	0.048	0.022	0.072	0.010
C20:3n-6	0.020	0.012	0.009	0.003
C20:4n-6	0.076	0.056	0.094	0.019
C20:4n-3	0.031	0.028	0.028	0.000
C20:5n-3	0.051	0.033	0.044	0.006
C20:6n-3	0.006	0.003	0.013	0.004
Total	0.448	0.259	**0.603**	0.200

It is evident that lamb meat with a total content of monounsaturated and polyunsaturated fatty acids (2.066 g) contains significantly more favourable fatty acids compared to unfavourable saturated fatty acids (1.730 g). *Cvrtila et al. (2007)* state that monounsaturated and polyunsaturated the fatty acids in lamb meat together account for about 70%, while the remaining 30% are saturated fatty acids. Ruminant meat is known to have a lower and less favourable ratio of unsaturated and saturated fatty acids (UFA/SFA) to non-ruminant meat. However, the ratio of n-6 and n-3 is low and is more favourable to human health than non-ruminant meat

(*Klir et al., 2012*). It is well known that a high content of saturated fatty acids in foods contributes to the onset of cardiovascular disease, arteriosclerosis, hypertension, hypercholesteremia (*McAffe et al., 2010*). The recommended value for the polyunsaturated to saturated fatty acids ratio is up to 0.45 and below 4.0 for the n-6 to n-3 fatty acids ratio (*Wood and Enser, 1997; Klir et al., 2012*). Therefore, the intention is to model the fatty acid profiles in lamb meat, through nutrition, and more precisely to optimize the ratio of unsaturated and saturated fatty acids.

Saturated fatty acids predominate in the meat of lambs whose primary food is milk, which is a consequence of the fatty acid composition of sheep's milk, which is dominated by this type of fatty acids (*Diáz et al., 2003).* Also, in this period, since these are non-functional ruminants, the biohydrogenation of fatty acids is negligible. When lambs are weaned and start eating more solid foods, the content of saturated fatty acids decreases (*Radzik-Rant et al., 2012).*

The influence of dietary management on fatty acid content of lamb meat has been studied by a number of authors. *Cividini et al. (2008, 2014)* find that grazing lamb meat hasd a significantly higher n-3 PUFA, CLA and more favourable n-6/n-3 fatty acid ratio ($P<0.001$), while the lamb meat originating from animals fed concentrated diet has a higher content of n-6 PUFAs and a higher ratio of n-6/n-3 fatty acids, exceeding the recommended value of 4.0. In the intramuscular fat of lambs fed concentrated feed, a higher share of oleic acid was found. According to some authors, the concentration of this acid may increase due to an increase in the activity of the Δ9 desaturase enzyme, which synthesizes oleic from stearic acid (*Griinari, 2000*). Examining the impact of 3 feeding systems: grazing, closed and combined, on the fatty acid profile of lambs, *Boughalmi and Araba (2016)* have found that meat of lambs fed a concentrated mixture in a closed system had higher contents of palmitic and oleic acids. Contrary to this, lambs in the grazing feeding system had more CLA, linolenic, n-6 and n-3 fatty acids ($P <0.01$), which can be explained by the fact that grass contains 1-3% fat, with C18: 3 n-3, are represented with 55-65%. The effect of grazing and concentrated fattening of lambs on the fatty acid profile of muscle and fat tissue was examined by *Rowe et al. (1999)* on samples of *m. longissimus dorsi*.

Table 2. Effect of diet on fatty acid content of MLD lambs (*Rowe et al., 1999*)

Fatty acids	Pasture	Fodder mixture	Significance
C18:0 stearic acid	30.11±0.42	23.51±0.36	p<0.01
C18:1n-9 oleic acid	30.73±0.40	38.21±0.44	p<0.01
C18:2 n-6 linoleic acid	2.63±0.14	3.85±0.13	p<0.01
C18:32 n- arachidonic acid	0.32±0.05	0.21±0.03	p<0.01
SFA	55.07±0.43	49.36±0.54	p<0.01
MUFA	31.73±0.35	40.68±0.49	p<0.01
PUFA	5.36±0.40	4.74±0.40	p<0.01

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

Meat of lambs fed solely on pasture had a higher content of saturated fatty acids (stearic and arachidonic), which can be explained by a higher intake of pasture food that promotes the activity of rumen microflora, which performs the biohydrogenation of fatty acids, increasing the content of SFA. Omega 3 fatty acids, which the pasture is rich in, is essential to the human body (they reduce the risk of cancer, inflammation, arthritis, they are important for the cognitive functions of the brain), but they are not synthesized. Particularly important is the content of ω -3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in meat, which have a positive effect on human health.

However, it must be taken into account that a higher amount of unsaturated fatty acids, especially PUFA, creates preconditions for easier oxidation, which adversely affects the organoleptic properties of meat (*Kaić et al., 2013*). In this case, the use of antioxidants is indicated, and fresh, voluminous nutrients are a rich source of antioxidants such as tocopherols, carotenoids, and phenolic compounds. *Petrova et al. (1994)* reports that concentrate-based diets lead to a greater amount of carbohydrate available, which shortens the retention time of food in rumen and reduces the biohydrogenation of polyunsaturated fatty acids. Also, the content of n-3 polyunsaturated fatty acids (PUFA) is higher in the meat of lambs fed hay (Table 3).

Table 3. Chemical composition of grass hay and concentrate pellet (*Demirel et al., 2006*)

Composition	**Grass hay**	**Concentrate**
Dry matter (%)	89.2	89.9
Crude protein (%)	10.2	16.4
Ether extract (%)	3.6	3.6
Ash (%)	8.8	7.9
NDF (%)	32.6	23.5
Metabolizable energy, MJ/kg DM[b]	8.28	10.45
Fatty acid content and composition, % fatty acids		
C14:0	2.7	0.4
C16:0	17.8	18.5
C16:1	1.9	0.5
C18:0	2.4	3.5
C18:1 *n* − 9	13.2	22.1
C18:2 *n* − 6	23.6	51.8
C18:3 *n* − 3	38.2	3.0

In lambs fed this diet, a 3.5-fold increase in linolenic acid (C18: 3 n-3) was observed in *m. longissimus thoracis*, relative to those fed with concentrate, which is a reflection of the high content of the same fatty acid in hay (Table 4). In the same study, the share of eicosapentaenoic acid EPA (C20: 5 n - 3) was 2.5 times higher, of docosapentaenoic acid - DPA (C22: 5 n - 3) 3 times and docosahexaenoic acid DHA 2.5 times higher in lambs fed diet based on hay. The levels of linoleic (C18: 2 n - 6) and arachidonic acids (C20: 4 n - 6) in lambs fed concentrated feed were higher compared to lambs fed hay because grain contains high levels of C18: 2 n - 6 and C20 : 4 n - 6, which are linoleic acid metabolites, obtained by enzymatic desaturation and elongation (*Gurr et al., 2008*). The share of C18: 2 n - 6 was almost twice as high and C20: 4 n - 6 was 1.5 times higher in lambs fed concentrated feed compared to lambs fed hay.

Table 4. Breed, feed and interaction effects on fatty acid composition of *longissimus thoracis* of lambs fed different forage: concentrate ratio (mg/100 g muscle) (*Demirel et al.., 2006*)

Fatty acid	FEED		BREED		SED	Breed	Feed	Breed × feed
	Grass hay	Concentrate	Kivircik	Sakiz				
C14:0	75	59.9	74.6	59.9	2.76	***	***	n.s.
C16:0	446	385	470	360	11.0	***	***	n.s.
C16:1	40.6	21.1	36.5	35.2	1.98	n.s.	***	n.s.
C18:0	424	321	419	325	14.1	***	***	*
C18:1	758	608	746	621	21.3	***	***	*
C18:2 *n* − 6	96.6	190	150	137	2.86	***	***	n.s.
C18:3 *n* − 3	46.7	13.0	31.9	27.9	0.92	***	***	n.s.
C20:4 *n* − 6	26.2	39.0	35.6	29.5	1.18	***	***	*
C20:5 *n* − 3	25.4	10.8	18.2	17.9	0.84	n.s.	***	n.s.
C22:5 *n* − 3	15.7	5.6	10.7	10.6	0.46	n.s.	***	n.s.
C22:6 *n* − 3	7.8	3.0	5.1	5.6	0.25	n.s.	***	*
Total FA	2086	1791	2128	1748	26.7	***	***	***

Modelling the fatty acid composition of lamb meat, through certain feeding strategies, with an emphasis on conjugated linoleic acid (CLA) has been the subject of research by a number of authors *(Schmid et al. 2006, Garcia et al. 2008, Serra et al. 2009)*. Conjugated linolenic acid consists of a group of geometric and positional isomers of linolenic acid, which are attributed with anticancer, antidiabetic effect, as well as effect on the immune system and bone metabolism. The highest concentrations of CLA have been found in lamb meat (4.3-19 mg/g fat). *Santos-Silva (2002)* finds higher concentrations of CLA in meat of lambs fed on pasture, compared with lambs fed with concentrated mixture (7.1 vs. 3.2 mg/g fat), which is attributed to the high content of PUFA in grass (especially n-3 18: 3 with a ratio n-6: n-3 of about 1: 3-5). *Noble et al. (1974)* find that in order to increase CLA in *m.semimembranosus* of lambs, after the grazing season, the best results are obtained by a nutritional treatment involving a combination of fish oil and linseed rich in linoleic fatty acid (C18: 3 n- 3). CLA has an anticancer, anti-diabetic effect, as well as a positive effect on the immune status, cardiovascular system and bone mineralization. According to *Dhiman et al. (2001)*, pasture-fed ruminant products have 300-500% more CLA compared to products from ruminants fed with 50%

hay and silage and 50% concentrated nutrients, which clearly indicates the importance of nutrition management that will result in an increase in CLA content in ruminant meat and even lambs.

Conclusion

The concentration and profile of fatty acids in meat, affect its nutritional value, as well as the health status of people, preventing or increasing the risks of cancer, cardiovascular disease, diabetes. Fat content and fatty acid content in lambs depend on: genotype, age, gender, diet and lambs rearing/housing system. Nutrition management has a significant place and provides the opportunity to model the composition of meat and fatty acids, which contributes to the improvement of fat content, the optimal ratio of saturated and polyunsaturated fatty acids, as well as a more favourable ratio of n-6/n-3. Nutrition of lambs consisting of pasture and hay compared to the concentrated diet will imply a lower carcass fat content and a higher concentration of n-3 PUFA, CLA and a more favourable n-6/n-3 fatty acid ratio. Omega 3 fatty acids, which the pasture is rich in, are essential for the human body (reduce the risk of cancer, inflammation, arthritis, they are important for cognitive brain function), but they are not synthesized. Conjugated linoleic acid (CLA) is of great importance, since it has an anticancer, antidiabetic effect as well as an effect on the immune system, indicating a direction for future research on lamb meat.

Menadžment u ishrani jagnjadi kao put modeliranja profila masnih kiselina u mesu

Dragana Ružić-Muslić, Milan P. Petrović, Zorica Bijelić, Violeta Caro Petrović, Nevena Maksimović, Bogdan Cekić, Ivan Ćosić

Rezime

Pored nutritivne vrednosti, veoma važan kriterijum pri odabiru mesa, za savremenog potrošača je i zdravstveni aspekt, odnosno sadržaj masnog tkiva i profil masnih kiselina u mesu. Sadržaj masti i masnih kiselina, je između ostalog uslovljen sistemom ishrane i načinom držanja. Jagnjad hranjena na paši imaju manji udeo masnog tkiva u trupu od grla hranjenih koncentrovanom smešom, u zatvorenom sistemu. Preporučena vrednost za odnos polinezasićenih i zasićenih masnih kiselina iznosi do 0.45 a ispod 4.0 za odnos između n-6 i n-3 masnih kiselina. Imajući u vidu da je uticaj ishrane jagnjadi na navedene odnose značajan,

modeliranje masnokiselinskog sastava treba usmeriti na sistem ishrane jagnjadi koji dovodi do smanjenja udela zasićenih a povećanje koncentracije polinezasićenih (PUFA) masnih kiselina u mesu. Strategija hranjenja koja podrazumeva pašni sistem ishrane jagnjadi, rezultira većim sadržajem n-3 PUFA, CLA i povoljnijim n-6/n-3 odnosom masnih kiselina, dok meso jagnjadi hranjene koncentrovanom hranom ima veći udeo n-6 PUFA i veći odnos n-6/n-3 masnih kiselina, što prevazilazi preporučenu vrednost od 4.0. Konjugovana linolenska kiselina (CLA) ima veliki značaj, obzirom da ima antikancerogeni, antidijabetični efekat kao i efekat na imuni sistem, što ukazuje na smernicu budućih istraživanja na jagnjećem mesu.

Ključne reči: jagnjad, masne kiseline, paša, n-3 PUFA, CLA

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MEAT QUALITY, CHEMICAL AND FATTY ACIDS COMPOSITION AND OXIDATIVE STABILITY OF PORK FROM ENTIRE MALES, SURGICAL CASTRATES AND GILTS AFTER BETAINE SUPPLEMENTATION TO DIET

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Abstract: This study was conducted to assess the effect of sex and betaine supplemented diet on chemical composition, cholesterol content, meat quality, fatty acids composition and oxidative stability of pork from entire males, surgical castrates and gilts. A total of forty-two pigs – (entire males - EM, surgical castrates – SC, and gilts - G, each of 14) progeny of Landrace sows and Hampshire x Pietrain boars were involved in the trial. Pigs were allocated to the control and experimental groups (each of 21 pigs – 7 EM, 7 SC and 7 G). Control pigs received standard diet without any supplement whereas experimental ones were fed the same diet with supplement of betaine (1.25 $g.kg^{-1}$ of feed) for thirty days prior to slaughter. Castrates had significantly higher intramuscular fat and cholesterol content ($P<0.05$) than entire males and gilts. Also, they had greater content of vaccenic, arachidonic ($P<0.05$), oleic, eicosanoic, and total monounsaturated fatty acids ($P<0.01$). Contrary, entire males had the highest level of linolenic, linoleic, total polyunsaturated and n-6 fatty acids ($P<0.05$). Sex of pigs did not have any effect on meat quality and oxidative stability of pork. Betaine supplementation increased cholesterol content in castrates compared to other two sexes ($P<0.05$). Drip loss value was reduced in group of entire males ($P<0.05$) and oxidative stability of muscle was improved in all three groups ($P<0.05$). Fatty acids profile was not influenced by betaine treatment. Interactions between sex and betaine supplementation were observed for cholesterol concentration, drip loss value, oleic, linolenic, total polyunsaturated and n-6 fatty acids as well as oxidative stability after 30 and 120 min. of incubation.

Key words: betaine, entire males, pork quality, fatty acids, oxidative capacity

Introduction

Surgical castration of piglets has been widespread procedure performed routinely in almost all European Union member states. The main reason for implementing this practice is to avoid the unpleasant odour so-called boar taint arising from adolescence of uncastrated males and releasing in the heat treatment of their meat *(Bonneau, 1998).* Recently, growing demands of animal rights organizations as well as public initiatives for livestock welfare put considerable pressure on union´s governing authorities towards to stopping the surgical castration of pigs in the EU after 2020. Therefore, rearing entire males might become the main practice in EU pig husbandry. It is well-known that entire males have several advantages compared to castrates, such as faster growth, better feed conversion, and higher lean meat content in the carcass. However, some studies have suggested several problems regarding meat quality of entire males, other than boar taint – higher incidence of DFD (dry-firm-dark) meat *(EFSA, 2004)*, worse toughness *(Pauly et al.,2008)*, lower ultimate pH *(Aluwé et al, 2013),* less intramuscular fat content *(Škrlep et al., 2010, 2012)*, softer fat due to higher proportion of unsaturated fatty acids *(Pauly et al., 2009)*, higher drip loss *(Aluwé et al., 2013).*

Sex differences in meat quality, fat content and fatty acid composition of fat tissue are well-known in the literature. Generally, entire males have lower fat content compared to castrates and females based on different hormonal status. Differences in fatty acid composition between entire males, castrates and gilts are mainly related to changes in the rate of subcutaneous fat accumulation in the carcass *(Wood et al., 2008; Pauly et al., 2009; Grela et al., 2013; Mackay et al., 2013).* Meta-analysis of *Pauly et al. (2012)* describing studies from 1990 until 2010 confirmed that entire males have a greater amount of polyunsaturated fatty acids (PUFA) than castrates but not gilts. They also have a lower content of saturated (SFA) and similar content of monounsaturated (MUFA) fatty acids, higher PUFA/SFA and lower n-6/n-3 ratios compared to castrates *(Pauly et al., 2009; Grela et al., 2013).* At the same time, analyses showed that entire males have a higher capacity for protein deposition in the body and higher turn-over *(Batorek et al., 2012; Pauly et al., 2012; Trefan et al., 2013).* Fat deposition and fatty acid profile in pigs are affected not only by hormones but also by genetics *(Canovas et al., 2009; Cho et al., 2011)*, which results in different enzymes activity *(Doran et al., 2006; Missotten et al., 2009; Mackay et al., 2013)*, dietary energy and protein intake *(Wood et al., 2004)*, and dietary fat composition as well *(Missotten et al., 2009; Benz et al., 2011).*

One of the possibilities to influence pork quality, its composition and other parameters is the addition of various supplements (vitamins, minerals, trace elements, creatine, betaine, etc.) to the pigs' nutrition *(Lauridsen et al., 1999; Apple et al., 2001; Swigert et al., 2004; Lahučký et al., 2007; Su et al., 2013;*

Madeira et al., 2015). Betaine is known as a product of choline degradation. It occurs naturally in many tissues where it plays a role in various metabolic processes as a methyl donor (e.g. formation of methionine). Other studies have suggested that betaine may influence the efficiency of energy utilization in pigs. Some reports have shown positive effect of dietary betaine supplementation on pork quality such as pH *(Matthews et al., 2001a, 2001b; Hur et al., 2007)*, colour *(Yang et al., 2009; Su et al., 2013);* whereas other studies have shown no effect on sensory properties *(Øverland et al., 1999),* subjective marbling and firmness-wetness, or even though, negative effect on subjective color of the loin muscle *(Matthews et al., 1998).* Recently, some studies reported positive impact of dietary betaine supplementation on reduction of fat deposition in pig carcasses *(Huang et al., 2006, 2008; Sales, 2011).* This decrease is associated with the increasing rate of lipolysis and decreasing rate of lipogenesis. Both of these biochemical processes are regulated by many enzymes *(Huang et al., 2006, 2009).*

Since there is a lot of studies in the literature related to the performance, pork quality and fat composition of castrated or female pigs but less of uncastrated males, the aim of this study was to evaluate the effect of betaine supplementation on chemical composition, meat quality, fatty acid profile and oxidative capacity of pork in entire males and to compare it to the surgical castrates and gilts.

Materials and Methods

Totally forty-two pigs (entire males - EM, surgical castrates – SC, and gilts - G, each of 14) – crosses between Landrace sows and Hampshire x Pietrain boars were involved in the experiment. Animals were housed in the pens at experimental farm of Research Institute for Animal Production (RIAP). They were located in test station at 20 – 25 kg live weight for timely adaptation to the new space and feed. Experiment started at 30 kg of live weight (age of 80 ± 7 days). Pigs were divided to the control and experimental groups (each of 21 pigs – 7 EM, 7 SC and 7 G). Control group received the standard diet (Table 1) without any supplement. Experimental group was fed standard diet with the same composition as in control group but with supplement of betaine (1.25 $g.kg^{-1}$ of feed) for thirty days prior to slaughter. Pigs were allowed free access to drinking water during whole experiment. Feed was provided on *ad libitum* basis. Pigs were regularly weighed in weekly intervals.

Table 1. Composition and nutritive value of diets

Ingredients[a], %	Control	Betaine	Chemical analysis	Control	Betaine
Barley	42.7	42.7	Dry matter, %	86.30	86.30
Wheat	21.0	21.0	Crude protein, %	16.84	16.84
Oat	15.0	15.0	Crude fat, %	2.43	2.43
Soybean meal	12.0	12.0	Crude fibre, %	4.86	4.86
Wheat brans	2.0	2.0	Ash, %	4.56	4.56
Meat and bone meal	2.0	2.0	N-free extract	57.68	57.68
Fodder yeast	1.7	1.7	Metabolizable energy (MJ)	12.31	12.31
Mineral supplement	2.5	2.5	Lysine, $g.kg^{-1}$	8.64	8.64
Biofactor supplement	0.6	0.6			
Fodder salt	0.5	0.5			
Betaine	-	0.125			

[a] Composition declared by producent of feed mixture

The experiment was performed in accordance with Act on animal veterinary care No. 39/2007 of Slovak Republic and approved by Animal Care Committee of the Research Institute for Animal Production. Animals were slaughtered at 110 ± 5 kg live weight (age of 174 ± 11 days) at the experimental slaughterhouse of RIAP by electrical stunning (90 - 100 V, 0.9 - 1.0 A, 50 Hz) followed by exsanguination. Evisceration was completed about 20 min *post mortem*. Chilling of the carcasses (air temperature 2 - 4 °C, velocity 0.5 – 1.0 $m.s^{-1}$) started approximately 60 min after slaughter and was continued overnight. After 24-hours chilling of carcasses at 4 °C, the *longissimus dorsi* muscle (150 g of sample) was removed from the right side of carcass and sliced into chops 2.5 cm thick for further meat quality (colour, drip loss) analysis. One wrapped sample was stored in the dark for 5 days at 4 °C for the shear force and colour analysis. Forty-five minutes and 24 h after slaughter, pH values were measured in the loin muscle (*musculus longissimus dorsi* - LD) from the right side of carcasses between 13th and 14th rib using device METTLER TOLEDO (pH meter FiveGo™, Columbus, USA) with combined electrode. Colour was measured by spectrophotometer MINISCAN XE Plus (Hunter Associates Laboratory, Inc., Reston, USA). Drip loss was assessed 24 h after slaughter by Honikel method *(Honikel, 1998).* Four days after slaughter, the shear force was measured using TEXTURE ANALYSER TA-XT2i device (Stable Micro Systems Ltd, Surrey, UK). Approximately, thirty min. *post mortem*, *longissimus dorsi* muscle samples (100 g) between 13th and 14th rib were taken, wrapped into aluminium foils and imposed in liquid nitrogen for 5 days for oxidative stability analysis. The oxidative capacity of *longissimus muscle* homogenate was determined as thiobarbituric acid reactive substances (TBARS) according to *Küchenmeister et al. (1999)*. TBARS values express as equivalents of malondialdehyde (MDA, $nM.mg^{-1}$ homogenate protein) which is breakdown product formed during peroxidation of lipids stimulated by Fe^{2+}/ascorbate. To

stimulate lipid peroxidation, 3 ml of muscle homogenate was incubated in 0.1 mM ascorbate and 5 mM $FeSO_4$. From this, 0.5 ml was immediately removed and pipetted into 0.25 ml of 20% trichloroacetic acid in 100 mM KCl. The remaining homogenate was placed in a water bath at 37 °C and after 30, 60, and 120 min, 0.5 ml each of this incubated homogenate were transferred into the trichloroacetic acid. Then samples were centrifuged at 10 000 g for 10 min and 0.5 ml of the supernatants were mixed with 0.5 ml of thiobarbituric acid (0.67%) and boiled for 15 min in a water bath. The absorbance at 535 nm was determined immediately after cooling. All samples determined for chemical and fatty acid compositions as well as cholesterol determination were transported to the Chemical laboratory of the Slovak Agricultural University. Each sample (50 g) was homogenized and subsequently analysed by the Fourier Transform Infrared (FTIR) method *(Carbonaro and Nucara, 2010)* using the device Nicolet 6700 (IET Ltd., Illinois, USA).

Data from the experiment were analysed by a two-way ANOVA with fixed effects of treatment (betaine or none) and sex (entire males, castrates or gilts) as well as corresponding interactions between treatment and sex using procedure GLM of the statistical program SAS-STAT, version 9.1.3 (SAS Institute Inc., Cary, N.C., USA, 2002-2003). Basic statistics was done using MEANS procedure.
The model used was:

$$y_{ijk} = \mu + B_i + D_j + B*D_{ij} + e_{ijk}$$

where y_{ijk} – characteristic of trait selected, μ – intercept, B_i – effect of sex (i = EM, SC, G), D_j – effect of diet (j = C, B), $B*D_{ij}$ – two-way interaction effect sex x diet, e_{ijk} – random error (k = 1.....n_{ijk}). Data in tables are expressed as Least Square Means (LSM) ± standard error of the mean (SEM). Comparisons between groups were done by Scheffe´s test and differences were considered to be statistically significant at the level of $P<0.05$.

Results and Discussion

Sex of pigs had significant effect on IMF and cholesterol content. Both of these parameters were the highest ($P<0.05$) in surgical castrates compared to entire males and gilts (Table 2). The same results regarding IMF have been reported in other studies *(Škrlep et al., 2010; Gispert et al., 2010).* Differences in IMF and cholesterol concentrations are associated with hormonal activity of sex steroids which influence lipid synthesis and metabolism. Surgical castration eliminates the effect of these steroids resulting in higher fatness of carcass including higher content of IMF. In general, betaine supplementation increased cholesterol content in experimental pigs compared to the control. This increase was due to significant enhancement in group of betaine supplemented surgical castrates which is showed

by interaction of sex and diet (Table 6), whereas entire males and gilts of control or supplemented groups had similar levels of cholesterol. This is in agreement with findings of *Albuquerque et al. (2017) and Li et al. (2017)* reporting increased cholesterol concentration in castrates or gilts, respectively. *Matthews et al. (2001b)* found higher plasma cholesterol concentration after 0.125 % of betaine supplementation to the pigs' diet. *Martins et al. (2010)* reported increased cholesterol content in subcutaneous dorsal fat deposition but not in the *musculus semimembranosus.* Authors suggested that betaine affects cholesterol metabolism in the liver by stimulating lipid mobilisation and enhancing hepatic lipoprotein secretion.

Table 2. Effects of sex and betaine treatment on chemical composition and cholesterol content of *longissimus dorsi* muscle

Trait	Sex (LSM)			Signif.	Diet (LSM)		Signif.	SEM	Sex x B
	EM	SC	G		C	B			
Total water, %	74.4	73.8	74.3	ns	74.1	74.2	ns	0.3	ns
Total protein, %	21.6	21.5	21.8	ns	21.7	21.6	ns	0.2	ns
Intramuscular fat, %	2.6[a]	3.5[b]	2.8[a]	*	3.0	3.1	ns	0.2	ns
Cholesterol, %	0.38[a]	0.58[b]	0.36[a]	*	0.41[a]	0.51[b]	*	0.2	*

[a,b] Different letters within row mean significant differences at * = $P<0.05$, EM – entire males, SC – surgical castrates, G – gilts, C – control group, B – betaine supplemented group, LSM – least square mean, SEM – standard error of means, ns = not significant ($P\geq0.05$)

Sex did not affected quality traits of *longissimus dorsi* muscle. There was only tendency (P = 0.054) of paler meat of entire males compared to castrates measured 24 h after slaughter but not after 5 days (Table 3).

Table 3. Effects of sex and betaine treatment on pork quality

Trait	Sex (LSM)			Signif.	Diet (LSM)		Signif.	SEM	Sex x B
	EM	SC	G		C	B			
pH_{45}	6.36	6.42	6.33	ns	6.42	6.29	ns	0.28	ns
pH_{24}	5.72	5.59	5.61	ns	5.68	5.52	ns	0.08	ns
$Colour_{24}$ L^*	50.68[ǂ]	49.81[ǂ]	50.03	0.054	49.97	49.37	ns	0.69	ns
a^*	1.75	1.86	1.85	ns	1.86	1.94	ns	0.16	ns
b^*	7.66	7.86	7.84	ns	7.75	7.78	ns	0.25	ns
$Colour_5$ L^*	51.67	52.06	51.82	ns	51.56	51.37	ns	0.72	ns
a^*	2.76	2.70	2.70	ns	2.65	2.61	ns	0.32	ns
b^*	8.28	8.47	8.38	ns	8.48	8.53	ns	0.31	ns
Drip loss, %	4.11	3.87	4.32	ns	4.21[a]	3.83[b]	*	0.33	*
Shear force, kg	5.36	5.07	5.08	ns	5.20	4.88	ns	0.25	ns

[a,b] Different letters within row mean significant differences at * = $P<0.05$, EM – entire males, SC – surgical castrates, G – gilts, C – control group, B – betaine supplemented group, LSM – least square

mean, SEM – standard error of means, Colour L* = lightness, a* = redness, b* = yellowness, ns = not significant (P≥0.05)

Betaine supplementation in present study had no significant effect on pH (45 min and 24 h), colour (24 h and 5 days) and shear force values. Some other studies reported a higher initial or ultimate pH after betaine supplementation *(Matthews et al., 2001a, 2001b; Hur et al., 2007).* This increase of pH may indicate that rate of pH decline after slaughter is slower in pigs fed betaine supplement. As known, meat pH is closely related to meat colour. This trait was not also affected by betaine supplementation. Similar results considering pork colour were reported by other authors *(Matthews et al., 2001a; Feng et al., 2006).* Positive effect of betaine on meat colour was found by *Yang et al. (2009).* In contrary, some studies reported reducing colour darkness *(Matthews et al., 2001b).* These discrepancies in experiments may be due to different genotypes and muscle types of used pigs. Betaine supplementation in our study significantly (P<0.01) decreased drip loss. Again, it was due to significant lowering only in one sex – entire males, which is presented by interaction between sex and betaine (Table 6). Similar results - decreasing by 11% after betaine administration – were found by *Matthews et al. (2001a).* Possible effect of betaine can be explained by stabilization of cell membranes against fluid loss from muscle.

Sex of pigs had considerable effect on several fatty acids concentrations in our study. Castrates had higher content of oleic and total MUFA than entire males (P<0.01); vaccenic and arachidonic acids than gilts (P<0.05); and eicosenoic acid (P<0.01) than both, entire males and gilts (Tab 4). On the other hand, entire males had higher content of linolenic acid than castrates (P<0.05); linoleic, total PUFA and n-6 fatty acids (P<0.05) than both, castrates and gilts. The same results in regard of oleic and linolenic acids were observed in study of *Grela et al. (2013). Cai et al. (2010)* also reported higher concentrations of PUFA in meat of entire males compared to castrates. Greater amount of PUFA and lower SFA in entire males compared to castrates (or gilts) have been reported in other studies *(Jaturasitha et al., 2006; Pauly et al., 2009, 2012).* These differences depend on different fatty acid composition of two basic lipid fractions – phospholipids, which are part of cell membranes (relatively constant with increasing fatness of body), and neutral lipids (triacylglycerols), which are accumulated in fat depots and their proportion increases with increasing body fatness *(De Smet et al., 2004; Wood et al., 2008).* Content of PUFA is higher in phospholipids (35-48%) than in triacylglycerols (5-14%) *(Cameron et al., 2000)* and increases slower than SFA and MUFA (contained in neutral lipids) during growth and fattening, which results in lower relative proportion of PUFA in pig body *(Riley et al., 2000).* Generally, carcass fatness/leanness has been found to be strongly associated with fatty acid composition. Increased backfat thickness has negative relationship to the degree of unsaturation *(Wood et al., 2008).* Recently, research in this area showed that also

other factors such as genes *(Gunawan et al., 2013)*, sex steroids and enzymes involving in lipid synthesis and metabolism *(Hallenstvedt et al., 2012; Corominas et al., 2013; Mackay et al., 2013)*, and nutrition *(Missotten et al., 2009; Benz et al., 2011)* as well, may all affect fat deposition and fatty acid composition of pig muscles and adipose tissue. Supplementation of betaine in the presented study had no effect on fatty acids profile of *longissimus dorsi* muscle. The same result was reported in the study of *Madeira et al. (2015). Albuquerque et al. (2017)* also did not find any influence of betaine on SFA, MUFA, PUFA and n-6/n-3 and PUFA/SFA ratios, as well. In contrary, study of *Madeira et al. (2016)* found decreasing concentrations of vaccenic, palmitoleic and total MUFA after betaine addition to the diet of entire male pigs. Interactions between sex and betaine in present study was observed for oleic, linolenic, total PUFA and n-6 fatty acids (Table 6). Differences within sex for control and supplemented groups were small and insignificant (P>0.05).

Table 4. Effects of sex and betaine supplementation on fatty acid composition of intramuscular fat (% of total fatty acids) in the *longissimus dorsi* muscle

Trait	Sex (LSM)			Signif.	Diet (LSM)		Signif.	SEM	Sex x B
	EM	SC	G		C	B			
Myristic C14:0	1.31	1.28	1.26	ns	1.28	1.28	ns	0.01	ns
Palmitic C16:0	24.50	24.57	24.46	ns	24.51	24.54	ns	0.04	ns
Stearic C18:0	11.30	11.28	11.31	ns	11.29	11.31	ns	0.07	ns
Total SFA	39.39	39.42	39.40	ns	39.41	39.40	ns	0.38	ns
Oleic C18:1n-9	44.66^{a}	47.42^{b}	45.80^{ab}	**	46.07	45.98	ns	0.65	*
Eicosenoic C20:1	0.61^{a}	0.69^{b}	0.60^{a}	**	0.64	0.66	ns	0.04	ns
Vaccenic C18:1t11	4.42^{ab}	4.50^{a}	4.39^{b}	*	4.45	4.46	ns	0.03	ns
Total MUFA	49.71^{a}	52.63^{b}	51.26^{ab}	**	51.32	51.58	ns	0.86	ns
Arachidonic C20:4n-6	1.38^{ab}	1.46^{a}	1.34^{b}	*	1.40	1.41	ns	0.08	ns
Linolenic C18:3n-3	2.07^{a}	1.92^{b}	2.05^{a}	*	1.97	2.01	ns	0.03	*
Linoleic C18:2n-6	9.36^{a}	8.33^{b}	8.60^{b}	*	8.89	8.96	ns	0.69	ns
Total PUFA	14.83^{a}	13.35^{b}	13.49^{b}	*	13.81	13.72	ns	0.78	*
n-3 FA	2.95	2.88	2.85	ns	2.91	2.89	ns	0.12	ns
n-6 FA	11.88^{a}	10.47^{b}	10.65^{b}	*	11.08	11.14	ns	0.56	*
n-6/n-3	4.05	3.64	3.76	ns	3.86	3.85	ns	0.43	ns
PUFA/SFA	0.37	0.34	0.34	ns	0.34	0.35	ns	0.13	ns

^{a,b} Different letters within row mean significant differences at * = P<0.05, ** = P<0.01, EM – entire males, SC – surgical castrates, G – gilts, C – control group, B – betaine supplemented group, LSM – least square mean, SEM – standard error of means, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, ns = not significant (P≥0.05)

An oxidative effect of sex and betaine supplementation is shown in Table 5. Sex of pigs did not affect oxidative stability of pigs muscle. As known, higher content of PUFA and lower of SFA (expressed as PUFA/SFA ratio) is beneficial from the human health point of view. On the other hand, fat containing a higher

amount of PUFA is softer and more liable to oxidative deterioration. Results suggest significant reducing of peroxidation – lower values of MDA production after incubation with Fe^{2+}/ascorbate mixture – in pigs fed betaine. This result is in accordance with the study of *Su et al. (2013).* Tendency of reducing the lipid oxidation (insignificant differences) in pork stored for 13 days were only outlined in the study of *Hur et al. (2007).* Significant interactions between sex and diet were found after 30 and 120 min incubation in the all three groups - entire males, gilts ($P<0.05$) and castrates ($P<0.05$, 0.01 resp.). So, positive effect of betaine supplementation on stabilization of cell membranes against oxidation was manifested in whole set as well as within each sex of pigs.

Table 5. Effects of sex and betaine supplementation on oxidative stability of *longissimus dorsi* muscle

Trait	Sex (LSM)			Signif.	Diet (LSM)		Signif.	SEM	Sex x B
	EM	SC	G		C	B			
TBARS (0 min)	0.06	0.05	0.06	ns	0.06	0.06	ns	0.002	ns
TBARS (30 min)	0.24	0.23	0.25	ns	0.28^{a}	0.21^{b}	*	0.02	*
TBARS (60 min)	0.30	0.32	0.29	ns	0.35^{a}	0.27^{b}	*	0.02	ns
TBARS (120 min)	0.38	0.39	0.36	ns	0.43^{a}	0.30^{b}	**	0.02	*

[a,b] Different letters within row mean significant differences at * = $P<0.05$, ** = $P<0.01$, EM – entire males, SC – surgical castrates, G – gilts, C – control group, B – betaine supplemented group, LSM – least square mean, SEM – standard error of means, TBARS - thiobarbituric acid reactive substances (nmol/mg protein), ns = not significant ($P\geq0.05$)

Table 6. Interactive effect of sex and betaine supplementation on cholesterol content, fatty acids and oxidative stability of *longissimus dorsi* muscle

Trait	EM (LSM)		SC (LSM)		G (LSM)		SEM
	C	B	C	B	C	B	
Cholesterol, %	0.36	0.39	0.46^{a}	0.73^{b}	0.38	0.33	0.10
Drip loss, %	4.48^{a}	3.65^{b}	3.95	3.76	4.41	4.20	0.28
Oleic C18:1n-9	44.61	44.68	47.46	47.40	45.83	45.78	0.54
Linolenic C18:3n-3	2.06	2.08	1.90	1.93	2.06	2.05	0.02
Total PUFA	14.76	14.91	13.44	13.24	13.53	13.43	0.76
n-6 FA	11.79	11.99	10.39	10.56	10.72	10.55	0.69
TBARS (30 min)	0.28^{a}	0.21^{b}	0.27^{a}	0.20^{b}	0.28^{a}	0.22^{b}	0.01
TBARS (120 min)	0.42^{a}	0.32^{b}	0.45^{A}	0.30^{B}	0.42^{a}	0.30^{b}	0.01

[a,b] Different letters with row mean significant differences at $P<0.05$, [A,B]Different letters with row means significant differences at $P<0.01$, EM – entire males, SC – surgical castrates, G – gilts, C – control group, B – betaine supplemented group, LSM – least square mean, SEM – standard error of means, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, TBARS - thiobarbituric acid reactive substances, ns = not significant ($P\geq0.05$)

Conclusion

In conclusion, sex of pigs had significant effect on IMF and cholesterol content as well as some monounsaturated and polyunsaturated fatty acids. Surgical castrates had highest IMF and cholesterol concentrations. Their IMF was formed significantly in greater amount of MUFA compared to gilts and entire males which had highest content of linoleic, linolenic, total PUFA and n-6 fatty acids. From the healthy human nutrition perspective, higher proportion of linolenic and total PUFA as well as less IMF and cholesterol in meat from entire males could be beneficial after an anticipated ban of surgical castration of piglets in the EU countries. However, higher content of n-6 PUFA, especially linoleic acid, could be problematic. Betaine supplementation significantly increased cholesterol content in surgical castrates, decreased drip loss in entire males and improved oxidative stability of pork in all three groups. Lower drip loss and better oxidative stability, especially in entire males, may be beneficial for meat industry, wholesale and retail, however further research is needed to evaluate different doses and time of supplementation of betaine.

Kvalitet mesa, hemijski sastav, profil masnih kiselina i oksidativna stabilnost svinjetine od nekastriranih mužjaka, hirurških kastrata i nazimica nakon dodavanja betaina u ishrani

Ivan Bahelka, Ondřej Bučko, Roman Stupka, Jaroslav Čítek, Katarína Hozáková

Rezime

Ova studija je sprovedena da bi se procenio uticaj pola i dodatka betaina u ishrani na hemijski sastav, sadržaj holesterola, kvalitet mesa, sastav masnih kiselina i oksidativnu stabilnost svinjskog mesa nekastriranih nerastova, hirurških kastrata i nazimica. U ispitivanje je bilo uključeno ukupno četrdeset i dva grla (nekastrirani nerastovi - EM, hirurški kastrati - SC, i nazimice - G, svaki po 14), potomci landras svinja i hempšir x pijetren nerastova. Svinje su raspoređene u kontrolne i eksperimentalne grupe (svaka po 21 grlo - 7 EM, 7 SC i 7 G). Kontrolne svinje su trideset dana pre klanja dobijale standardnu ishranu bez ikakvih dodataka, dok su eksperimentalne hranjene istom hranom sa dodatkom betaina (1,25 g.kg-1 hrane). Kastrati su imali znatno viši sadržaj intramuskularne masti i holesterola ($P<0,05$) u odnosu na nekastrirane nerastove i nazimice. Takođe, imali su veći sadržaj

vakcenične, arahidonske (P<0,05), oleinske, eikosanojske i ukupnih mononezasićenih masnih kiselina (P <0,01). Suprotno tome, nekastrirani nerastovi su imali najviši nivo linolenske, linolne, ukupnih polinezasićenih i n-6 masnih kiselina (P<0,05). Pol svinja nije imao uticaj na kvalitet mesa i oksidativnu stabilnost svinjskog mesa. Dodatak betaina povećao je sadržaj holesterola u kastratima u poređenju sa ostala dva pola (P<0,05). Vrednost kala ceđenja smanjena je u grupi nekastriranih nerastova (P<0,05), a oksidaciona stabilnost mišića poboljšana je u sve tri grupe (P<0,05). Tretman betainom nije uticao na profil masnih kiselina. Primećene su interakcije između pola i dodavanja betaina kod koncentracije holesterola, vrednosti kala ceđenja, oleinske, linolenske, ukupnih polinezasićenih i n-6 masnih kiselina, kao i oksidativnu stabilnost posle 30 i 120 min. inkubacije.

Ključne reči: betain, nekastrirani mužjaci, kvalitet mesa svinja, masne kiseline, oksidativni kapacitet

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THE STUDY OF THE SHARE OF TISSUES IN BOVINE CARCASS PARTS UNDER THE INFLUENCE OF THE FLAXSEED DIET

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Abstract: The aim of the experiment was to investigate the effect of adding flax seed to the cattle diet in the final stage of fattening. A total of 30 male Simmental cattle of uniform initial weight were selected for the trial, which were divided into 2 groups (KON (control) and LS (experimental)). Animals in the control group did not consume flax seed as a dietary supplement, and animals in the experimental group consumed flax seeds in the amount of 3.75% of the concentrated portion of the meal in the last 90 days of fattening, i.e 300 g per day. After slaughtering and cooling, the left carcass side was cut into basic parts according to the Regulation. The study included examination of the tissue fraction of parts of the carcass of the young, determined by dissection. The results of the study showed that the addition of flax seed in the diet had no statistically significant effect on the composition of the carcass parts of the young bulls at the end of the experiment.

Key words: flaxseed diet, young bulls, Simmental breed

Introduction

The quality of carcasses of slaughtered animals is a subject of interest, both in primary production and in the meat industry *(Petrović et al., 2016*). On the basis of the estimated value of the carcasses of slaughtered animals and their classification into classes, it is possible to make adequate financial compensation to the producers, i.e. to the animal owners and thus to stimulate them to produce the highest quality animals for slaughter. The criteria for evaluating beef carcass most often are its weight, conformation, carcass coverage by fat tissue, and muscle to fat ratio. The pre-slaughter body weight of young bulls has a significant impact on carcass yield, carcass meat yield and meat quality.

Aleksić et al. (2001) state that meat quantity and quality are phenotypic characteristics in function of the genotype and diet. *Pečiulaitienė et al. (2015)* state that with increasing body weight and age of the animal the yield and increases carcass meat yield also improve. The amount of fat tissue and its distribution plays a significant role in the value of the carcass, since too much fat can have a negative economic effect. Excess of intermuscular or subcutaneous fat tissue is removed when processing the carcass or parts of the carcass and it is an economic loss for manufacturers and processors *(Harper et al., 2001).* As a consequence, the amount of carcass fat tissue has decreased over time, with a negative effect on the physico-chemical characteristics of the fat tissue *(Allen and Foegeding, 1981*). The ratio of muscle to fat is very important for the quality of the carcass. However, since fat tissue has an effect on improving the meat's tenderness, succulence and aroma, an increase in the share of fat tissue in the carcass is preferable since it enhances the sensory quality of the meat *(Aleksić et al., 2005).*

One of the most important factors that significantly affect the quality of beef and meat is the diet (*Abrahão et al., 2005; Prado et al., 2008*). Diet for fattening beef must be balanced in terms of dry matter content, energy, protein, minerals and vitamins. Grains are a major source of energy in the final stages of nutrition, but oils and fats can also be used as alternative components *(Rotta et al., 2009).* It is very important that the meal is tasty so that animals can better consume it.

Flaxseed is present in animals' diet because of the specific and nutritional aspect of the desired fatty acid composition. Due to its high oil content, flaxseed is used in the bovine diet as a source of fat. This characteristic makes flaxseed an extremely interesting raw material for the production of functional nutrients that can increase the intake of essential fatty acids in animals, and thus change the fatty acid composition of fats and meat, and increasing the intake of essential fatty acids in humans. The positive effect of flaxseed on the fatty acid composition of the meat of animals fed flaxseed, especially on the increase in the amount of α-linolenic fatty acid (ALA) was established in the research of *Larsen et al. (2012).* The increase in the content of essential fatty acids in meat depends on the animal species and the amount of flaxseed added to the diet. However, since the fat content in bovine diet is limited to a maximum of 5% in dry matter, the maximum amount of flaxseed in bovine diet may not exceed 12 to 14% of dry matter, depending on the chemical composition of the mixture (*Byers and Schelling, 1988*). Flaxseed can also be used as an alternative source of protein in ruminant diets, but in limited quantities due to its high oil content (*Lardy and Anderson, 1999*). Flaxseed thermally treated (toasting, extruding) has a greater impact on meat yield than untreated seeds *(Maddock et al., 2004).* The study was carried out in order to determine that the use of heat treated flaxseed in the cattle diet has no detrimental effect on the carcass composition.

Materials and Methods

The study was performed on the experimental farm and in the experimental slaughterhouse of the Institute of Animal Husbandry in Zemun (Serbia). The experiment used male young bulls, domestic Simmental breed. Total of 30 animals of uniform body weight were selected for the experiment. They consumed food of the same composition until they reached the age of 390 days. The feeding of beef before the experiment was performed according to the valid recipes for fattening cattle used on the farm of the Institute for Animal Husbandry (whole maize plant silage and concentrate mixture with 12% of total proteins). The fattening of the beef was carried out in a free system. In order to fulfil the aim of the experiment it was necessary to prevent the movement of the animal in the clamp while consuming the concentrated portion of the meal, so that it can be safely claimed that each animal consumed the intended amount of concentrate. At the age of 390 days, two groups of 15 young bulls were formed: control group (KON) in which the animals did not consume thermally treated flax seeds and experimental group (LS) in which part of the concentrate was replaced by thermally treated flax seeds, so that each animal consumed 300 grams of flax seed per day.

The final weight before slaughter was about 570 kg. One day before slaughter, the bulls did not receive food but had free access to water. Slaughter and primary processing were carried out at the Experimental slaughterhouse of the Institute of Animal Husbandry. Animals were weighed immediately before slaughter and then slaughtered according to standard commercial procedures. After primary treatment, the carcasses were refrigerated at 4^0C for the next 24 hours. After chilling, the carcasses were measured and split along the vertebral column in two halves. The left side of each carcass was divided into the twelve anatomical regions: round, beefsteak, loin, shoulder, back, neck, chest/brisket, chuck, ribs, abdomen, fore shank and rear shank, using a standard technique. The carcass sides were dissected according to the Regulation ("Official Gazette of the SFRY", No. 34/74, 26/75, 13/78 - other regulations, 1/81 - other regulations and 2/85 - other regulations). The procedure for dissecting and categorizing carcass sides into parts was as follows: The leg is separated from the loin/flank by a cut between the last lumbar and the first sacral vertebra, and from the knee in the knee joint; the lumbar portion is separated from the back by a cut between the 12th and 13th ribs, and from the round by a cut between the last lumbar and the first sacral vertebra; the flank is separated from the loin by a cut that runs parallel to the spinal column so that it begins at the point which is farthest away from the tips of the transverse lumbar processes of the loin vertebra to their length; the back is separated from the chunk by a cut between the 6th and 7th ribs and from the loin by a cut between the 12th and 13th vertebrae and the 12th and 13th ribs; the ribs are separated from the back by a cut transversely to the direction of the ribs so that the top third of the

associated ribs remains on the back; the shoulder is separated from the chunk and brisket/chest by a natural muscle connection; the fore shank is separated from the shoulder at the elbow joint; the chunk is separated from the neck by a cut between the last cervical and the first thoracic vertebrae; the chest/brisket is separated from the shoulder by a transverse incision in the direction of the ribs, so that only the ends of the first six ribs remain on the brisket/chest.

The obtained data were processed by analysis of variance in one-way ANOVA program SPSS Statistics 20, and all results are displayed as the mean value ± standard deviation. The statistical significance of the difference between mean values was determined by t-test.

Results and Discussion

The share of the main carcass parts is shown in Table 1. The shares of the most valuable carcass parts (steak and round) were approximately the same between the groups and did not differ significantly between the groups ($p > 0.05$).

Table 1. Effect of flax seed supplement in beef diet on the share of main carcass parts *

(%)	KON	LS	p
Extra category carcass parts			
Beef steak	2.41 ± 0.45	2.41 ± 0.25	ns
I category carcass parts			
Round	28.05 ± 1.21	28.97 ± 0.29	ns
II category carcass parts			
Loin	4.84 ± 1.15	5.32 ± 0.61	ns
Back	5.48 ± 0.69	5.32 ± 0.33	ns
Shoulder	12.60 ± 0.73	11.63 ± 0.24	ns
III category carcass parts			
Rear shank	3.66 ± 0.52	3.91 ± 0.10	ns
Fore-shank	2.78 ± 0.26	3.16 ± 0.14	ns
Neck	10.14 ± 1.01	9.96 ± 0.57	ns
Chest/Brisket	5.18 ± 0.64	5.26 ± 0.38	ns
Chuck	11.90 ± 0.43	12.26 ± 0.59	ns
Ribs	6.75 ± 1.43	6.03 ± 0.62	ns
Flank	6.16 ± 0.77	5.70 ± 0.60	ns

*In relation to processed carcass; ns – not significant

In the KON and LS groups, the same value was found for the share of steaks. The round content ranged from 28.05% in KON to 28.97% in LS. The shares of the loin, back, shoulder and chuck did not differ significantly ($p > 0.05$) between the groups influenced by the examined factor. The share of the back ranged from 4.84% to 5.32%. A higher share of back was found in KON group. LS had a lower shoulder share, while the share of chuck was higher. *Petričević et al.*

(2015) report the following shares of main carcass parts of bovines that did not consume flaxseed in the diet: round (28.36%), shoulder (12.20%), rear shank (3.59%) and fore shank (2.73%).

The effect of feeding young bulls flaxseeds on the composition of beefsteak and rounds is shown in Table 2. The processing of the obtained data did not reveal significant differences in the composition of carcass parts of extra and I category.

Table 2. Effect of flax seed supplement in the bovine diet on the share of individual tissues in the beefsteak and round

(%)	KON	LS	p
Beefsteak (extra category parts)			
Processed beef steak	57.76 ± 6.65	51.91 ± 10.50	ns
Remaining muscle tissue	18.57 ± 5.73	28.93 ± 9.84	ns
Fat tissue	23.56 ± 5.91	19.03 ± 3.88	ns
Round (I category parts)			
Muscle tissue			
Dorsal part of the round	9.30 ± 0.34	10.39 ± 2.23	ns
Medial part of the round	20.96 ± 1.97	22.17 ± 0.96	ns
Caudal part of the round	16.32 ± 0.68	16.85 ± 0.64	ns
Cranial part of the round	11.59 ± 1.80	12.87 ± 0.58	ns
*Semitendinosus m*uscle	6.24 ± 0.36	6.76 ± 0.13	ns
Remaining muscle tissue	13.74 ± 2.86	11.14 ± 1.23	ns
Fat tissue	9.42 ± 2.19	7.36 ± 1.29	ns
Bones	12.38 ± 1.33	12.44 ± 1.04	ns

ns – not significant

The share of beefsteak muscle tissue was higher in the KON group. Similar values were found between groups for the shares of all parts of the muscular tissue of the thigh (Dorsal part of the round, medial part of the round, caudal part of the round, cranial part of the round and *Semitendinosus*). The fat content of beefsteak and round under the influence of flaxseed diet was lower compared to the KON group. These differences were not statistically significant ($p>0.05$). The share of bones of the round e were similar between the groups.

Aleksić et al. (2009) have found that at an average pre-slaughter weight of young bulls of 597 kg, the share of muscle tissue in the round was about 86% and in the carcass parts of the II category (shoulder) about 78%. *Petričević et al. (2011)* have found the share of muscle tissue in the round of 81.52%, while *Karolyi et al. (2008)* reports lower values (76.97%).

The effect of feeding flax seed on the composition of the bovine loin, back and shoulder is shown in Table 3. The share of muscle, fat and bone in the category II carcass parts did not differ significantly ($p> 0.05$) between the studied groups.

Table 3. Effect of flax seed supplement in the bovine diet on the share of individual tissues in the category II carcass parts

(%)	KON	LS	p
Loin			
Muscle tissue			
MLD	48.31 ± 3.53	47.05 ± 2.64	ns
Remaining muscle tissue	15.68 ± 4.85	18.41 ± 4.38	ns
Fat tissue	13.64 ± 3.52	12.22 ± 2.83	ns
Bones	22.26 ± 3.48	22.21 ± 0.93	ns
Back			
Muscle tissue			
MLD	37.18 ± 3.27	31.02 ± 1.07	ns
Remaining muscle tissue	26.96 ± 6.48	40.56 ± 1.85	ns
Fat tissue	10.72 ± 1.80	8.40 ± 0.16	ns
Connective tissue	0.94 ± 0.33	0.67 ± 0.03	ns
Bones	24.14 ± 3.61	19.23 ± 3.11	ns
Shoulder			
Muscle tissue	75.40 ± 3.02	76.06 ± 2.47	ns
Fat tissue	8.36 ± 2.17	6.35 ± 1.07	ns
Bones	16.20 ± 1.80	17.53 ± 1.78	ns

ns – not significant

The share of total muscle tissue of the loin (65.46%), back (71.58%) and shoulder (76.06%) were higher in the LS group, while young bulls of the KON group had higher fat content for all category II parts.

The results of *Ragni et al. (2014)* obtained by dissection of the loin show significantly lower fat content, significantly higher muscle content, and significantly lower bone content in young bulls that consumed flax seed in the diet.

The effect of the addition of flax seed in the final stage of fattening on the composition of Category III carcass parts is shown in Table 4. The addition of flax seed had no significant effect on the composition of chest/brisket, ribs, neck, chuck, flank, rear shank and fore shank.

For the LS group, higher content of muscle tissue was found in the chest/brisket (59.96%), ribs (66.95%), neck (81.98%) and chuck (70.74%). The lowest values were recorded for the neck, chuck and flank in the KON group (76.12%, 67.78%, 58.52%, respectively). The share of muscle tissue of rear shank (41.44%) and fore shank (48.93%) was higher in the KON group. A lower share of fatty tissue in the chest/brisket, ribs, neck, chuck and flank was recorded in beef of the LS group. In their research, *Karolyi et al. (2008)* report the share of chest/brisket muscle tissue of 59.99%, ribs 57.21%, neck 77.46% and flank 74.40% for young Simmental bulls over 500 kg. *Petričević et al. (2011)* state that the share of neck bones for Simmental breed bovines is 11.30%.

Table 4. Effect of flax seed supplement in the bovine diet on the share of individual tissues in the Category III carcass parts

%	KON	LS	p
Chest/Brisket			
Muscle tissue	55.03 ± 1.75	59.96 ± 4.10	ns
Fat tissue	30.42 ± 3.40	24.82 ± 1.90	ns
Bones	14.16 ± 2.32	15.11 ± 2.33	ns
Ribs			
Muscle tissue	65.34 ± 8.04	66.95 ± 2.88	ns
Fat tissue	17.65 ± 3.00	16.94 ± 1.86	ns
Bones	16.94 ± 4.83	16.10 ± 1.10	ns
Neck			
Muscle tissue	76.12 ± 1.65	81.98 ± 3.68	ns
Fat tissue	11.21 ± 0.83	9.82 ± 2.90	ns
Connective tissue	1.34 ± 0.36	1.21 ± 0.23	ns
Bones	11.13 ± 1.58	6.93 ± 0.75	ns
Chuck			
Muscle tissue	67.78 ± 3.98	70.74 ± 2.03	ns
Fat tissue	14.88 ± 3.69	13.91 ± 0.84	ns
Connective tissue	1.05 ± 0.27	1.29 ± 0.33	ns
Bones	16.24 ± 3.03	14.02 ± 1.19	ns
Flank			
Muscle tissue	58.52 ± 8.57	65.74 ± 6.49	ns
Fat tissue	39.02 ± 8.59	31.50 ± 7.31	ns
Connective tissue	1.32 ± 0.41	1.53 ± 0.89	ns
Bones	1.04 ± 0.14	1.13 ± 0.06	ns
Rear shank			
Muscle tissue	41.44 ± 1.23	40.36 ± 5.44	ns
Fat tissue	4.84 ± 0.91	8.20 ± 2.14	ns
Connective tissue	4.83 ± 0.15	4.05 ± 2.20	ns
Bones	48.85 ± 1.66	47.24 ± 2.75	ns
Fore shank			
Muscle tissue	48.93 ± 1.31	48.32 ± 5.85	ns
Fat tissue	6.46 ± 1.00	6.77 ± 1.87	ns
Connective tissue	1.92 ± 0.45	2.26 ± 0.34	ns
Bones	42.59 ± 3.01	42.58 ± 4.03	ns

ns – not significant

Table 5 shows the effect of the consumption of flax seed in the bovine diet on the composition of the categories II and III carcass parts.

Table 5. Effect of flax seed supplement in the bovine diet on the composition of the carcass parts

%	KON	LS	p
Category II carcass parts			
Muscle tissue	70.38 ± 3.23	72.21 ± 2.64	ns
Fat tissue	10.43 ± 2.73	8.24 ± 1.26	ns
Connective tissue	0.21 ± 0.08	0.21 ± 0.08	ns
Bones	18.92 ± 2.04	19.27 ± 1.68	ns
Category III carcass parts			
Muscle tissue	62.37 ± 2.87	66.77 ± 2.93	ns
Fat tissue	19.30 ± 4.82	15.81 ± 1.95	ns
Connective tissue	1.00 ± 0.15	1.29 ± 0.04	ns
Bones	17.32 ± 2.12	16.07 ± 1.06	ns

ns – not significant

The LS group had higher shares of muscle tissue in the carcass parts of both categories. The differences found in the carcass parts for both categories did not differ significantly between the groups. A higher share of muscle tissue and a lower share of fat tissue in category II and III carcass parts of animals in the LS group, caused the ratio of muscle to fat tissue to be higher compared to the KON group. *Karolyi et al. (2008)* report the share of connective tissue in category II and III carcass parts of 4.76% and 6.55%, respectively, for bovines weighing over 500 kg of the Simmental breed, while the share of muscle and fat tissue, bone and connective tissue in the carcass of 70.45%, 7.46%, 16.33% and 5.76%, respectively.

Conclusion

The addition of flaxseed to the diet during the final stage of fattening had no statistically significant ($p>0.05$) effect on the share of carcass parts. The results of the study confirm that the use of thermally treated flax seed in the bovine diet does not have a negative effect on the carcass composition as determined by dissection of carcass parts.

Ispitivanje udela tkiva delova trupa junadi pod uticajem ishrane sa semenom lana

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Rezime

Ogled je postavljen sa ciljem da se ispita efekat dodavanja semena lana, u ishranu junadi, u završnoj fazi tova. Za ogled je odabrano 30 muških junadi simentalske rase ujednačenih početnih telesnih masa, koja su podeljena u 2 grupe (KON (kontrolna) i LS (ogledna)). Kontrolna grupa junadi nije konzumirala seme lana kao dodatak ishrani. Junad ogledne grupe su konzumirala seme lana u količini od 3,75% koncentrovanog dela obroka u poslednjih 90 dana tova, tj. 300 g dnevno. Nakon klanja i hlađenja leva polutka je rasecana u osnovne delove prema Pravilniku. Istraživanje je obuhvatilo ispitivanje udela tkiva delova trupa junadi, koje je utvrđeno disekcijom. Rezultati istraživanja su pokazali da dodatak semena lana u ishrani nije imao statistički značajan uticaj na sastav delova trupa junadi na kraju ogleda.

Ključne reči: ishrana lanom, junad, Simentalska rasa

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FATTY AND AMINO ACID PROFILE OF MEALWORM LARVAE (*TENEBRIO MOLITOR* L.)

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Original scientific paper

Abstract: The yellow mealworm (*Tenebrio molitor* L., *Coleoptera*: *Tenebrionidae*) is an edible insect, distributed worldwide and a convenient candidate for industrial-scale production. Mealworms could be commercially used for the substitution of conventional protein sources. In our previous study, it was found that *T. molitor* larvae predominantly contained crude protein (55.83%) and crude fat (25.19%), as well as low content of nitrogen-free extract (based on dry weight). Mealworm specimens were maintained in an incubator under controlled conditions in plastic containers. Insects were sieved and put into the container with boiling water and cooked for 180 seconds. Moisture content was determined as weight loss after drying of larvae. Amino acids were determined on an Agilent Technologies 1260 series HPLC system. Fatty acid composition was determined on a Thermo Scientific TRACE 1300 gas chromatograph equipped with a flame ionization detector using TR-FAME column. The results showed that the content of unsaturated fatty acid is very high, i.e. oleic acid (C18:1) formed the major lipid component in 40.83%, which was followed by linoleic acid (C18:2, omega-6 fatty acid) with 29.80% and linolenic acid (C18:3) with 1.08%. The essential amino acids are highly represented in the samples (in % dry matter). This primarily refers to isoleucine (4.12), tyrosine (3.86), phenylalanine (3.06), leucine (2.96), lysine (2.67) and methionine (1.76). The differences in essential fatty and amino acid content between our results and discussed literature data, could be the consequence of different substrates used for rearing of insects. After everything stated above, the biological value of *T. molitor* larvae proves that it could be suitable as animal feed.

Key words: edible insects, *Tenebrionidae*, chemical composition

Introduction

The world population is anticipated to reach 9 billion by 2050 and the challenge to feed the increasing population is huge, given the limited agricultural resources of land and water in the climate-change era (*FAO, 2009*). Thus, safeguarding sustainability has become the most pertinent challenge of today. In this context, insects could be viewed as an alternative source of animal feed and human food in terms of micro-livestock. Edible insects have played an important role in the long history of human nutrition in Africa, Asia and Latin America *(Aletor, 1995)*. More than 1000 species of insects that are edible in a certain stage of their life cycle have been reported worldwide as traditional foods by humans and has been an important part of the nutritional intake and economic value of many societies *(Illgner and Nel, 2000)*. Not every edible insect species contains every nutrient. There is scope for selective breeding and possibilities exist to fortify species with nutrient components they are lacking or to prepare a mixture of different insects as an ideal nutritional source used in insect flours or as food supplements. Inclusion of insects in the animal meat product industry should ease the excessive pressure on conventional livestock production and in turn on the environment *(Gnosh et al., 2017)*.

The yellow mealworm (*Tenebrio molitor* L., *Coleoptera*: *Tenebrionidae*) is an edible insect, distributed worldwide and a convenient candidate for industrial-scale production (*Van Huis, 2013; EFSA, 2015)*. Mealworm larvae are commercially used as animal feed and in some countries, such as Asia and Africa, for human nutrition because of their high fat, protein and mineral content *(Finke, 2002; Rumpold and Schluter, 2013).* Even though *T. molitor* are insects that infest stored products, many people do not consider them as pests, but culture their larvae in large quantities for sale as food for insectivorous animals (such as birds and aquarium fishes) raised in captivity *(Ng et al., 2001). T. molitor* are the most widely reared insects as human food in Europe *(Caparros Megido et al., 2014). Ravzanaadii et al. (2012)* suggested that mealworms could be commercially used for the substitution of conventional protein sources (as they contain approximately 46% proteins based on dry weight). The authors also indicated that these insects contain approximately 33% lipids (dry weight basis). According to the results of *Siemianowska et al. (2013)* the fresh mealworm larvae contained more total protein, total fat and ash in comparison to traditional meats i.e. chicken, pork, beef, fish and eggs. In our previous study, it was found that *T. molitor* larvae predominantly contained crude protein (55.83%) and crude fat (25.19%), as well as low content of nitrogen-free extract in dry matter *(Jajić et al., 2019).*

High content of fatty acids in diets affects their antioxidant activity, which is highly desirable in human diets *(Wojciak and Dolatowski, 2012). Yang et al. (2006)* found that edible insects contained good quality fatty acid especially long

chain omega-3 fatty acids such as alpha-linolenic acid, eicosapentaenoic acid and that different kinds of insects had different fatty acid profiles. Mealworms contain high amounts of unsaturated fatty acids, mainly linoleic acid and oleic acid, and palmitic acid as saturated fatty acid *(Lenaerts et al., 2018). Paul et al. (2017)* concluded that oleic and linoleic acids formed the major fatty component of *T. molitor* larvae and *Acheta domesticus* lipids, respectively. These results were in good agreement with the findings of *Ravzanaadii et al. (2012) and Tzompa-Sosa et al. (2014)*. Even though both these insect species were fed with a similar diet (containing wheat flour, wheat bran and brewer's yeast), they exhibited a different fatty acid composition. This indicates that fatty acid profile also varies with individual species. Almost all insects are able to biosynthesize palmitic, stearic and oleic acids *(Stanley-Samuelson et al., 1988; Grapes et al., 1989)*. *T. molitor* larvae lipids exhibit a very high n-6/n-3 ratio *(Bendová et al., 1991).* In the study of *Nielsen (2016),* it was indicated that omega-3 fatty acids in *T. molitor* larvae lipids could be enhanced by omega-3 fatty acid supplementation in the diet. In the study of *Janssen et. al. (2017)*, the fatty acid analysis showed an extraordinary composition of long chain fatty acids (C18-C24), oleic acid (C18:1) being the main component followed by linolenic acid (C18:2) and palmitic acid (C16). Significant amounts of linolenic (C18:3) were also found. *Nielsen (2016)* indicated that omega-3 fatty acids in *T. molitor* larvae lipids could be enhanced by omega-3 fatty acid supplementation in the diet. *Ravzanaadii et al. (2012*) found remarkable content of long chain fatty acids (C18-C22) with oleic acid (C18:1), linoleic acid (C18:2) and palmitic acid (C16) being the highest components. In addition, comparatively high amounts of omega-3 fatty acids were found in larvae. These essential fatty acids are mostly available in sea species, where mealworms are demonstrating that it can be used for many other purposes such as feeding of domestic animals, food supplement and recycling supplement, etc. (*Nettleton, 1995; Ravzanaadii et al., 2012). Ravzanaadii et al. (2012)* found considerable amounts of unsaturated fatty acids (77.74%). In terms of degree of unsaturation of fatty acids, insects have composition similar to poultry and fish. In some groups, such as essential fatty acids, linolenic acids and linoleic were even higher than fish and poultry *(Defoliart, 1991). T. molitor* larvae were characterized by favorable proportion of n-6/n-3 fatty acids in comparison to pork meat. A very good n-6/n-3 acids ratio (6.76) in mealworm larvae may be taken as another determinant of their high quality and nutritive value.

All insect species considered as animal feed have high levels of protein with amino acid profiles suitable to be used as feedstuffs *(Makkar et al., 2014; Henry et al., 2015; Veldkamp and Bosch, 2015; Van Huis and Tomberlin, 2017)*. Furthermore, insects can be used as a natural nutrient source for poultry *(Jozefiak et al., 2016)*. The amino acid composition of proteins determines their quality as animal feed. The optimal amino acid composition may vary among different species due to different feed requirements and some amino acids may need to be

supplemented *(Müller et al., 2017)*. It has already been proven that edible insects may provide sizable amounts of protein, including all indispensable amino acids *(Ramos-Elorduy et al., 1997; Verkerk et al., 2007; Chen et al., 2008)*. Overall, insects contain higher amounts of lysine and threonine which are deficient in most commonly used wheat, rice, cassava and maize, but lower amounts of methionine and cysteine *(Defoliart, 1992)*. *Finke (2004)* stated that adult specimens of *T. molitor* contain 653 g protein per kg dry matter (DM), while larvae of the same species contain 49.1 g protein per kg DM. In addition, the digestibility of insect protein is comparable to that of meat protein *(Verkerk et al., 2007; Longvah et al., 2011)*. Insects are therefore presented by the Food and Agriculture Organization of the United Nations (FAO) as a valuable alternative to meat *(FAO, 2013)*. Conventional meat contains high levels of purines *(Choi, 2010)*, which are very important to the human body, as two of the nucleic acids, adenine and guanine, are purines *(Bednarova et al., 2013)*. In the research of *Aguilar-Miranda (2002)* the amino acid content of *Tenebrio* larvae powder showed the requirements of essential amino acids as reported by *FAO/WHO/UNU (1986)*. Larval powder had high phenylalanine + tyrosine (7.7 g/100 g of protein) and tryptophan contents (1.8 g/100 g of protein).

The aim of the present study was to determine the fatty and amino acid composition of the powdered *T. molitor* larvae.

Material and Methods

Rearing of insects

The insects were obtained from the Department of Plant and Environmental Protection, Faculty of Agriculture, University of Novi Sad, Serbia. Mealworm specimens were maintained in an incubator under controlled conditions (temperature: 27±1 °C, photoperiod: 0 h light - 24 h dark, relative humidity: 55%) in 12 L plastic containers (20 cm x 40 cm x 15 cm). The insects were grown on a food mixture which contained 400 g of wheat bran, 250 g of dried barley germs, 200 g of dried oat germs, 50 g of barley flakes, 50 g oat flakes and 50 g of powdered beer yeast. Pieces of apple were spread over the food mixture to provide additional moisture to the insects.

Preparing insects for drying and cooking

Insects were sieved (2.5 mm pore diameter) and the remaining insect parts were removed with weak wind flow produced by hair dryer. Sieved larvae were moved to the sieve with smaller holes and with weaker windflaw remains of insect bodies were removed. Afterwards cleaned larvae were transferred into the 2 L

plastic container, and gently washed under the water jet. After that insects were put into the container with boiling water and cooked for 180 seconds. Thereafter, the entire content of the cooking pot was filtered through sieve in order to remove water, and then, the larvae were spread onto the filter paper in a thin layer in order to evaporate excessive water during 24 h. Dried insects were collected and put on a new filter paper and left to dry for another 24 h.

Chemical analysis

Dry matter content (DM) was determined after drying (AOAC Official Method 934.01). Crude protein (CP) was analyzed according to standard Kjeldahl method (AOAC Official Method 2001.11), while crude fat content (EE) was determined as petroleum ether extract (AOAC Official Method 991.36).

Amino acids were determined on an Agilent Technologies 1260 series HPLC system (Agilent Technologies, USA) by applying previously established analytical conditions *(Jajić et al., 2013)*. Fatty acid composition was determined on a Thermo Scientific TRACE 1300 gas chromatograph equipped with a flame ionization detector (Thermo Scientific, USA) using TR-FAME (length 30 m, inner diameter 0.32 mm, film thickness 0.25 μm) column (Thermo Scientific, USA). The injector and detector temperatures were 200 °C. Helium was used as carrier gas with a flow rate of 1.3 mL/min. Sample and standard were diluted in n-heptane (analytical purity). 1 μl of sample was injected into the injector. The fatty acid composition was calculated based on the area of the peaks. Prior to GC analysis, fat was extracted from samples using Soxhlet extractor. About 20 mg of fat was weighted in 5 cm^3 v-vial (Sigma-Aldrich, Switzerland) and 0.5 ml of 0.5 M NaOH was added. Vial was then heated to 70 °C for 10 minutes and cooled to room temperature. Then, 0.5 ml of boron trifluoride (Sigma-Aldrich, Switzerland) was added and again heated to 70 °C for 10 minutes and cooled to room temperature. Finally, 1 ml of saturated NaCl solution and 1 ml n-heptane was added and gently mixed. Upper (heptane) layer was transferred into 1 ml tube containing anhydrous sodium-sulfate. After holding for 30 minutes, heptane layer was transferred into GC vial and then analyzed.

Results and Discussion

We were able to identify nine fatty acids present in different proportions. The results in Table 1 were presented in different units for easier comparison with results from previous research.

As can be seen, the content of unsaturated fatty acid is very high, i.e. oleic acid (C18:1) formed the major lipid component in 40.83%, which was followed by linoleic acid (C18:2, omega-6 fatty acid) with 29.8% and linolenic acid (C18:3)

with 1.08%. On the contrary, the amount of saturated fatty acids is generally lower than above mentioned and belonged predominantly to palmitic acid (C16:0) at 16.2% and stearic acid (C18:0) with 2.21%. Other fatty acids from both groups were in individual quantities of less than 1%.

Table 1. Fatty acid profile

Fatty acids	**g/100 g fat or % fat**	**% in sample**	**% DM or g/100 g DM**	**g/100 g protein**	**mg/g protein**
Palmitic acid C16:0	16.20±0.47	4.08±0.12	4.16±0.12	7.44±0.22	74.45±2.16
Stearic acid C18:0	2.21±0.03	0.56±0.01	0.57±0.01	1.02±0.01	10.16±0.14
Oleic acid C18:1	40.83±0.57	10.29±0.14	10.48±0.15	18.76±0.26	187.64±2.60
Linoleic acid C18:2 (omega-6)	29.80±0.46	7.51±0.11	7.65±0.12	13.69±0.21	136.95±2.09
Linolenic acid C18:3	1.08±0.02	0.27±0.01	0.28±0.01	0.50±0.01	4.96±0.11
Eicosanoic acid C20:0	0.09±0.01	0.02±0.00	0.02±0.00	0.04±0.00	0.41±0.03
Docosanoic acid C22:0	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.09±0.00
Eicosapentaenoic acid (EPA); C20:5	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.09±0.00
Docosahexaenoic acid (DHA), C22:6	0.07±0.01	0.02±0.00	0.02±0.00	0.03±0.00	0.32±0.04
Other	9.68±0.30	2.44±0.08	2.48±0.08	4.45±0.14	44.48±1.38

Our results of fatty acid profile are in accordance with the findings of *Zielinska et al. (2015)*, who compiled the nutritional composition of mealworm larvae and found content of oleic acid (40.86%) and linoleic acid (29.68%) as well as palmitic acid (18.0%). Similar results have also been found by *Mlcek et al. (2019)* for palmitic (18.6%) and oleic acid (36.9%), while the content of linoleic acid was much higher (30.9%). The contents of palmitic, linoleic and eicosanoic acid were in close agreement with previous report of *Gnosh et al. (2017)* that amounted to 4.71; 7.57 and 0.2 g/100 g DM, respectively.

In investigation of *Paul et al. (2017)*, the contents of oleic (35.83%), linoleic (22.83%) and γ-linolenic acid (0.11%) were considerably lower when compared to our results. Furthermore, the authors didn't find the presence of eicosanoic, docosanoic, eicosapentaenoic and docosahexaenoic acid. Their results were similar to research of *Ravzanaadii et al. (2012)* who reported of 0.05 g/100 g protein of γ-linoleic acid and found no Eicosapentaenoic and docosahexaenoic acid. This applies also for investigation of *Gnosh et al. (2017*) who reported only about 0.08 g/100 g DM of stearic acid. *Aguilar-Miranda et al. (2002)* also studied the fatty acid content, and they reported following values of fatty acid content (mg/100 mg of sample): palmitic (6.76); stearic (1.46), oleic (19.77) and eicosanoic acid (0.08). This and fatty acid content in the research from *Ravzanaadii et al. (2012)* were considerably higher when compared with our results (in g/100 g of protein) for palmitic acid (16.72), stearic acid (2.49), oleic acid (43.17), linoleic acid (30.23) and eicosanoic acid (0.24). *Paul et al. (2017)* also found higher values of palmitic acid (21.33) and stearic acid (7.92) in comparison with our results. The comparison between our results and other authors showed that considerable differences in the levels of fatty as well as amino acids in chemical composition of *T. molitor* larvae still existed. According to the earlier reports *(Ramos-Elorduy et al., 2002; Wyss, 2012; Finke and Oonincx, 2013; Broekhoven et al., 2015)* the most important reason for this could be feeding insects with different substrates. In this context, there are differences in the feeding regime of the above-mentioned authors. *Aguilar-Miranda et al. (2002)* reported that the larvae were grown on a sawdust bed, containing oat and corn flakes, dry bread, and pieces of vegetables as water supplement. In the research of *Bednarova et al. (2013*) all larvae and nymphs were fed a combination of wheat bran and carrots in the ratio 3:1 (w/w) for a 14-day period. *Gnosh et al. (2017)* raised mealworms on wheat bran with few leaves of Chinese cabbage as a source for water uptake and being nocturnal were kept in a dark environment. *Ravzanaadii et al. (2012)* stated that wheat bran was the main food for mealworm and vegetables such as cabbage, reddish and carrots were added as water source twice a week. In the investigation of *Siemianowska et al. (2013)* insects were fed oat flakes with addition of vegetables as a source of water. In the experiment of *Paul et al. (2017) T. molitor* larvae were reared on a diet containing wheat flour, wheat bran and brewer's yeast.

It can be seen from Table 2 that essential amino acids are highly represented in our samples. This primarily refers to isoleucine, tyrosine, phenylalanine, leucine, lysine and methionine.

Our results are similar to the amino acid profile published earlier by *Bednarova et al. (2013).* They reported that protein contain the following amounts of amino acids on dry weight: glutamate (6.51%), alanine (3.04%) and lysine (3.04%). *Zielinska et al. (2015)* observed a similar trend in the amino acid composition during their investigations on *T. molitor*. They reported that protein

contained (mg/g of protein) 26.1, 45.8 and 43.4 of threonine, leucine, and proline, respectively.

Table 2. The amino acids composition in mealworm

Amino acids	% DM or g/100 g DM	g/100 g protein	mg/g protein
ASP	4.30	7.71	77.13
GLU	6.44	11.54	115.44
SER	2.38	4.28	42.79
GLY	3.67	6.58	65.87
THR	1.47	2.63	26.36
ARG	3.60	6.45	64.54
ALA	4.53	8.11	81.14
TYR	3.86	6.92	69.28
VAL	0.65	1.17	11.76
MET	1.76	3.16	31.61
PHE	3.06	5.48	54.87
ILE	4.12	7.38	73.88
LEU	2.96	5.31	53.12
LYS	2.67	4.79	47.94
PRO	2.67	4.79	47.94

Abbreviations: Asp = Aspartate; Glu = Glutamate; Ser = Serine; Gly = Glycine; Thr = Threonine; Arg = Arginine; Ala = Alanine; Tyr = Tyrosine; Val = Valine; Met = Methionine; Phe = Phenylalanine; Ile = Isoleucine; Leu = Leucine; Lys = Lysine; Pro = Proline

Furthermore, we found it important to compare our results with literature data that showed dissimilar findings when compared to ours. For this reason, we needed to recalculate our results into different units found in literature (Table 2). A good example for this is the publication of *Ravzanaadi et al. (2012)* with results which almost all (except valine) were considerably less than our findings (in g/100

g of protein): aspartate (3.59), glutamate (5.67), serine (2.09), glycine (2.41), threonine (1.80), arginine (2.43), alanine (3.68), tyrosine (3.46), methionine (0.67), phenylalanine (1.75), isoleucine (3.55), leucine (3.40), lysine (2.90) and proline (3.01). In the research of *Bednarova et al. (2013)*, they obtained the results of following content of amino acids (dry matter basis) arginine (3.03%), methionine (0.99%), phenylalanine (0.89%) and isoleucine (3.03%). The amino acid content was less when compared with our results. It is evident from table 2 that protein from the *T. molitor* larvae contained much higher levels of amino acids when compared with results in investigation of *Zielinska et al. (2015)*. They reported the following amounts (mg/g of protein) glutamate (79.7), glycine (31.8), arginine (25.6), alanine (44.3), tyrosine (28.8), methionine (9.6), phenylalanine (16.1), isoleucine (21.4) and lysine (26.7). *Gnosh et al. (2017)* studied the amino acid profile of *T. molitor* and reported that protein contained 2.76% aspartate, 5.78% glutamate, 2.20% serine, 2.61% glycine, 2.23% arginine, 3.96% alanine, 1.76% phenylalanine, 1.98% isoleucine, 2.01% lysine and 1.66% proline (dry matter basis). Their results also showed considerably lower amino acid content when compared to our study.

In contrast to previous comparisons, some of the above-mentioned authors obtained the results of amino acid content which were considerably higher than our results. *Bednarova et al. (2013)* published the following results (% DM): aspartate (4.66), serine (2.83), glycine (5.07), threonine (2.30), alanine (5.01), valine (3.42), leucine (5.47) and proline (3.92). Similar results were also reported from *Gnosh et al. (2017)*, threonine (1.83), tyrosine (3.45), valine (2.94) and leucine (3.37) in g/100 g DM. Furthermore, the report of *Janssen et al. (2017)* who found the following results (in g/100g of protein): aspartate (9.21), serine (5.03), threonine (4.52), valine (6.42), leucine (8.33), lysine (6.14) and proline (7.96).

Conclusion

In addition to the fact that *T. molitor* is a very rich source of protein and fat, our results showed a very good fatty acid and amino acid profile. Furthermore, high levels of essential fatty acids (linoleic), as well as essential amino acids (lysine, methionine, and threonine) make this a nutrient of high biological value. The differences in essential fatty and amino acid content between our results and discussed literature data, could be the consequence of different substrates used for rearing of insects. By substrate change, protein, and fat content, as well as amino and fatty acid profile could be modified, which we proved in our unpublished research. After everything stated above, the biological value of *T. molitor* larvae proves that it could be suitable as animal feed.

Masnokiselinski i aminokiselinski profil larvi crva brašnara (*Tenebrio molitor* L.)

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Rezime

Crv brašnar (*Tenebrio molitor* L., *Coleoptera*: *Tenebrionidae*) je jestivi insekt, rasprostranjen po celom svetu i pogodan za proizvodnju na industrijskom nivou. Brašnar se može koristiti i u komercijalne svrhe kao zamena za konvencionalne izvore proteina. U našem prethodnom istraživanju utvrđeno je da se suva materija larvi *T. molitor* pretežno sastoji od sirovih proteina (55,83%) i sirovih masti (25,19%), kao i malog sadržaja bezazotnih ekstraktivnih materija. Crvi su gajeni u inkubatoru pod kontrolisanim uslovima u plastičnim kutijama. Insekti su prosejani, a zatim stavljeni u posudu sa ključalom vodom i kuvani 180 sekundi. Sadržaj vlage je određen kao gubitak težine nakon sušenja larvi. Amino kiseline su analizirane na HPLC sistemu "Agilent Technologies 1260 series". Sadržaj masnih kiselina je utvrđen pomoću gasnog hromatografa "Thermo Scientific TRACE 1300" opremljenog sa plamenim jonizujućim detektorom korišćenjem "TR-FAME" kolona. Rezultati su pokazali visoki sadržaj nezasićenih masnih kiselina npr. oleinske kiseline (C18:1) koja čini većinsku lipidnu komponentu od 40,83%, za kojom sledi linolna kiselina (C18:2, omega-6 masna kiselina) sa 29,80% i linoleinska kiselina (C18:3) sa 1,08%. Esencijalne aminokiseline su visoko zastupljene u uzorcima (u % suve materije). To se pre svega odnosi na izoleucin (4,12), tirozin (3,86), fenilalanin (3,06), leucin (2,96), lizin (2,67) i metionin (1,76). Razlike koje su konstatovane u sadržaju esencijalnih masnih i amino kiselina u našim rezultatima u odnosu na analizirane literaturne podatke mogle bi biti posledica upotrebe različitih hraniva za gajenje insekata. Na osnovu svega navedenog, pokazalo se da bi larve *T. molitor* mogle biti pogodne kao hrana za životinje zbog svoje visoke biološke vrednosti.

Ključne reči: jestivi insekti, *Tenebridae*, hemijski sastav

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QUALITY OF EGGS FROM PASTURE REARING LAYERS OF DIFFERENT GENOTYPES

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Abstract: The comparison of physical quality properties of table eggs of commercial hybrid Tetra SL and two native indigenous breeds of hens, Banat Naked Neck and Svrljig hen, was performed in conditions of alternative production in the pasture system in portable cages without the floor. The experiment was conducted during the vegetation period, at the age of hens of 53-57 weeks. In order to produce good quality eggs, the diet was designed with a complete mixture based on maize and soybean. In addition, constant availability of pastures was ensured. Egg sampling was performed in the morning and the initial egg quality was examined based on egg weight, shape index, egg white, egg yolk and shell quality parameters. Layer hens of the commercial hybrid Tetra SL had significantly higher egg weight and better internal egg quality ($p<0.01$) compared to hens of Banat Naked Neck and Svrljig hens, except for the colour of the yolk, which was more intense in the same feeding and breeding conditions and the age of laying hens, in eggs of native breeds ($p<0.01$). Native breeds in relation to the commercial hybrid had lighter shell colour ($p<0.01$). Deformation and egg shell thickness differed ($p<0.01$) between all three genotypes. The determined rank of layer hen genotypes for shell thickness was: Tetra SL, Banat Naked Neck, Svrljig hen. However, the breaking force was without statistically confirmed difference between hen genotypes.

Key words: egg quality, genotype, native breeds, alternative production

Introduction

The acceptability of table eggs on the market is increasingly influenced by the level of welfare of laying hens, as well as the quality and safety of the product itself for consumers. Related to this is the consumer interest in poultry farming systems (*Rodić et al., 2010; Tolimir et al., 2019*). Free range, according to the results of survey research of consumer attitudes, is a more acceptable breeding system compared to commercial cage and other systems because it provides more natural conditions for egg production that are more desirable (*Pavlovski et al., 2011*). Numerous studies of the effect of breeding systems on egg quality have different, often inconsistent results (*Sekeroglu et al., 2010; Englmaierova et al., 2014; Yenice et al., 2016; Kucukkoyuncu et al., 2017; Škrbić et al., 2019*). Accordingly, *Holt et al. (2011)* find that a number of factors, including breed or genotype, should be considered in terms of understanding the effects on egg quality and safety before switching to any of the alternative farming systems.

Genetic conditionality of egg quality traits is known. Hybrid layer hens in intensive and controlled growing conditions show a high level of genetic potential for egg weight, egg shell and egg white quality. However, certain traits of egg quality, in addition to the genetic basis, are under the primary influence of diet and breeding conditions (*Rakonjac et al., 2014*). Native, unselected hens are characterized by higher adaptive abilities necessary for free range production and thus lower requirements in terms of breeding conditions. Their genetic potential for production is significantly lower compared to hybrid layers (*Škrbić et al., 2011*).

Alternative production systems have long been in the focus of researchers, as well as finding opportunities for the self-sustainability of native, indigenous breeds through the production of biologically valuable food. In this regard, research on local native breeds of hens is increasingly present, both in terms of morphological and production characterization *(Milošević et al., 2013; Teneva et al., 2015)*, and in terms of various aspects of product quality *(Pavlovski et al., 2011; 2013; Mitrović et al., 2011; Stanišić et al., 2015)*, while few studies (*Pavlovski et al., 2012; Sokolowicz et al., 2018)* investigate the physical characteristics of egg quality of native hen breeds.

Starting from the known effects of layer genotype and breeding system on individual egg quality traits, the aim of the study was to compare the physical quality traits of eggs from hybrid layers and two native, indigenous hen breeds and determine the level of these differences in one of the forms of alternative production, i.e. pasture breeding system in portable cages.

Material and Methods

The hens of the Tetra SL hybrid and two native, autochthonous breeds: the Banat Naked Neck and Svrljig hens, were reared in portable cages, without a floor, on natural pasture. The experiment was conducted during the vegetation period, May-June, at the Institute of Animal Husbandry in Belgrade, at the age of hens of 53-57 weeks. The area of the cage was 4.5 m^2 and the stocking density was 3 layers/ m^2. There were a total of 6 cages, or 30 layer hens per each genotype. The cages were equipped with a feeder, drinker, nests and perches. The birds in the cages were protected from adverse weather conditions. The layers were exposed to natural light for the duration of 15 hours. Given the nutrient requirements of layer hens, necessary for the production of good quality eggs, and limiting pasture area, the diet was adapted to the most demanding, hybrid hen and was made with a complete mixture based on maize and soybean, with the metabolic energy content of 11.5MJ/kg, 16,4% crude protein content, 0.8% lysine, 0.4% methionine, 3.9% calcium and 0.38% digestible phosphorus. In addition, in order to ensure constant availability of pastures, the cages were moved over the pasture surface. Food and water were available to the layers ad libitum. Egg sampling was performed in the morning and the initial egg quality was examined based on the properties of external and internal egg quality *(Pavlovski et al., 1997)* and egg shell quality *(Pavlovski and Vitorović, 1996)* in 3 replications with 30 eggs per treatment.

Statistical data processing was performed by variance analysis One-way ANOVA using the statistical software package STATISTICA, version 8, StatSoft, Inc. (www.statsoft.com).

Results and Discussion

The effect of genotype on egg weight, shape index and internal egg quality parameters in the three age periods of layer hens, as well as the average for the examined period, is shown in Table 1. The presented egg quality parameters indicate significant difference between hybrid Tetra SL and native hen breeds.

Differences in quality traits that are under the dominant influence of genotype, i.e. selection progress of hybrid layers, are more pronounced in relation to native, unselected hens. The egg weight, on average for the test period, was 64.62 g in Tetra SL laying hens, and in native hens 55.1 and 55.6 g, respectively. The shape index indicates a more rounded shape of the eggs from hybrid layers compared to the native breeds, which is the result of genetic improvement and aims to reduce egg breakage. Haugh Units, according to egg white height, were with insignificant differences between Svrljig hen and Banat Naked Neck (68.48 vs. 67.82) and significantly ($p < 0.01$) higher in Tetra SL eggs. Eggs of the examined

native breeds of hens had significantly ($p<0.01$) more intense coloration of the yolk compared to Tetra SL laying hens in pasture feeding conditions.

Tabela 1. Egg quality parameters (mean ± SD)

Genotype/ Age/ Parameter	Tetra SL				Banat Naked Neck				Svrljig hen			
	53	55	57	**53-57**	53	55	57	**53-57**	53	55	57	**53-57**
Egg weight, g	66.97 5.31	66.53 5.89	60.35 4.41	64.62^{A} 5.98	55.38 2.72	55.02 3.56	54.96 2.57	55.10^{B} 2.96	55.75 5.27	55.53 6.72	54.81 5.32	55.60^{B} 5.72
Shape index	77.35 2.58	76.75 2.45	77.40 2.95	77.17^{A} 2.64	73.50 2.09	74.55 3.03	74.74 1.91	74.31^{B} 2.44	74.50 2.46	73.25 2.02	74.05 2.87	73.93^{B} 2.49
Albumen height 0.1mm	82.05 15.04	79.00 10.02	74.25 17.95	78.43^{A} 14.81	52.50 19.17	53.50 17.48	47.47 8.55	51.13^{B} 15.53	52.45 10.39	48.20 12.11	47.45 11.61	49.37^{B} 11.42
Yolk colour (Roche)	13.25 0.64	12.85 0.59	11.15 0.49	12.42^{B} 1.08	13.50 0.73	13.25 2.07	13.58 0.69	13.44^{A} 1.36	13.30 0.66	13.45 0.51	13.25 0.64	13.33^{A} 0.60
Egg white/yolk ratio	2.02 0.22	2.11 0.21	1.99 0.19	2.04^{A} 0.21	1.56 0.17	1.51 0.19	1.56 0.16	1.54^{B} 0.17	1.44 0.28	1.50 0.24	1.42 0.25	1.45^{B} 0.25
HU	87.20 9.66	86.20 5.80	83.85 11.55	85.75^{A} 9.27	69.44 15.75	66.95 17.01	67.37 7.75	67.82^{B} 13.85	71.10 8.94	67.50 9.06	66.85 9.59	68.48^{B} 9.24

A-B - average values of parameters for examined period in each row without a common designation are significantly different at the level of 1%

These results show consistency with the research of *Svobodova et al. (2014)* who examined the quality of Czech hen and Lohmann White eggs, in a cage and floor rearing system, found a significant genotype effect and significantly higher values of egg weight, egg shell weight and better egg white quality of eggs from Lohmann White hens. Contrary to our results, the Czech hen shape index is higher compared to Lohmann White. According to *Sokolowicz et al. (2018),* the internal quality of eggs of hybrid layer hens in the free range system shows a significantly higher value compared to the eggs of the Polish native breed of hens (Greenleg Partridge hens) in the same breeding system. *Krawczyk (2009)* states that in unselected native hens, the quality of eggs changes in relation to the level of production and age of the layer hens and that there is a difference in relation to the patterns present in commercial hybrid layer hens. Accordingly, the research of *Škrbić et al. (2011)* shows that, despite the generally poorer quality of eggs of Banat Naked Neck hen, the internal quality of eggs is more stable in relation to the age of the laying hens and that the differences in the level of correlation of certain egg quality traits and laying age conditions favour theirprolonged exploitation in conditions of alternative production compared to hybrid layers.

Significantly higher ($p<0.01$) egg white to yolk ratio of Tetra SL hens (2.04) compared to hens of Banat Naked neck (1.54) and Svrljig hens (1.45) indicates a significantly higher share of yolks in eggs of native breeds of hens. It is known that native breeds of hens accumulate higher fat deposits compared to hybrid layers *(Stanišić et al., 2015),* which is associated with differences in productivity of these layers (*Rizzi and Chiericato, 2010).* The more intense coloration of egg yolks of Polish native hens *(Sokolowicz et al., 2018)* in the free range system compared to hybrid layers from the same systems, as well as Czech hen *(Svobodova et al., 2014)* in the cage and floor breeding system, confirm the effect of genotype, in accordance with our results, and indicates that this trait of yolk quality is not necessarily related to the availability of pastures, i.e. nutrition.

The effect of genotype on egg shell quality parameters in three age periods of laying hens, as well as the average for the examined period, are shown in Table 2. Based on the presented results, it can be concluded that, in addition to differences between Tetra SL and native hens, significant differences in egg shell quality were also found between the autochthonous breeds of Banat Naked neck and Svrljig hens.

The egg shell weight was significantly higher ($p<0.01$) in Tetra SL eggs compared to eggs of native hens, in line with the egg weight. Genetically determined, the colour of the egg shell of native hens was significantly lighter compared to hybrid layer hens of brown eggs (1.64 and 1.80 to 3.57). The cleanliness of the shell in all three hen genotypes was at a satisfactory level with established differences between Tetra SL laying hens and Svrljig hens at the level of $p<0.05$, i.e. Tetra SL and Banat Naked Neck, at the level of $p<0.01$. According to the legal provisions *(Rulebook on the quality of eggs, 2019),* eggs for consumption placed on the market must not be washed or cleaned in any other way, which emphasizes the importance of achieving a high level of egg cleanliness from alternative systems. The results of the experiment confirm the possibility that a satisfactory level of shell cleanliness can be achieved in the pasture system, which was over 4 points for all three hen genotypes, but with significant differences in the manifestation of this trait.

Other properties of egg shell quality, apart from the breaking force, showed differences between all three examined genotypes. Eggs from Svrljig hens had significantly lower shell deformation compared to Banat Naked Neck ($p<0.01$) and on the other hand, significantly higher compared to Tetra SL hens ($p<0.01$). Accordingly, the egg shell thickness was determined, on the basis of which the following rank of the examined hen genotypes can be determined: Tetra SL, Svrljig hen, Banat Naked Neck.

Table 2. Egg shell quality parameters (mean ± SD)

Genotype/ Age/ Parameter	Tetra SL				Banat Naked neck				Svrljig hen			
	53	55	57	**53-57**	53	55	57	**53-57**	53	55	57	**53-57**
Egg shell colour points	3.70 0.57	3.45 0.51	3.55 0.60	3.57^{A} 0.56	1.50 0.63	1.75 0.72	1.63 0.68	1.64^{B} 0.68	1.90 0.55	1.90 0.55	1.60 0.60	1.80^{B} 0.58
Egg shell cleaniness points	4.70 0.73	5.00 0.00	4.75 0.91	4.82^{Aa} 0.68	4.25 1.29	4.65 0.75	3.74 1.41	4.22^{Bb} 1.21	4.50 0.95	4.60 0.94	4.90 0.31	4.67^{ABb} 0.80
Egg shell deformation 0.001mm	22.05 4.14	21.25 2.63	21.60 2.50	21.63^{C} 3.14	27.88 5.84	31.84 6.45	33.00 7.73	31.07^{A} 6.98	24.35 4.23	25.20 3.49	28.58 5.82	26.00^{B} 4.87
Egg shell weight, g	9.45 0.77	9.14 1.08	8.30 0.98	8.96^{A} 1.05	6.93 0.77	6.64 0.81	6.12 0.53	6.54^{B} 0.77	6.95 0.74	6.98 0.84	6.57 0.64	6.83^{B} 0.75
Egg shell thickness 0.01mm	33.70 3.40	32.55 7.23	33.95 2.42	33.40^{A} 4.78	29.44 2.71	27.85 2.81	27.47 2.37	28.18^{C} 2.72	30.50 2.56	30.90 2.73	28.60 2.41	30.00^{B} 2.72
Breaking force, kg	2.40 0.41	2.46 0.35	2.44 0.51	2.43^{A} 0.42	2.17 0.41	1.93 0.34	1.88 0.28	1.98^{B} 0.36	2.29 0.33	2.16 0.36	1.96 0.65	2.14^{B} 0.48

a-b - average values of parameters for examined period in each row without a common designation are significantly different at the level of 5%; A-B - average values of parameters for examined period in each row without a common designation are significantly different at the level of 1%

The breaking force, which is also an indicator of the structural quality of the egg shell, was 2.43; 2.14 and 1.98 kg for Tetra SL, Svrljig hen and Banat Naked Neck. The obtained results confirm the poorer quality of the eggshell of the Banat Naked Neck in relation to other native breeds of hens (*Pavlovski et al., 2012*). *Škrbić et al. (2011),* in a previous study, has found similar values of the quality parameters of the eggshell of a Banat Naked Neck aged 52 weeks. Contrary to this, the results of *Sokolowicz et al. (2018)* on the determined higher values of egg shell thickness of native breed eggs in relation to hybrid layer hens, while differences in shell strength were not statistically confirmed.

Conclusion

In the alternative production of table eggs, in pasture conditions, the laying hens of the commercial hybrid Tetra SL had significantly higher egg weight and better egg quality compared to Banat Naked Neck and Svrljig hen, except for the egg yolk colour which, in the same conditions of feeding and rearing, as well as layer age, was more intensive in eggs of native breeds. The genetically conditioned lighter color of the egg shell of native unselected hen breeds compared to hybrid layer hens has been confirmed. Differences between all three genotypes were found

in egg shell deformation and thickness. The determined rank of layer genotypes for egg shell thickness was: Tetra SL, Banat Naked neck, Svrljig hen. However, the breaking force was without statistically confirmed difference between hen genotypes.

The results of the research indicate the possibility of producing table eggs of good quality in conditions of limited pasture areas, as well as the use of commercial hybrid layer hens in the mentioned alternative breeding system. From the aspect of native hen breeds, the results represent a contribution to the study of physical characteristics of egg quality of these breeds in the general perception and undestanding of their the needs and possibilities for their improvement, in order to increase acceptability from consumers and producers. Based on that, in the future, it would be possible to base the conservation of genetic resources on the principle of self-sustainability through the production of eggs for consumption.

Kvalitet jaja kokoši nosilja različitog genotipa gajenih na pašnjaku

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Rezime

Komparacija fizičkih osobina kvaliteta konzumnih jaja kokoši komercijalnog hibrida Tetra SL i dve nativne autohtone rase kokoši, banatska gološijanka i svrljiška kokoš, izvršena je u uslovima alternativne proizvodnje u pašnjačkom sistemu gajenja u portabl kavezima bez poda. Ogled je sproveden u toku vegetacionog perioda, u uzrastu kokoši 53-57 nedelja. U cilju proizvodnje jaja dobrog kvaliteta, ishrana je vršena kompletnom smešom na bazi kukuruza i soje. Pored toga, obezbeđena je konstantna dostupnost pašnjaka. Uzorkovanje jaja je bilo u jutarnjim satima i ispitivan je inicijalni kvalitet jaja baziran na masi jajeta, indeksu oblika, parametrima kvaliteta belanca, žumanca i ljuske. Nosilje komercijalnog hibrida Tetra SL su imale značajno veću masu jajeta i bolji unutrašnji kvalitet jaja ($p<0.01$) u odnosu na kokoši banatske gološijanke i svrljiške kokoši, osim boje žumanca koja je u istim uslovima ishrane i gajenja, kao i uzrasta nosilja, bila intenzivnija kod jaja nativnih rasa ($p<0.01$). Nativne rase u odnosu na komercijalni hibrid imaju svetliju boju ljuske ($p<0.01$). Deformacija i debljina ljuske su se razlikovale ($p<0.01$) između sva tri genotipa. Utvrđeni rang genotipova nosilja za debljinu ljuske je bio: Tetra SL, banatska gološijanka, svrljiška kokoš.

Međutim, sila loma je bila bez statistički potvrđene razlike između genotipova kokoši.

Ključne reči: kvalitet jaja, genotip, nativne rase, alternativna proizvodnja

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MEAT QUALITY TRAITS OF VIETNAMESE INDIGENOUS NOI CHICKEN AT 91 DAYS OLD

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Abstract: Indigenous chicken breeds have showed slower growth rate and yield lower meat production, compared to commercial broilers. However, their meat quality is valued by modern consumers. The present study aimed at analyzing the quality traits of breast meat samples of Noi broilers, one of the famous indigenous chicken breeds in Vietnam. A total of 355 breast fillet samples were collected to evaluate quality meat traits such as pH, surface color, drip loss, and cooking loss at different time points of 3, 24, and 48 hours after slaughtering as well as to analyze chemical compositions such as dry matter, crude protein and ether extract. As result, sex and cold-storage time significant affect some of quality traits of breast meat, whereas their interaction did not associate among the observed properties. After 3 hour-storage, the pH value was determined at 5.63, then decrease to 5.56 and 5.55 after 24 and 48 hours, respectively. The color values (L^*, a^*, and b^*) were in the normal range reported from previous studies. Meat samples of two sexes did not vary in the cooking loss and drip loss values, whereas it was significantly different due to cold-storage time. The ether extract content of the meat was found negatively correlated with the cooking loss. The higher dry matter content of breast meat resulted in the lower drip loss value after 3h cold-storage ($r=-0.12$, $P<0.05$). There is a negative relationship between L^* and a^*. The variation demonstrated in this study can be used in breeding schemes in order to improve meat quality of Noi chicken lines.

Key words: Noi chickens, breast meat, cold-storage, quality traits, correlation.

Introduction

Ingestion of healthy and nutritious food materials play a crucial role in maintaining the human health, as advised by doctors and nutritionists (*Farrell, 2010; Ahmad et al., 2018*). This concern has gained globally contributing to the raising awareness regarding the remarkable development of poultry production. Regarding nutritional aspects, poultry meat has been considered as "functional foods" of a well-balanced diet. It is well fit the current consumer preference for a meat type which is high in protein and low in saturated fatty acids. According to *Ahmad et al. (2018),* the average value of the meat protein is about 22%, but it could range from high protein value of 34.5% in chicken breast to as low as 12.3% protein in duck meat. The amino acid score adjusted for protein digestibility, an indicator of protein quality, reveals that chicken meat has high efficiency of 69,79% compared to other meat sources including shrimp and turkey (52.58% and 63.49%, respectively) (*Barron-Hoyos et al., 2013*). In addition, chicken meat may also provide other bioactive substances with favorable effects on human health, such as conjugated linoleic acid, vitamins and antioxidants (*Petracci and Cavani, 2012*).

The growing consumption and consumer demands for chicken meat have resulted in pressure on intense selection processes in poultry production to enhanced animal growth rate, feed efficiency, size and quality of breast muscle as well as reduction in abdominal fatness (*Baracho et al., 2006*). The age at which poultry is slaughtered has been continually reducing halved to five weeks, while the yield of breast meat was significantly improved by 10% compared with poultry production 50 years ago (*Anthony, 1998; Havenstein, 2006*). Sensory characteristics and functional properties of poultry meat are critical not only for consumer's initial selection but also for final product satisfaction and the most important quality attributes are appearance and texture. Major appearance quality issues are skin and meat colors while meat tenderness, juiciness and flavor are primarily associated with texture traits (*Fletcher, 2002; Ismail and Joo, 2017*). Quality traits of chicken meat along with nutritional compositions are dependent upon animal genetics, feeding source, rearing systems, handling and slaughter techniques (*Ahmad et al., 2018*). Interestingly, meat of indigenous chicken has been found to possess a unique taste, with tough muscles, whereas the meat of commercial broiler chicken obtains an over-tender characteristic (*Wattanachant, 2008; Assan, 2015*).

The meat quality properties of indigenous chickens have been widely researched. Korean native chicken contained higher percentage of protein but a

lower fat and moisture contents than broilers (*Jayasena et al., 2013*). The color score of breast Korean chicken meat was also higher than that of silky fowl, an imported poultry breed from China owning special nutritive and medicinal values (*Choo et al., 2014*). For Leung Hang Khao (the Thai indigenous chicken), *Molee et al. (2018)* found a significant correlation between animal weight and cooking loss of breast meat. It was also found that the drip loss of the meat may be negatively affected when growth performance is improved. However, breast and thigh muscles of Thai chickens were higher in shear force value and collagen content, the cholesterol and triglyceride contents as well as n-3 fatty acid were also more favorable compared to those of broiler breeds and their crossbreds (*Jaturasitha et al., 2008*). *Raphulu et al. (2015)* obtained that the meat of Venda chickens (an indigenous breed in South Africa) containing less fat and more crude protein than the meat of commercial broilers. The available information might assure the opportunity to improve the production of native chicken. However, our knowledge of the quality variables of breast meat of indigenous Noi chickens in Vietnam is limited. It is also necessary to consider potential interactions between sex and cold-storage time on meat quality of Noi chickens. The objective of this study was to investigate the meat quality traits of Noi chickens, a famous indigenous breed originated in Vietnam in order to provide substantially data for commercial/industrial scale-up production.

Materials and Methods

Based on the previous experimental layout of *Do et al. (2019)* as well as continuing the research work of *Nguyen et al. (2020)*, a total of 355 fillet samples of breast meat from 164 males and 191 female Noi chickens at 91 days old (weighed 1.43±0.29 kg/bird) were collected to evaluate their quality traits.

Generally, the samples were stored at 4^{o}C after slaughtering to analyze (1) their chemical compositions such as dry matter (DM) and ether extract **(**EE, by using ANKOM XT15 Extractor, ANKOM Technology, USA) according to AOAC protocols (*AOAC, 2005*), followed by crude protein (CP, by using a DLK8 Fully Auto Digester and a UDK149 Automatic Kjeldahl Distillation Unit manufactured by VELP Scientifica, Italy) as guidance of Kjeldahl's method, (2) their quality traits such as pH value by using a pH meter (Hanna Instruments, HI 8314, Padova, Italy; *Cömert et al., 2016*), surface color (L^*, a^* and b^*) by using a CI60 colorimeter (Lovibond, UK; *C.I.E., 1978*), cooking loss (*Bertram et al., 2003*) and drip loss (*Guan et al., 2013*) at three different time points 3, 24 and 48 hours.

The differences between quality traits of breast meat data were analyzed with analysis of variance (ANOVA), the significance of each pair wise comparisons and Pearson's correlation coefficients of traits were estimated with Minitab 16 software. A probability value of less than 0.05 was considered to be

significant. Mean ± standard deviation (SD) was used to measure all the parameters.

Results and Discussion

Chemical compositions

Nutritionally, chicken meat is a rich source of high value protein compared to vegetable proteins (*Soriano-Santos, 2010*). In addition, the low energy content with a high proportion of omega-3 polyunsaturated fatty acids, and other nutrients including zinc, iron, selenium, potassium, magnesium, sodium, vitamin A, B-complex vitamins and folic acid, places chicken meat as a healthy food, indicated for use in healthy diets, when compared to other meat sources (*Ahmad et al., 2018*). The chemical compositions of Noi chickens (average weight 1.43±0.29 kg/bird) are shown in Table 1.

Table 1. Difference of chemical compositions of breast meat

Traits	Sex				Overall
	Male (n = 164)	**Female** (n = 191)	**SEM**	**P**	
DM (%)	25.04 ± 1.05	25.20 ± 1.20	0.09	0.212	25.13 ± 1.14
EE (%)	0.53 ± 0.24	0.52 ± 0.21	0.02	0.705	0.53 ± 0.23
CP (%)	23.77 ± 1.21	23.93 ± 1.02	0.08	0.203	23.86 ± 1.11

In overall, the contents of DM, EE and CP of the breast muscle were 25.13%, 0.53%, and 23.86%, respectively. The results also indicated that there were no differences between sexes in relation to proximate compositions of DM, EE and CP (P>0.05). This observation is in agreement with *Bogosavljevic-Boskovic et al. (2010)*, who found no significant effects of rearing system and sex on dry matter and ash contents of breast muscle of Hybro G, a fast-growing broiler strain. In regards to indigenous chickens, *Pambuwa and Tanganyika (2017)* reported a non-significant difference between sexes found in proximate composition of Malawi indigenous chickens. *Jeon et al. (2010)* indicated that the protein content of breast meat in North Korean native chickens ranged from 24.13% to 24.63%, higher than the result of this study. Noi chicken muscle also contained less fat than Korean native chickens (0.53% and 1.31%, respectively). Bearded chickens, another indigenous chicken breed in the North of Vietnam, showed higher contents in dry matter (27.47-28.57%) and crude fat (0.54-0.91%) but lower in crude protein (19.36-20.25%) (*Nguyen et al., 2012*), as compared to the results of this study.

Meat quality traits

After slaughtering, postmortem glycolysis is activated and accumulation of lactic acid in the muscle is increased, which results in a pH decline (*Wideman et al., 2016*). A dramatic pH decline is associated with protein denaturation and negatively affects the meat quality, causing paleness, low water-holding capacity, and soft texture (*Petracci and Cavani, 2012*). Different traits of breast quality between sex, cold-storage time and their interaction as well as the descriptive statistics of these variables were shown in Table 2. In overall, there were significant effects (P<0.05) of sex and cold-storage time to some of quality traits of breast meat. However, regarding their interaction; there were no significant effects (P>0.05) for all the observed properties.

Table 2. Quality traits of breast meat at different time of measurement

Traits		pH value	Lightness, L^*	Redness, a^*	Yellowness, b^*	Cooking loss (%)	Drip loss (%)
Sex effect							
Male		$5.61^{a} \pm 0.18$	57.40 ± 4.44	1.44 ± 1.51	$11.14^{a} \pm 3.05$	29.01 ± 8.40	2.08 ± 1.04
Female		$5.55^{b} \pm 0.14$	56.91 ± 4.33	1.48 ± 2.21	$12.64^{b} \pm 3.32$	28.69 ± 8.38	2.16 ± 1.10
Time effect							
3h		$5.63^{a} \pm 0.17$	57.44 ± 4.58	$1.11^{a} \pm 2.13$	$12.27^{a} \pm 3.18$	$25.52^{a} \pm 7.12$	$2.70^{a} \pm 1.09$
24h		$5.56^{b} \pm 0.16$	57.27 ± 4.46	$1.48^{ab} \pm 2.28$	$11.34^{b} \pm 3.38$	$30.54^{b} \pm 8.70$	$2.06^{b} \pm 0.91$
48h		$5.55^{b} \pm 0.15$	56.76 ± 4.09	$1.78^{b} \pm 2.58$	$12.06^{a} \pm 3.23$	$30.49^{b} \pm 8.26$	$1.61^{c} \pm 0.92$
Interaction effect							
Male	3h	5.65 ± 0.19	57.80 ± 4.43	1.10 ± 2.36	11.54 ± 3.12	25.91 ± 7.16	2.69 ± 1.02
	24h	5.60 ± 0.19	57.65 ± 4.50	1.43 ± 2.36	10.61 ± 3.08	30.64 ± 8.13	2.04 ± 0.96
	48h	5.58 ± 0.16	56.75 ± 4.34	1.78 ± 2.75	11.28 ± 2.90	30.49 ± 8.99	1.52 ± 0.79
Female	3h	5.61 ± 0.14	57.08 ± 4.69	1.12 ± 1.92	13.00 ± 3.09	25.14 ± 7.08	2.70 ± 1.16
	24h	5.53 ± 0.12	56.89 ± 4.41	1.54 ± 2.21	12.07 ± 3.49	30.43 ± 9.19	2.07 ± 0.87
	48h	5.52 ± 0.14	56.77 ± 3.87	1.78 ± 2.44	12.84 ± 3.32	30.49 ± 7.61	1.70 ± 1.01
SEM							
Sex		0.01	0.19	0.10	0.14	0.35	0.04
Time		0.01	0.23	0.12	0.17	0.43	0.05
Sex × Time		0.61	0.07	0.01	0.33	0.18	0.24
P-value							
Sex		0.000	0.070	0.762	0.000	0.512	0.228
Time		0.000	0.101	0.001	0.000	0.000	0.000
Sex × Time		0.326	0.422	0.945	0.973	0.805	0.481

[a-c] Superscripts on different means in a column within a factor differ significantly (p<0.05).

Regarding muscle pH, the ultimate-pH value of male chickens was higher ($p<0.05$) as compared to the females (5.61 *vs*. 5.55, respectively). After 3 hour-storage, the pH value was determined at 5.63, then decreased to 5.56 and 5.55 after 24 and 48 hours, respectively (Table 2). However, interaction was not observed between sex and cold-storage time for pH values. According to *Marcinkowska-Lesiak et al. (2016)*, the declining in pH values are probably due to the chances in glycogen content according preservative time. The findings are in line with other authors who found significant differences on the ultimate pH between different sexes of chickens (*Schneider et al., 2012; Khan et al., 2018*). Meat color is highly correlated to the amount of heme containing compounds such as myoglobin, haemoglobin, and cytochrome c. The breast muscle is almost entirely composed of white fibres which are low in myoglobin as compared to red fibres of the thigh/leg muscle. Therefore, chicken breast fillet generally appears to have a pink color, which is a desirable characteristic for the consumer (*Wideman et al., 2016*). Meat color is generally influenced by animal related factors, mainly genotype (*Barbosa-Filho et al., 2017*) and age (*Michalczuk et al., 2016*) of the birds. The color values of this study (L^*=56.75-67.80; a^*=1.10-1.78; b^*=10.61-13.00) were in the normal range reported from different broiler experiments (*Wideman et al., 2016*). Sex only affected b^* value of muscle color, whereas showed an independence relationship with the values of L^* and a^*. As shown in Table 1, the mean values of redness scoring (a^*) obtained greater standard deviations, probably indicating a high variation in the population of Noi chickens.

This result is consistent with the finding of *Khan et al. (2018)*, who reported that female Aseel chickens exhibited a higher yellowness (b^*) value than males. The results also confirmed that cold-storage time had an effects on meat color, a^* and b^*. The interaction between cold-storage time and sex do not affect breast muscle colors (L^*, a^* and b^*) in Noi chickens. Regarding the a^* value, it showed a reverse tendency which previously reported by *Suwattitanun and Wattanachant (2014)*, who stated that the redness of breast meat would be steadily decreased with storage. Within the local breeds, Korean local chickens have significantly lighter, darker red, and yellowish breast meat than that of silky fowl, which is assumed to be due to genetic difference (*Choo et al., 2014*). This finding was in agreement with that of *Jaturasitha et al. (2008)* who reported the similar color values in Thai native chickens. Comparing to commercial broilers, breast fillet of Noi chickens was lighter, yellower but less red than that of Cobb 500 (*Al-Marzooqi et al., 2019*).

Water holding capacity is defined as ability of the raw meat to retain its water during the application of external forces, such as transporting, processing and cooking. The water released can be described as cooking loss, drip loss, which is inversely related to water holding capacity (*Warner, 2017*). Meat samples of two sexes did not vary in the cooking loss and drip loss values ($P>0.05$), whereas it was significantly different due to cold-storage time ($P<0.05$). The interactions between

the factors also have no influences (P>0.05) on the percentages of cooking loss and drip loss (Table 2). At 4ºC condition, the increasing storage time positively increased the cooking loss (25.52% to 30.54%) but reduced the drip loss (2.70% to 1.61%). The opposite effect of the storage time on drip loss was observed by other authors. *Suwattitanun and Wattanachant (2014)* found that longer storage time (under conditions of 0-4ºC and 12-15ºC) was induced greater drip loss. It was, additionally, confirmed that storage time had a negative effect on meat quality and caused greater drip loss regardless of the packaging methods (*Marcinkowska-Lesiak et al., 2016*).

Correlation between chemical compositions and other meat quality traits

The correlation coefficients among physicochemical parameters of breast meat are presented in Table 3. Regarding the chemical compositions, there is a positive correlation between dry matter content and ether extract (r=0.23, P<0.001) crude protein (r=0.20, P<0.001). Dry matter content of the breast muscle also correlated with the meat color b^* (r=0.13, P<0.05) and L^* but in different manner (r=-0.11, P<0.05). The ether extract content of the meat was found negatively correlated with the cooking loss percentage (r=-0.15, P<0.01). The higher breast meat DM content resulted in the lower drip loss value after 3h cold-storage (r=-0.12, P<0.05). According to former studies, there was an effect of surface pH on water holding capacity (*Baéza et al., 2012, Suwattitanun and Wattanachant, 2014; Marcinkowska-Lesiak et al., 2016*). However, that correlation was less obvious, as in the current study. At 48h post-mortem, the pH value also seems to have negative influence on the color of the meat, with higher pH values resulting in a lighter meat color (r=-0.18, P<0.001). At all investigation points, there was a negative relationship between L^* and a^*, determining that a^* values were higher in fillets showing lower L^* values. Similar to this study, *Kralik et al. (2014)* found a negative correlation between L^* and pH value as well as the negative correlation coefficient between L^* and a^*.

Table 3. Correlation coefficients (r) among meat quality traits at 3h – 48h post-mortem

Traits	DM	EE	CP	pH	L^*	a^*	b^*	CL	DL
3h post-mortem									
DM	**1.00**								
EE	0.23^{***}	**1.00**							
CP	0.20^{***}	0.09^{NS}	**1.00**						
pH	0.01^{NS}	0.01^{NS}	-0.02^{NS}	**1.00**					
L^*	-0.11^{*}	-0.06^{NS}	0.05^{NS}	-0.06^{NS}	**1.00**				
a^*	0.07^{NS}	0.002^{NS}	-0.01^{NS}	-0.03^{NS}	-0.15^{**}	**1.00**			
b^*	0.13^{*}	-0.02^{NS}	0.09^{NS}	-0.05^{NS}	-0.03^{NS}	0.43^{***}	**1.00**		
CL	-0.12^{*}	-0.15^{**}	0.05^{NS}	-0.11^{*}	0.08^{NS}	0.03^{NS}	0.11^{*}	**1.00**	
DL	-0.04^{NS}	0.01^{NS}	0.00^{NS}	0.002^{NS}	-0.13^{**}	0.14^{**}	0.06^{NS}	-0.01^{NS}	**1.00**
24h post-mortem									
DM	**1.00**								
EE	0.23^{***}	**1.00**							
CP	0.20^{***}	0.09^{NS}	**1.00**						
pH	-0.09^{NS}	0.00^{NS}	0.04^{NS}	**1.00**					
L^*	-0.13^{*}	0.04^{NS}	-0.08^{NS}	0.01^{NS}	**1.00**				
a^*	0.06^{NS}	0.03^{NS}	-0.03^{NS}	-0.07^{NS}	-0.28^{***}	**1.00**			
b^*	0.10^{NS}	0.13^{*}	0.04^{NS}	-0.05^{NS}	-0.05^{NS}	0.17^{***}	**1.00**		
CL	-0.04^{NS}	-0.10^{*}	-0.01^{NS}	0.02^{NS}	-0.01^{NS}	-0.03^{NS}	0.06^{NS}	**1.00**	
DL	-0.03^{NS}	-0.07^{NS}	-0.12^{*}	-0.02^{NS}	0.09^{NS}	0.004^{NS}	0.09^{NS}	0.07^{NS}	**1.00**
48h post-mortem									
DM	**1.00**								
EE	0.23^{***}	**1.00**							
CP	0.20^{***}	0.09^{NS}	**1.00**						
pH	-0.002^{NS}	-0.07^{NS}	0.12^{*}	**1.00**					
L^*	-0.17^{***}	0.03^{NS}	-0.09^{NS}	-0.18^{***}	**1.00**				
a^*	0.01^{NS}	0.004^{NS}	0.01^{NS}	0.01^{NS}	-0.31^{***}	**1.00**			
b^*	0.08^{NS}	0.06^{NS}	0.04^{NS}	-0.08^{NS}	-0.04^{NS}	0.09^{NS}	**1.00**		
CL	0.02^{NS}	-0.02^{NS}	0.02^{NS}	0.05^{NS}	-0.04^{NS}	0.01^{NS}	-0.002^{NS}	**1.00**	
DL	-0.09^{NS}	0.01^{NS}	-0.03^{NS}	0.01^{NS}	0.10^{*}	-0.02^{NS}	0.07^{NS}	-0.09^{NS}	**1.00**

CL= Cooking loss (%), DL= Drip loss (%), L^*= lightness, a^* = redness, b^* = yellowness.

*=significant at P<0.05, **=significant at P<0.01, ***=significant at P<0.001, NS= non-significant.

Conclusion

The chemical compositions of Noi chicken meat might fully meet the expectation of modern consumers. There were significant effects of sex and cold-storage time to some of quality traits of breast meat. It is also suggested that there are relationships among the variables of meat quality in Noi chickens although the interactions showed less significant effects. In conclusion, the variation in meat quality can be used in breeding schemes in order to improve meat quality of Noi chicken lines.

Osobine kvaliteta mesa vijetnamske autohtone rase živine Noi starosti 91 dan

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Rezime

Autohtone rase živine su pokazale sporiju stopu rasta i nižu proizvodnju mesa, u poređenju s komercijalnim brojlerima. Međutim, njihov kvalitet mesa je cenjen od strane savremenih potrošača. Ova studija imala je za cilj da analizira osobine kvaliteta uzoraka mesa grudi pilića rase Noi, jedne od poznatih autohtonih rasa živine u Vijetnamu. Ukupno je prikupljeno 355 uzoraka fileta grudi za procenu kvaliteta svojstava mesa kao što su pH, boja, kalo ceđenja i kalo kuvanja, u različitim vremenskim tačkama 3, 24 i 48 sati nakon klanja, kao i analiza hemijskog sastava, kao što su suva materija, sirovi protein i ekstrakt etra. Kao rezultat toga, pol pilića i vreme skladištenja na hladnom značajno utiču na neke osobine kvaliteta mesa grudi, dok njihova interakcija nije povezana sa posmatranim svojstvima. Posle skladištenja od 3 sata, pH vrednost je određena na 5,63, a zatim je pala na 5,56 i 5,55, posle 24 i 48 sati. Vrednosti boje (L *, a * i b *) bile su u normalnom rasponu koji je zabeležen u prethodnim studijama. Uzorci mesa od dva pola pilića nisu se razlikovali u vrednostima za kalo kuvanja i kalo ceđenja, dok se značajno razlikuju kao rezultat vremena skladištenja u hladnom. Sadržaj ekstrakta etra u mesu je u negativnoj korelaciji sa kalom kuvanja. Viši sadržaj suve materije u mesu grudi rezultirao je nižom vrednosti za kalo ceđenja nakon 3h skladištenja u hladnom (r = -0,12, P <0,05). Postoji negativan odnos između L * i a *. Varijacija koja je prikazana u ovoj studiji može se koristiti u šemama uzgoja u cilju poboljšanja kvaliteta mesa linija Noi pilića.

Ključne reči: Noi pilići, meso grudi, hladno skladištenje, osobine kvaliteta, korelacija.

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INTERRELATION BETWEEN BODY WEIGHTS OF SIRE, DAM AND THEIR LAMBS AT EARLY STAGE OF GROWTH

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Abstract: Records of female lambs and their parents of the Mis sheep breed have used. All animals are approximately have weaned at 90 days of age. Descriptive statistics, paired sample test, paired differences, measures of association, correlations and regression of body weights between female lambs and their parents have done. A complementary least body weights at 30 days and weaning between dams and lambs but utmost weight at 30 days, the lambs were higher while at weaning, the dams had higher weight. It can observe that the averages on body weights the rams were the highest, followed by lambs and the lowest the dams' body weights. The coefficient of determination of R^2 varies from low to high, indicating that the lamb's body weight has more influenced by other factors that we have not considered.There were significant correlations between lamb body weight at birth and sire/dam body weight at birth. The results showed highly significant correlations of lamb's body weight at 30 days with dams but with sires, positive and very low. There had positive but no significant correlation between lamb body weight at weaning and sire body weight at weaning. Lamb body weight at weaning and dam body weight at weaning are highly correlated.

Key words: sire, dam, lambs, body weight, birth, weaning, early stage growth, association, correlation

Introduction

In sheep selection, it is very imperative to get the right information about the quality of future parents at an early age *(Petrović et al., 2018).* "Progeny test"

lasts a long result about the value of the parents we get when several years pass. In this regard, it is important to find out the relationship between the body development characteristics of parents at an early age, and the growth traits of their offspring. This research so far has not received much attention. The lamb's body weight at different ages has a deterministic outcome on the expediency of sheep production enterprises *(Mokhtari et al.,* 2013). As well, bodyweight is one of the relevant selection criteria for the enhancement of meat animals such as sheep *(Afolayan et al., 2006).* The phenotypic information of both parents can approximately predict how the offspring will perform. However, the observed performance of each animal in each trait is the result of the heredity that it receives from both parents, and the environment in which it raised and even when an attempt is made to provide a uniform environment, there are still accidental and unknown environmental differences between animals *(Babar et al., 2004).* The live weight considered most important to monitor in animals since it serves as an indicator to accurately meet it uses either the purpose of reproduction and or market specifications. The meat production primary parameter is body weights and has influenced by genetic and environmental factors *(Aksoy et al., 2016).* Body weights also help or even to guide breeders to determine the ideal management practices to maintain the gain at an optimum level *(Lalit et al., 2016).* Weight information could also use in determining the value of animals and the efficiency of rearing *(Shirzeyli et al., 2013).*The body weights reflect the phenotypic characteristics of the animals; it's a source for standard determination of certain breed. It has a major role to accomplish profitable effects *(Petrović et al., 2015).* The success of genetic improvement has based on expectations that the descendants by their phenotypic values will be above the average values of parents *(Caro Petrovic et al., 2018).* The body weight and growth performance is an important character which determines the overall productivity of the flock and the economic return from sheep production enterprises with the main objective for meat production *(Yiheyis et al., 2012; Zidane et al., 2015; Caro Petrovic et al., 2017*).

The study aimed to determine associations/correlations of sires, dams' body weights at an early stage of growth with their female lambs. This paper is to shed light on this issue of great importance in sheep science and practice.

Material and Methods

The study pertained to the early growth of the Mis sheep breed of the Institute for Animal Husbandry, Belgrade-Zemun, Serbia. The collected records of 100 female lambs and their parents have used. The animals are approximately have weaned at 90 days of age. All the animals have the same feeding and housing management and have reared intensively. The sires and dams have conditioned before the premating. The dams feeding and management during

the gestation period treated uniformly. Bodyweight controls of the lambs have performed at birth, at 30 and 90 days of age. The statistical analysis of body weights of sires (4) at birth (PBWB), at 30 days (PBW30), at weaning (PBWW); dams (100) at birth MBWB, at 30 days MBW30, at weaning (MBWW), and their female lambs (100) at birth (LBWB), at 30 days (LBW30), at weaning (LBWW) performed using SPSS software package program on the following: Descriptive statistics, paired sample test, paired differences and measures of association, correlations and regression of body weights between female lambs and their parents.

Results and Discussion

The sires, dams, and their lamb's body weights at different early growth stages regardless, of their birth type, have presented in the table below.

Table 1. Means, Standard Deviation, Variance of body weights (sires, dams and their female lambs)

Traits	Minimum	Maximum	Mean	St. Error	Std. Deviation	Variance
PBWB	5.30	6.30	5.9250	.03917	.39167	.153
MBWB	2.80	6.50	4.3390	.07914	.79135	.626
LBWB	2.60	6.50	4.4560	.09055	.90546	.820
PBW30	16.00	19.00	18.0000	.12309	1.23091	1.515
MBW30	10.00	19.00	13.3330	.18463	1.84627	3.409
LBW30	10.00	20.50	13.6400	.22969	2.29686	5.276
PWW	26.00	35.00	31.0000	.34082	3.40825	11.616
MWW	20.00	34.00	23.6480	.24420	2.44195	5.963
LWW	20.00	32.00	24.1520	.28135	2.81348	7.916

It is known that ewe size, pregnancy nutrition and pregnancy rank are known to affect the productive performance of ewes and their offspring (*Petrovic et al., 2013*). In can notice (table 1) that dams and lambs showed a similar minimum body weight at 30 days and weaning. On the other hand, at the maximum weight at 30 days, the lambs were higher for 1.50 kg while in weaning weight, the dams higher for 2 kg. It can observe that the averages on body weights the rams were the highest, followed by lambs and the lowest the dams' body weights. We can see that the body weight of lambs is moving within normal limits for this population *(Petrovic, 2006; Caro Petrovic et al., 2013).*

In Table 2, the degree of relationship of the observed samples in the population has estimated.

Table 2. Measures of Association of lambs body weights between lambs and sires; lambs and dams

Traits	R	R Squared	Eta	Eta Squared
LBWB * PBWB	.204	.042	.224	.050
LBWB * MBWB	.481	.231	.680	.463
LBW30 * PBW30	.099	.010	.119	.014
LBW30 * MBW30	.353	.124	.646	.417
LWW * PWW	.127	.016	.191	.037
LWW * MWW	.315	.099	.621	.386

The results of the performed analysis show that the estimated regression model for testing the influence of parents' body weight at an early age expressed by the coefficient of determination of R^2 varies from 1.0% - LBW30 * PBW30 to 23.1% - LBWB * MBWB. In the context of this analysis, this indicates that the weight of the lamb's body has more influenced by other factors that we have not considered.

Eta Squared- η^2 is the proportion of total variance that could attribute to the influence of the observed factor. From the table we can see that the values of this parameter range from .014- LBW30 * PBW30 to .463- LBWB * MBWB. With this, it means that the influences related to the body weight of the lambs in the table ranged from small to very large.

Aman et al. (2013) have used a nonlinear regression model to predict lamb body weight. He obtained high values of the coefficient of determination of R^2.

Other authors like *Lambe et al. (2008)* have utilized exponential models and linear regression models for growth analysis of two breeds of lambs from birth to slaughter.

Table 3. Paired Samples Test of Differences of body weights (lamb vs. rams; lamb vs. dams)

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	LBWB – PBWB	-1.46900	.91007	.09101	-16.142	99	.000
Pair 2	LBWB – MBWB	.11700	.87005	.08700	1.345	99	.182
Pair 3	LBW30 - PBW30	-4.36000	2.49622	.24962	-17.466	99	.000
Pair 4	LBW30 - MBW30	.30700	2.38574	.23857	1.287	99	.201
Pair 5	LWW – PWW	-6.84800	4.13369	.41337	-16.566	99	.000
Pair 6	LWW – MWW	.50400	3.09048	.30905	1.631	99	.106

The paired samples test of body weights differences (Table 3) was highly significant ($P<0,01$) on body weights between lambs vs. sires from birth weight to their weaning weight (LBWB-PBWB; LBW30-PBW30; LWW-PWW while

between lambs vs. dams had no significant differences (P>0,05) in tested body weights.

Table 4. Correlations of lambs body weight at birth with sires and with dams

		LBWB	PBWB	MBWB
LBWB	Pearson Correlation	1	.204*	.481**
	Sig. (2-tailed)		.041	.000
	Sum of Squares and Cross-products	81.166	7.180	34.112
	Covariance	.820	.073	.345
	N	100	100	100
PBWB	Pearson Correlation	.204*	1	.011
	Sig. (2-tailed)	.041		.917
	Sum of Squares and Cross-products	7.180	15.188	.322
	Covariance	.073	.153	.003
	N	100	100	100
MBWB	Pearson Correlation	.481**	.011	1
	Sig. (2-tailed)	.000	.917	
	Sum of Squares and Cross-products	34.112	.322	61.998
	Covariance	.345	.003	.626
	N	100	100	100

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 4 shows significant correlations (P<0.05) between lamb body weight at birth (LBWB) and sire body weight at birth (PBWB) (P<0.01) between lamb body weight at birth (LBWB) and dam body weight at birth (MBWB).

Table 5. Correlations of lambs body weight at 30 days with sires and with dams

		LBW30	PBW30	MBW30
LBW30	Pearson Correlation	1	.099	.353**
	Sig. (2-tailed)		.327	.000
	Sum of Squares and Cross-products	522.280	27.700	148.128
	Covariance	5.276	.280	1.496
	N	100	100	100
PBW30	Pearson Correlation	.099	1	-.068
	Sig. (2-tailed)	.327		.504
	Sum of Squares and Cross-products	27.700	150.000	-15.200
	Covariance	.280	1.515	-.154
	N	100	100	100
MBW30	Pearson Correlation	.353**	-.068	1
	Sig. (2-tailed)	.000	.504	
	Sum of Squares and Cross-products	148.128	-15.200	337.461
	Covariance	1.496	-.154	3.409
	N	100	100	100

** Correlation is significant at the 0.01 level (2-tailed).

The results showed correlations highly significance of lambs body weight at 30 days with dams (Table 5) with sires, positive and very low correlations.

Table 6. Correlations of lambs body weight at weaning with sires and with dams

		LWW	PWW	MWW
LWW	Pearson Correlation	1	.127	.315**
	Sig. (2-tailed)		.206	.001
	Sum of Squares and Cross-products	783.650	121.000	214.220
	Covariance	7.916	1.222	2.164
	N	100	100	100
PWW	Pearson Correlation	.127	1	.072
	Sig. (2-tailed)	.206		.474
	Sum of Squares and Cross-products	121.000	1150.000	59.600
	Covariance	1.222	11.616	.602
	N	100	100	100
MWW	Pearson Correlation	.315**	.072	1
	Sig. (2-tailed)	.001	.474	
	Sum of Squares and Cross-products	214.220	59.600	590.350
	Covariance	2.164	.602	5.963
	N	100	100	100

** Correlation is significant at the 0.01 level (2-tailed).

In table 6, it showed positive but no significant correlation between lamb body weight at weaning (LWW) and sire body weight at weaning (PWW). On the other hand, between lamb body weight at weaning (LWW) and dam body weight at weaning (MWW) have shown very significant correlations. Interesting research of *Matika et al. (2001)* that may be related to ours is that lamb weight at birth has a mean genetic correlation with maternal weight. Furthermore, these authors stated correlations between birth weight and other weights to 18 months were high (0.75-0.85). Total weight of lamb weaned was moderately correlated to birth weight (rg = 0.46 ± 0.15) but tended to be highly correlated with 18 month weight (0.92 ± 0.10) and ewe weights (0.75 ± 0.09-0.91 ±0.07).

Ali et al. (2006) stated that correlation and regression coefficients between the above mentioned two traits were 0.37 and 0.025 ± 0.0001, respectively. Analysis of variance of dam age at service and birth weight of lambs due to regression revealed that this regression was statistically significant (P<0.01).

Ghafouri et al. (2008) informed that genetic correlations among growth traits of Mehraban sheep were positive, indicating that selection for WW would also increase BW and other weights.

Studies of the relationship between the body weight of lambs have been examined by other authors (*El Fadili, 2000; Bromley, 2001),* in different sheep population with the results on the existence and importance of examining the interrelation of weight for selection in sheep breeding.

Conclusion

Our research has shown that there is a significant association between lamb weight and their parents. The results showed correlations of high significance with regards to the body weight of lambs over 30 days compared with mothers and low in comparison with fathers. There was a positive but not significant correlation between lamb body weight at weaning and father body weight at weaning. The body weight of lambs at weaning and the weight of mothers at weaning are strongly and closely related. The coefficient of determination of R^2 varies from low to high, indicating that the lamb's body weight has more influenced by other factors that we have not considered. These studies are rare in the literature and have great practical relevance for the selection of future breeding sheep at an early age, which is an eternal goal in breeding domestic animals.

Interrelacija između mase tela očeva, majki i njihovih jagnjadi u ranom stadijumu telesnog razvoja

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Rezime

Istraživanja su obavljena kod ženke jagnjadi i njihovih roditelja u populaciji ovaca rase Mis. Sve životinje su odbijene u starosti od 90 dana. Ispitivane su komparativne mase telesnog razvoja jagnjadi i njihovih roditelja, da bi se ustanovila eventualna povezanost, a u cilju efikasnije selekcije u ranom uzrastu jedinki. Analiza je obuhvatila pored mase od rođenja do zalučenja, korelacije i regresiju mase između jagnjadi i njihovih roditelja. Majke i jagnjad su imali sličnu minimalnu telesnu masu sa 30 dana i pri odbijanju, ali maksimalna masa jagnjadi sa 30 dana bila je veća dok su kod odbijanja majke imale veću težinu. Može se primetiti da su prosečni telesni parametri ovnova bili veći, u poređenju sa masom jagnjadi, ali manji u komparaciji sa masom tela u razvojnom uzrastu majki. Koeficijent determinacije R^2 varira od niskog do visokog, što ukazuje da na masu tela više utiču drugi faktori koje nismo razmatrali. Postoje značajne korelacije između telesne mase janjadi pri rođenju i mase tela ovnova, ali i ovaca majki pri njihovom rođenju. Rezultati su pokazali korelacije visokog značaja u pogledu telesne mase jagnjadi tokom 30 dana u poređenju sa majkama i niskog stepena u komparaciji sa očevima. Postoji pozitivna, ali ne značajna korelacije između telesne mase jagnjadi pri odbijanju i mase tela očeva prilikom njihovog odbijanja.

Mase tela jagnjadi pri odbijanju i mase majki pri odbijanju su jako i pozitivno povezane. Ova istraživanja su retka i škrta u literaturi, ali imaju veliki praktični značaj za selekciju budućih priplodnih ovaca u ranom uzrastu, što je večiti cilj u oplemenjivanju domaćih životinja.

Ključne reči: mužjak, ženka, jagnjad, telesna masa, rođenje, zalučenje, rani porast, povezanost, korelacija

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EXPRESSION OF ISG15 AND CONJUGATING ENZYME DURING PERI-IMPLANATION PERIOD IN SHEEP

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Abstract: Pregnancy recognition in ruminant affects numerous genes regulation. Interferon-stimulatory gene (*ISG15*), an ubiquitin-like protein that mediates theconjugation of different proteins through its ISGylation enzymes *UBE1L* and *UBCH8,* is also differentially expressed during early pregnancy. The purpose of this study was to investigate the role of *ISG-15* in the establishment of pregnancy and conceptus elongation during early post conception periods and to ascertain the presence of ISGylation enzymes *UBE1L* and *UBCH8*. Therefore, sheep were synchronized through cloprostenol sodium and gonadotrophin releasing hormone-1 and serviced by rams. The blood was collected on the post-mating days 6, 11, 12, 13, 14, 16, 17, 21, 23 and 25. The *ISG-15*, *UBE1L* and *UBCH8* primers were used to amplify the corresponding transcriptomic region using PCR. Recovery rate of each transcriptomic fragment was compared with the housekeeping gene *GAPDH*. ISG-15 expression was higher on day 12, contrary to *UBE1L* were higher on day 6 and *UBCH8* on day 21. Furthermore, the *ISG-15* is ubiquitin-like protein, mediates *UBE1L* and *UBCH8* enzymes to guard the conceptus against viral pathogenicity during early pregnancy.

Key words: ISG-15, sheep, pregnancy, ISGylation, conception, ubiquitination.

Introduction

In bovine and ovine, embryonic loses occur most of the times during pre-implantation stages. These embryonic losses occur due to lack of biochemical communications between the embryo and the uterus *(Li and Winuthayanon, 2017)*. Interferon-tau (IFN-τ) is from type I IFNs family, and is a basic cytokine in

establishment of ruminant's pregnancy, it is produced by the ruminant's conceptus around the time of implantation *(Ka et al., 2018*). IFN-τ also represents immune modulatory action towards leukocytes by changing their proliferative responses and cytokine production. This cytokine action has been extensively studied for the past ten years *(Sanlorenzo et al. 2017)*. It has been viewed as a potential pathway in improving the performance and genetics for ruminant production. Furthermore, the high antiviral effectiveness and low cytotoxicity of IFN-τ in contrast with IFN-α has hired this cytokine in the development of possible therapeutic agent in humans and animals (*Kiladjian et al., 2016*).

ISG-15 is an IFN-τ stimulated gene, encodes for a 15 *kDa* ubiquitin-like protein (UBL) that was first identified in mouse and generated from IFN stimulated murine tumor cell RNA (*Cella et al., 2019*). It was the first ubiquitin-like modifier to be identified, initially named as a ubiquitin cross-reactive protein (UCRP) (*Lin, 2017*). This cross-reactivity is explained by the fact that ISG-15 consists of two domains, each domain stands high sequence homology to ubiquitin (addition of ubiquitin to a substrate protein is called ubiquitination). The main functions of ISG-15 are still unknown (*Bogunovic et al., 2012*). However, it is suggested that ISG-15 and the modification system have significant roles in innate immunity systems responses, interferon regulation signaling system, pregnancy, and cancer *(Xiao et al. 2018*).

ISG-15 role in pregnancy has been well studied in ruminant species. The establishment of early pregnancy in ruminants is due to IFN-τ activation *(Chandrakar et al., 2020)*. The ISG-15 expression is enhanced in the endometrium of humans, baboons (*Chandrakar et al., 2020*), in response to IFNs activated by embryos. In addition, the placenta is accumulated with macrophages which produce IFNs when activated in embryos. The expression of bovine ISG-15 is enhanced in the endometrium during early pregnancy (*Ruhmann et al., 2017*). Attack on receptive uterine epithelium by the conceptus during the origination of pregnancy makes the deicidal responses and this event is categorized by the initiation of angiogenesis and inflammation. The phenomenon that ISG-15 expression enhances during pregnancy is essential for embryo implantation and maintenance of early pregnancy *(Yaginuma et al., 2019)*. The expression profile of ISG-15 was not investigated in sheep, the present study will focus on the expression and role of the ISG-15 during peri-implantation period.

Materials and Methods

Blood was collected from sheep at the sheep resources center of The University of Agriculture, Peshawar (Pakistan), according to guidelines of the University. Twelve open Kari sheep were naturally impregnated by rams on day 0 of standing estrous cycle and four sheep were selected as control. Blood were

collected from jugular vein of all sheep at day 6, 11, 12, 13, 14, 16, 17, 21, 23 and 25 and transported to the laboratory using ice box.

Isolation of PBMC (peripheral blood monocyte) and RNA extraction

The blood samples were diluted with equal volume of PBS and the suspension was layered onto Ficoll solution, centrifuged at 6.000 g for 25 min at 4 °C. The samples were incubated for 5 min at 37 °C and then centrifuged at 300 g for 10 min. The supernatant was discarded, and the pellets were washed with 10 mL of PBS and centrifuged for 10 min at 300 g. After removal of the supernatant, the pellets were lysed with lysis buffer for protein extraction or TRIzol reagent (Life Technologies, Grand Island, NY, USA) for RNA extraction. The samples were stored at -80 °C until RNA extraction.

Real-time RT-PCR

The cDNA samples were synthesized by annealing the $oligo_{dT\text{-}18}$ primer at 72°C for 5 min followed by cooling of the samples on ice. Thereafter, 5 x reverse transcriptase (RT) buffer, 10 mM dNTPs, 20 U RNase inhibitor (Thermo Scientific) and 200 U of M-MLV reverse transcriptase (Thermo Scientific) were added to a final reaction volume of 20 mL. The samples were incubated at 42 °C for 60 min and then at 70 °C for 15 min to terminate the reaction. The amplification reactions were performed in a 20 µL reaction volume containing 1 µL of cDNA, 10 µL of dreamTaq green PCR Master Mix (2x), 7 mL of sterile water and 1 µL each of the forward and reverse gene-specific primers (10 µM). The PCR samples were then analyzed on 2 % gel. The primer sequences and GenBank accession numbers for each gene are listed in Table 1.

Statistical Analysis

The relative mRNA levels of the different genes were analyzed using latin square design (LSD) and one-way ANOVA. The effects of the different treatments and endpoints (control vs natural breeding, ISG-15 treatment, pregnancy status, days) on the expression of ISG-15, UBE1L and UBCH8 were analyzed using one-way ANOVA, SPSS (SPSS16.0, Inc.).

Table 1. The primer sequences and GenBank accession numbers for each gene
Primer pairs (F= forward; R= reverse) used for PCR

Gene symbol	Primer sequence 5'-3'	bp	Accession no
ISG-15	F: CCATGACGGTATCCGAGCTA R: GGGCCTCCCTTCAAAAGACA	317bp	NM_174366.1
UBE1L	F:GTGTTCATACCGCACGTGAC R:GGTTGTGGCAGGAATGTACC	109bp	NM_001012284.1
UBCH8	F:AGAATTCAGAAGGAACTTGCAG R:AAGGTGACCTTGGGGGGTTTA	195bp	NM_001191190.1
GAPDH	F:CTCCCAACGTGTCTGTTGTG R:TGAGCTTGACAAAGTGGTCG	222bp	NM_001034034.2

Results

Expression of ISG-15 on successive days of pregnancy

The graph indicates the expression of ISG-15 in PBMC on different days after conception (Figure 1.1). The relative expression of ISG-15 was only seen in pregnant ewes. ISG-15 expression was observed from early days of pregnancy after conceptus elongation (starting from day 11). The highest expression was observed on day 12 after insemination and was also seen on following days (day 13 till 21) while the lower expression was found on day 6 and later days (day 23 and 25). Similarly, the expression on day 16 was also significantly higher compared to day 11. After day 21 no expression of ISG-15 gene was found in pregnant ewes. The control and non-pregnant animals exhibit minimal or no expression compared to the pregnant ones.

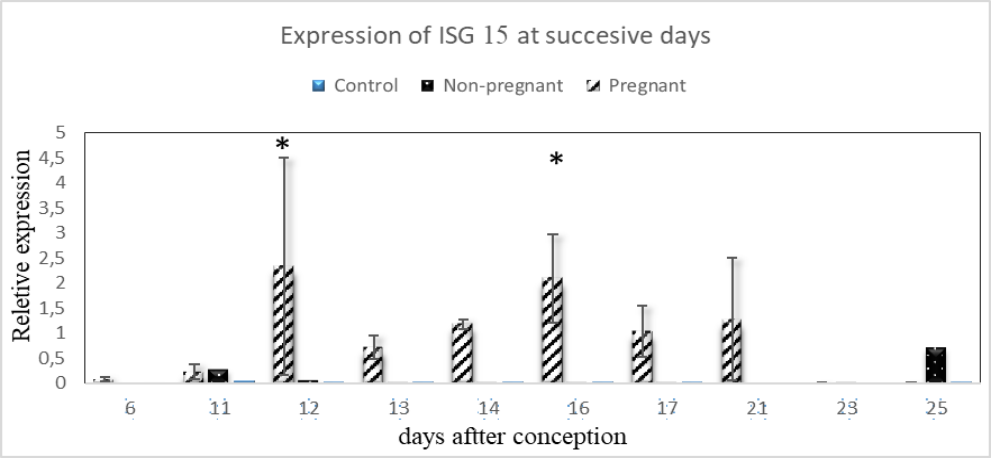

Figure 1.1 Chart of ISG-15 on important days (* indicates P < 0.05)

Expression of UBE1Lgene on successive days of pregnancy

Figure 1.2 demonstrates the UBE1L expression on progressive long stretches of pregnancy. The UBE1L expression was observed in control, pregnant and non-pregnant sheep at day 6. Generally, the expression was checked in pregnant sheep. The most surprising expression was recorded on day 6 after matting. Then expression of UBE1L was observed at day 11 followed by day 14, afterwards no expression was observed in pregnant and non-pregnant sheep.

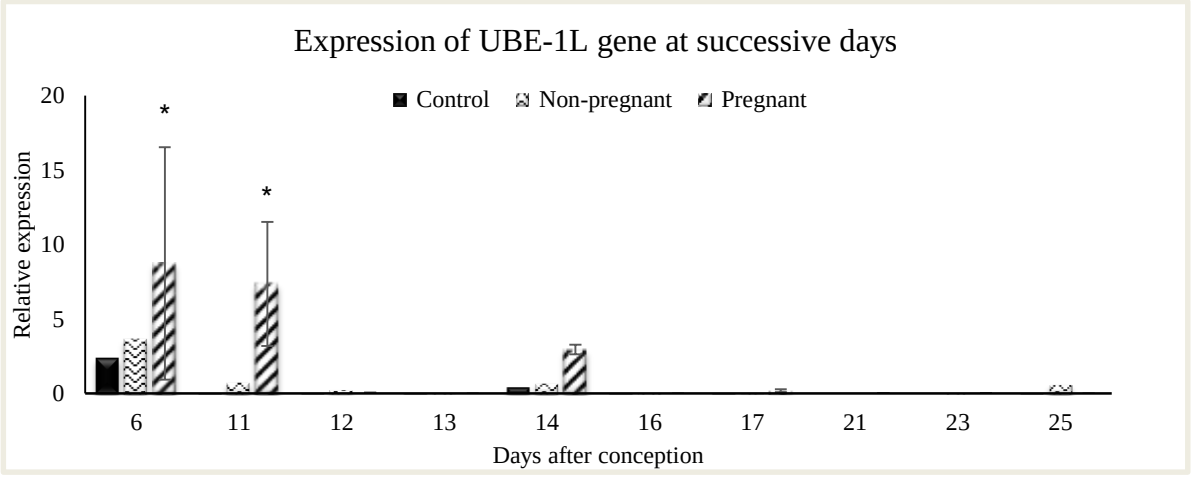

Figure 1.2 UBE1L on selected day* indicates significance difference compared to day 6, p< 0.05, * indicates significance difference compared to day 11, p< 0.05

Expression of UBCH8 gene on successive days of pregnancy

The Figure 1.3 demonstrates the UBCH8 expression in PBMC on various pregnancy days. The expression was only observed in pregnant sheep. The highest expression was observed on day 21 and moderate expression was recorded on day 17 and 23. While, no expression of UBCH8 were found in control and non-pregnant ewes.

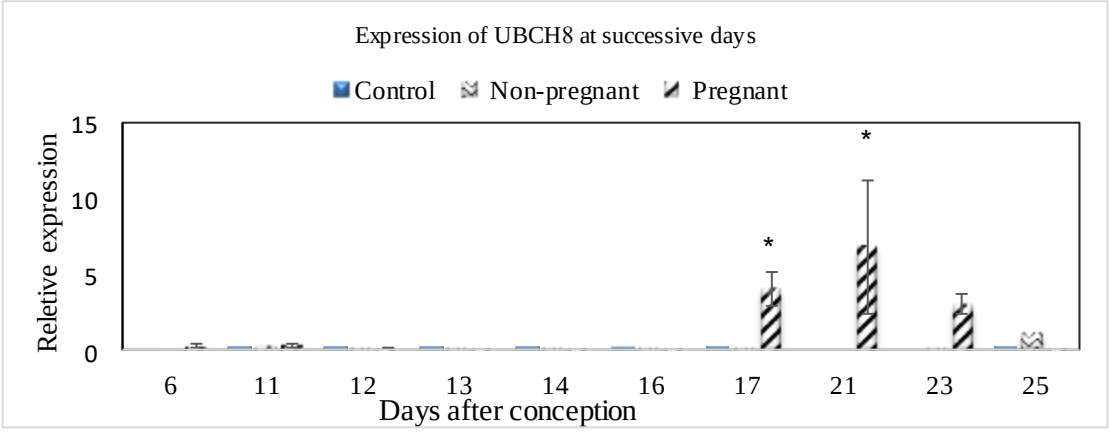

Figure 1.3 Chart of UBCH8 on important days* indicates higher expression in pregnant sheep P<0.05 while minimal expression in non-pregnant and control indicates P<0.005

Discussion

The expression of ISG-15 was observed in early day (from day 12 till 21) of the pregnancy after matting only in pregnant ewes. The expression of ISG-15 ISGylation enzyme UBE1L and UBCH8 in PBMC was observed at early days of pregnancy, in which the higher expression of UBE1L were found on day 6. However, the expression of UBE1L was observed in pregnant, non-pregnant and control sheep. The expression of UBCH8 was also found significantly different from UBE1L, which was found only in pregnant ewes. There was no expression of UBCH8 in non-pregnant and control ewes. Similar to our results, *Haq et al. (2016)* investigated this work in bovine in which they found the high expression of ISG-15 and its ISGylation enzymes UBE1L and UBCH8 in early pregnant cows. They found these genes have role in the establishment of early pregnancy in cow, and also investigated that ISG-15 conjugates to target protein through UBE1L and UBCH8 while further E3 enzyme to control pathogenicity. *Kiyma et al. (2016)* likewise explored the outflow of ISG-15 is exceptionally up-regulated in the

endometrium of early pregnant ewes and they found the high accumulation of ISG-15 on day 13 after developing life implantation. In the current study, the most noteworthy ISG-15 expression was observed on day 12 of the pregnancy, which may due to the exceptional shorter gestation length of Kari sheep *(Reagan-Shaw et al., 2008). Ling et al. (2017)* examined this work in bone marrow of ewes during early pregnancy. They investigated that ISG-15 is highly expressed in early pregnant ewes during embryo implantation. They have also shown that ISG-15 ISGylation enzymes UBE1L and UBCH8 were expressed during early pregnancy and were involved in the establishment of early pregnancy, basically they are anti-pathogenic. Similarly, this work shows the expression of ISG-15, UBE1L and UBCH8 during early days of pregnancy in Kari sheep. *Yang et al. (2010)* worked on finding ISG-15 and related proteins in bovine endometrium during early pregnancy. ISG-15 was highly expressed in early pregnant cows and the expression of ISG-15 mediates the conjugation of some genes that helped ISG-15 conjugate to target protein during embryo implantation. They also investigated that the ISGylation gene UBE1L are expressed in all cyclic and pregnant cows.

Conclusion

In the light of above findings, we concluded and suggest that ISG-15 was highly expressed in PBMC in early pregnant ewes and the ISG-15 ISGylation enzyme UBE1L and UBCH8 are involved in the establishment of early pregnancy. Furthermore, ISG-15 is ubiquitin-like protein (*Zhang et al., 2005*) stimulates its ISGylation enzyme UBE1L and UBCH8 to control pathogenicity during early implantation of embryo.

Ekspresija ISG15 i enzima za konjugaciju tokom perioda peri- implantacije kod ovaca

Shahin Shah Khan, Haidar Ali, Sher Hayat, Sohail Ahmad, Muhammad Ibrahim, Syed Adnan haider, Ijaz Ahmad, Ihtesham Ul Haq

Rezime

Uspostavljanje graviditeta kod preživara utiče na regulaciju brojnih gena. Interferonski stimulativni gen (ISG15), ubikvitinu-sličan protein koji posreduje u konjugaciji različitih proteina preko ISGilacionih enzima UBE1L i UBCH8, takođe se različito izražava tokom ranog graviditeta. Svrha ove studije bila je istražiti ulogu ISG-15 u uspostavljanju graviditeta i elongacije zametka tokom ranih perioda nakon začeća i utvrditi prisustvo ISG enzima UBE1L i UBCH8. Zbog toga

su ovce sinhronizovane korišćenjem kloprostenol natrijuma i gonadotropin oslobađajućeg hormona-1 i pripuštene ovnovima. Krv je sakupljana u danima nakon parenja 6, 11, 12, 13, 14, 16, 17, 21, 23 i 25. Prajmeri ISG-15, UBE1L i UBCH8 korišćeni su za amplifikaciju odgovarajućeg transkriptomskog regiona korišćenjem PCR. Brzina oporavka svakog transkriptomskog fragmenta je upoređena sa GAPDH. Ekspresija ISG-15 bila je veća 12. dana, suprotno od UBE1L gde je bila viša 6., a UBCH8 21. dana. Štaviše, ISG-15 je protein koji je sličan ubikvitinu, posreduje enzimima UBE1L i UBCH8 za zaštitu zametka od virusne patogenosti tokom rane trudnoće.

Ključne reči: ISG-15, ovce, graviditet, ISG, koncepcija, ubikvitinacija

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CHANGE OF ANTLERS MORPHO-METRIC PARAMETERS AND TOTAL TROPHY SCORE IN ROE DEER (*CAPREOLUS CAPREOLUS* L.) IN RELATION TO AGE

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Original scientific paper

Abstract: The aim of this study was to determine the change in the value of morpho-metric parameters of antlers and the total trophy score in relation to the age of roe deer (*Capreolus capreolus L.*). The study was conducted on 228 roe deer trophies from the "Barajevska reka" hunting ground in Serbia, aged one to seven years. From the second to the fourth year, the growth of antlers was the most intense, and the differences compared to one year old animals were significant (P <0.001). The highest average length of the branches was monitored in six years old animals (22.4 ± 2.05 cm), with significant differences (P <0.001) compared to younger animals, except for the three years old animals (P>0.05). From the second to the fifth year, a gradual increase in antler weight (from 192.2 ± 30.41 g to 221.9 ± 61.86 g) and antler volume (from 74 cm^3 to 90 cm^3) was observed, with the highest values of antler weight (291.8 ± 58.43 g and 319.1 ± 98.89 g, respectively) and antler volume (123.8 cm3 and 121.2 cm3) in six and seven year old animals. Overall trophy scores increased from year one to year seven (42.6 ± 7.86 vs 97.4 ± 27.40), with differences in trophy scores for animals aged six and seven years significantly greater than for animals aged one to five years (P <0.001). The highest quality antlers have six and seven year olds, at which time their hunting should be conducted.

Key words: *Capreolus capreolus* L., antlers, age, trophy

Introduction

According to the Law on Wild Game and Hunting ("Official Gazette of the Republic of Serbia" No. 18/2010), roe deer (*Capreolus capreolus* L.) belongs to big game species hunted only in restricted time periods during year (*Beuković and Popović, 2014*). Roe deer inhabits parts of the Asian continent and almost all of Europe, with the exception of some islands in the Mediterranean Sea and Ireland. In our country, roe deer inhabits the whole territory, from the Pannonian Plain to the mountain areas, but its abundance varies in different areas (*Gajić and Popović, 2010*).

The quality of hunting trophies is the indicator of health of population, environmental conditions in the hunting ground and breeding manipulations. Growth of the first antlers in roe deer ends with 7 – 9 months of age. After the end of the mating season and the decrease in testosterone concentration, which also coincides with the period of deposition of minerals into the bone extensions of horns, antlers gradually begin to separate from the bone extensions (*Nečas, 1972*). Healthy animals discard antlers during late autumn, after which the growth of new antlers begins immediately during winter (*Sempéré et al., 1998*). Growth and rejection of antlers is a very complex process, which comes as a result of achieving the balance between hormonal factors (*Price and Allen, 2004*), genetic factors (*Scribner et al., 1989*), environmental factors – nutrition, length of light period, conditions in the habitat and population density (*Bán and Fodor, 1982; Pélabon and Breukelen, 1998; Czyżowski et al., 2018*). Hormonal growth control is a result of the balance of testosterone, growth hormone (GH), insulin-like growth factor I (IGF-I) and thyroid hormones, as well as parathyroid and adrenal hormones that play a role in controlling the metabolism of minerals (*Bartos et al., 2012*). Genetic factors are of primary importance in the expression of antlers shape, while nutrition helps them to develop to their full genetic potential (*Harmel et al., 1988*). If sufficient quantity of good quality food is not available, smaller and narrower antlers will develop, and protein-deficient diets lead to the development of antlers with fewer branches (*Ullrey, 1983*). Roe deer in lowland habitats (agrobiotopes) consume easily digestible and energy-rich foods, which can have a direct impact on the quality of antlers (*Kruuk et al., 2002*). These individuals have faster body development and are of better body condition, so the morpho-metric elements and overall trophy score sooner culminate compared to roe deer from forest habitats (*Pételis and Brazaitis, 2003; Janiszewski et al., 2009*). Research of *Czyżowski et al. (2018)* conducted on 518 European roe deer animals (*Capreolus capreolus* L.) aged 4 to 7 years, originating from the hunting complexes in Lublin (Poland) showed that individuals from areas with denser forest cover have lower mean values of ontogenetic quality parameters (carcass weight, renal fat index, breast circumference, antler weight) compared to roe deer from typical agricultural areas.

In roe deer hunting populations, from an economic and biological point of view, it is highly important to reliably determine when males culminate in the growth of trophies and trophy values. *Hell and Holý (1988)* state that the age of roe deer at trophy shooting should not be taken uniquely for the whole country, but should be specifically determined for different areas depending on the biotopic potential of each region. The optimal management age, as an important factor in the management of roe deer populations, should be shorter for animals from agroecosystems compared to animals from typical forest habitats (*Gačić, 2006*).

Determination of roe deer trophy quality is performed according to formula suggested by *The International Council for Game and Wildlife Conservation (CIC*). The highest importance for the final trophy score, according to this formula, is given to the morpho-metric elements of assessment: length, weight and volume of antlers, while the participation of characteristics such as antlers range and external appearance makes only 12.60% of the trophy score (*Popović, 2000*).

Urosević et al. (2017) compared different systems for evaluating trophy value of roe deer and found out that there was a stronger and more pronounced correlation between antlers volume and other antler value indicators, than between antlers weight and those indicators.

The aim of this study was to investigate the influence of roe deer age on morpho-metric elements of trophy score: the length of left and right antler, average length of antler, weight and volume of antlers, as well as on total trophy score (number of achieved CIC points).

Materials and Methods

Investigation on influence of roe deer age on morpho-metric parameters of trophy included trophies from 228 animals from "Barajevska reka" hounting ground, age one (N=13/228; 5.7%), two (N=10/228; 4.4%), three (N=23/228; 10.1%), four (N=32/228; 14.0%), five (N=51/228; 22.4%), six (N=86/228; 37.7%) and seven years (N=13/228; 5.7%). Determination of age was done based on incisor and molar teeth wear (*Lehmann and Sägesser, 1986; Lochman, 1987*).

The evaluation of roe deer trophy value was performed according to formula given by *The International Council for Game and Wildlife Conservation* (*Varićak, 1998*). The length of the antlers was measured using a patch, across the middle of the outside edge of the antler, from the bottom of the rose to the top of the tine ends. To calculate the total trophy score, the length of both antlers were summed, divided by 2, and the mean obtained was then multiplied by 0.5. The weight of the antlers was expressed in grams, and the calculation of the number of points for this parameter was obtained by subtracting 65 - 90 grams from the weight of the dry trophy without lower jar (more than 3 months old) and multiplying by a factor 0.1. The volume of the antler is determined by the

hydrostatic balance, measuring the amount of water that antlers extrude from the vessel when immersed in it. The difference between the weight of the antler outside the water and the weight of the antlers immersed in water represents the volume of the antler expressed in cm^3. The resulting value is multiplied by 0.3 to get the number of points that will be used in the formula for calculating the total trophy score.

Determination of statistically significant differences between the individual parameters of the trophy score within the examined age groups for normally distributed variables (the left and right antler length, average antler length, antler weight and total number of CIC points achieved) was performed by analysis of variance (ANOVA) and Tukey tests. A non parametric Kruskal-Wallis H Test and Mann-Whitney U test with a corresponding correction for the number of comparisons were used for the variable that did not follow the normal distribution (change in antler volume depending on the age of roe deer). Significant differences were considered for $P < 0.05$. The Sperman correlation analysis examined whether there was a significant correlation between age and the morpho-metric elements of the trophy score and the total trophy score, also the corresponding regression models and equations describing the influence of age on the parameters tested were defined.

Calculations were performed with software STATISTICA 8.0. (StatSoft, Inc. 2007) and Microsoft Office EXCEL 2007.

Results and Discussion

Table 1 shows the changes in the value of the morpho-metric elements of the antlers score - lenght of left antler, length of right antler and average length in relation to the age of roe deer.

Table 1. Changes in the value of the lenght of left antler, length of right antler and average length in relation to the age of roe deer (mean±SD)

Age, years	No	LLA, cm	LRA, cm	ALA, cm
1	13	15.6±1.86^{a}	15.7±1.94^{a}	15.6±1.88^{a}
2	10	19.4±2.23^{b}	19.2±2.07^{b}	19.3±2.11^{b}
3	23	21.6±2.07^{b,v}	21.9±2.48^{b,v}	21.8±2.22^{b,v}
4	32	21.0±2.61^{b,v}	20.7±2.60^{b,v}	20.6±3.31^{b}
5	51	20.8±2.70^{b,v}	20.7±3.01^{b,g}	20.5±3.02^{b}
6	86	22.5±2.19^{v}	22.3±2.21^{v}	22.4±2.05^{v}
7	13	21.6±4.60^{b,v}	22.8±2.76^{v,g}	22.2±3.53^{b,v}

LLA – lenght of left antler; LRA – length of right antler; ALA – average length of antler
The data is shown as Mean values ± Standard Deviation
Values by letters (^{a,b,v,g}) in one column describe significant differences ($P<0.001$)

The lowest values of left and right antler lengths (15.6 ± 1.86 cm and 15.7 ± 1.94 cm), as well as average antler length (15.6 ± 1.88 cm) were observed in one year old males. The first statistically significant change in the length of the right and left, and therefore in the average length of the antlers ($P<0.001$), were observed in two years old animals. This was followed by a slight increase in length, a plateau of growth was achieved and the length of antlers did not change significantly until six years of age. The second sudden jump in antler length was observed in six years old roe deer, which corresponds to the maximum achieved average length of the antlers (22.4 ± 2.05 cm). A slight decrease in the length of the left branch (21.6 ± 4.60 cm vs 22.5 ± 2.19 cm) and average branch length (22.2 ± 3.53 cm vs 22.4 ± 2.05 cm) was observed in seven years old animals compared to six years old, but the decrease was not statistically significant ($P>0.05$). Obtained length of the branches (left and right), as well as the average length of antlers of seven years old roe deer were significantly higher only from one year old animals, and the length of the right branch compared to the value recorded in two years old animals ($P<0.001$).

Between age and investigated parameters there was a statistically significant positive correlation, showing that with age also the values of morpho-metric parameters of trophy increase. Based on calculated correlation coefficients, a medium strenght correlation was found between age of animals and lenght of left antler ($\rho=0.389$), length of right antler ($\rho=0.373$), as well as average lenght of antler ($\rho=0.383$). Regression equations describe that the influence of age on length of left antler makes 30.42% of its variability, 28.22% of variability in length of right antler, and 29.04% variability for the average length of antlers.

Observed trend of growth and decline in antler length is also in line with the results of the *Gačić (2006)* survey, which included 546 roe deer animals from 12 hunting grounds in Bačka and Banat area, caught from 1998 till 2005. This author has determined that the highest quality antlers were found in six years old animals, with some individuals reaching these values as early as the fifth year, while the length of antlers shows a gradual decline from the age of seven. *Popović and Bogdanović (2004)* also reported intense antler growth between the first and fourth year of age, and maximum antler length at six years, as well as equalization the length of antlers at eight years with the length achieved in the fourth year. The observed trend of increasing and then decreasing the length of the antlers to the age of seven obtained in this research is in correspondance with the results of *Pélabon and Breukelen (1998)*, with slightly lower average values by years. *Bán and Fodor (1982)* found that maximal lengths of antlers were evenly maintained up to the age of 10 to 13 years.

Table 2 shows the changes in the value of the morpho-metric elements of the antlers score - weight and volume of antler, as well as the total score of the trophies (number of CIC points achieved) in relation to the age of roe deer.

Table 2. Changes in the value of the weight of antler, volume of antler and total score of the trophies in relation to the age of roe deer (mean±SD)

Age, years	No	Weight, g	Volume, cm^{3*}	*CIC* points
1	13	156.6 ± 29.79^{a}	52.0 $(34.0\text{-}56.0)^{a}$	42.6 ± 7.86^{a}
2	10	$192.2\pm30.41^{a,b}$	74.0 $(62.5\text{-}83.5)^{b}$	$60.9\pm7.57^{a,b}$
3	23	225.3 ± 51.58^{b}	92.0 $(72.0\text{-}102.0)^{b}$	72.0 ± 15.00^{b}
4	32	227.0 ± 80.95^{b}	97.6 $(68.5\text{-}109.0)^{b,g}$	74.1 ± 19.10^{b}
5	51	221.9 ± 61.86^{b}	90.0 $(79.4\text{-}110.2)^{b}$	74.3 ± 16.04^{b}
6	86	291.8 ± 58.43^{v}	123.8 $(101.5\text{-}141.9)^{v,g}$	92.4 ± 16.26^{v}
7	13	319.1 ± 98.89^{v}	121.2 $(105.1\text{-}160.3)^{g}$	97.4 ± 27.40^{v}

The data is shown as Mean values ± Standard Deviation; *median (interquartile range)
Values by letters (^{a,b,v,g}) in one column describe significant differences ($P<0.001$)

The lowest measured weight of antler was observed in one year old roe deer (156.6 ± 29.79 g). Afterwards, there was a gradual increase in the weight of antler, and a significant increase was observed in three years old animals (225.3 ± 51.58 g; $P<0.001$). Another intense growth of antler weight was observed in roe deer at six years of age (291.8 ± 58.43 g), when the values recorded were significantly higher than in all younger individuals ($P<0.001$). Unlike to the length of antlers, the weight of antlers continued to increase at the age of seven, but the difference found was not statistically significant with respect to the values measured in the six year olds ($P>0.05$).

The correlation between the age of animals and antler weight was very strong, with coefficient of correlation 0.608. The simple third degree regression equation and coefficient of determination calculated by regression analysis explain that proportion of total variability in roe deer antlers weight caused by age was 29.1%.

The first significant increase in the volume of antlers was observed in two years old roe deer (74.0 cm^3). A slight increase in volume continued in the three year olds (92.0 cm^3), but with no statistical significance compared to the two year old animals. After that period, a plateau followed, i.e. slowing down in increasing trophy volume. Other intense growth was observed in males aged six years, which represented the highest measured value of antler volume (123.8 cm^3), and the value was significantly higher than in all the younger tested categories ($P<0.001$). The volume of antlers in seven years old roe deer declined (121.2 cm^3) compared to six

years old animals, and the measured values were significantly higher only than one-, two-, three- and five year old roe deer (P<0.001).

The strongest correlation was obtained between age and trophy volume (ρ=0.696). Based on fourth degree regression equation and coefficient of determination, 28,94% of changes in trophy volume could be explained by changes in age of animals.

The obtained results are in accordance with research by *Popović and Bogdanović (2004),* who found that the increase in weight (about 50 g per year) and antler volume was the most intense between the first and fourth year, and that maximum values of these parameters were reached in seven years old roe deer. Thereafter, there is a slight trend of decrease in these values. The weight of antlers in the ninth year is equal to the weight of the antlers in the fifth year, its weight in the tenth year is below the average value achieved in the fifth year, while the volume of the antlers in the eighth year is slightly above the level determined in the fifth year. *Danilkin (1999)* found that the heaviest antlers were determined in animals between the ages of four and eight, and then the volume decreases, followed by a decrease in the size and weight. *Gačić (2006)* and *Urošević et al. (2016)* also states that growth of roe deer antlers culminates in the sixth year of age. However, several authors have found that maximum values of antlers weight and volume are reached only at the age of eleven (*Bakkay et al., 1978; Bán and Fodor, 1982*). *Krapinec et al. (2014; 2019)* found that roe deer antlers in Croatia have a significantly lower weight, and therefore lower proportion of trophies compared to large number of countries (Hungary, Romania, Serbia, Switzerland and the United Kingdom), but also significantly higher than in Bosnia and Herzegovina. At the same time, animals from the studies of *Krapinec et al. (2014; 2019)* had significantly higher share of antlers in the total value of trophies than those in Slovenia and Switzerland, and lower than roe deer in Bosnia and Herzegovina, Poland, Romania, Slovakia and United Kingdom. *Balčiauskas et al. (2017)* compared the different hunting strategies of the three Baltic States and examined the impact of age-restricted roe deer hunting, conducted in Lithuania for over 40 years, on improving the value of trophy morpho-metric parameters. These authors found that the largest roe deer trophies are from Lithuania, compared to trophies from Latvia and Estonia, in which roe deer hunting is not age-restricted, they also had significantly higher weight (538.54 g vs 391.63 g and 380.97 g), volume (231.71 cm^3 vs 167.40 cm^3 and 167.06 cm^3), left antler length (26.14 cm vs 24.67 cm and 25.07 cm) and right antler length (26.59 cm vs 24.82 cm and 24.88 cm). The application of the restriction model in Lithuania has allowed the trophy value of 3 - 4 year old animals to be at the level of the most valuable mature individuals in Estonia and Latvia, despite lower availability of good quality food.

One year old roe deer trophies had the lowest grade, ie. the lowest CIC score (42.6 ± 7.86). Significant changes in the total trophy score were observed in three years and older animals, compared with one year olds (P<0.001). Intense

growth is accompanied by a plateau, i.e. by slowing the growth of the antlers, so that the total trophy ratings in animals 3 to 5 years old (from 72.0 ± 15.00 to 74.3 ± 16.04) did not differ significantly (P>0.05). The highest value was recorded for the oldest individuals caught at the age of seven, although it was only slightly different from the values achieved at the age of six. Six and seven year old roe deer trophies were considered as ones with the highest quality, and received a significantly higher overall score (92.4 ± 16.26 and 97.4 ± 27.40; P<0.001) compared to trophies of younger animals (one to five years old).

Also a strong correlation was found between age and parameters of trophy score (ρ=0.601). A more complex fourth degree regression equation and coefficient of determination are indicating that age of animals contributes 40.99% to variability in trophy score.

Obtained results are in accordance with the results of *Popović and Bogdanović (2004)*, who noted that the increase in the total trophy score was most pronounced by the age of four, and that from the fourth to sixth year, the growth was more moderate. These authors also found that maximum average value of the trophy score is reached in the seventh year, primarily due to the influence of weight and volume, whose values culminate in the seventh year, while the other parameters reach the maximum mainly in the fifth or sixth year. *Rihter (1997)* points to a large jump in trophy score between the second and third year of age, since the largest increase in the length and volume of the antlers, as well as the antler weight, appear in this period. In the study conducted by *Urošević et al. (2013)* on 66 trophies from three hunting seasons (2006/07, 2007/08 and 2008/09) from the area of Žagubica Hunting Authority, low mean CIC points were determined, ranging from 46.33 to 52.23. Five years later, *Urosević et al. (2018)* found that in 2013 and 2014, 61 animals whose trophy value also had an unsatisfactory CIC score were shot in the same hunting area. The animals were too young, about 3 years old. Such inappropriate hunting scheme did not allow the animals to develop antlers to their maximum trophy value, and a very high coefficient of variation for all observed traits indicated the inhomogeneity of the population and the necessity of planned selection procedures. The results obtained in our study are in accordance with results of *Hell and Hollý (1988)*, who reported that in lowland habitats of southwestern Slovakia the total trophy score culminates at the age of 6 years. The values found were significantly higher compared to the trophy score for the entire state, which was highest for seven years old animals. *Gačić (2006)* stated that in Vojvodina field hunting grounds, the culmination of the antler trophy value is reached in the sixth year (105.7±17.7 points for the total trophy score, 146.8±32.2 cm^3 for the volume of the antlers, 363.7±63.6 g for the antlers weight and 24.4±2.1 cm for the length of the antlers). This author has determined that individual animals can reach maximum values at four or five years, while from the age of seven, the first signs of over-aging and the decline of trophy value are observed. *Urošević et al. (2016)* founded highest trophy value for six

year old roe deer from the hunting ground in Slavonia (97.0±18.1 points for the total trophy score). Also, all parameters observed during this assessment were maximally developed (23.7±3.0 cm for lenght of left antler, 24.1±3.1 cm for length of right antler, 364.7±53.8 g for the antlers weight and 9.3±3.2 cm for inside span). *Sadiković et al. (2019)* analyzed 154 trophies from three localities in Bosnia and Herzegovina and also found that roe deer reach maximum trophy quality between the sixth and seventh year, after which this value gradually declines. The average age of hunted roe deer in this investigation was 4.4 years and the average trophy value was only 69.6 CIC points, which indicates a very low quality of trophies. The authors considered management, a premature shooting that prevented the prospective animals from reaching their body and trophy maximum, the absence of breeding and selective shooting as the reasons for such a low trophy value.

According to *Nečas (1972)*, the total trophy score culminates in the age of 5 to7 years. *Popović and Bogdanović (2004)* believe that from the view point of management, the quality of trophies is the highest between the sixth and eighth year, and that trophy shooting should not take place before the age of five. These authors stated that the increase in the overall trophy score is most pronounced by the age of four and that this fact must be taken into account in the selection of roe deer. Proper utilization of compensatory hunting model in Lithuania, banning roe deer hunting under the age of five and if the weight of antlers is less than 300 - 320 g, has led to an increase in the quality of antlers and the attainment of trophy maturity in roe deer animals five to seven years old (*Balčiauskas et al., 2017*). *Hromas (1982), Vach (1993)* and *Gačić (2006)* consider that animals with very good body condition, living in an adequate environment with the best hunting management should not be hunted before six years of age. However, reaching the culmination of the overall trophy score at seven years of age in our study is significantly earlier than results obtained by *Bakkay et al. (1978).*

Conclusion

Performed analysis of roe deer trophies show that:

- The most intense growth of antlers appears between year 2 to year 4
- The weight of trophy increases all the way up to 7th year of age
- The highest volume of antlers is achieved in the 6th year
- The total trophy score is the highest in 7th year of age. Somewhat lower score is achieved with six years, however there is no significant difference in the total trophy score between 6th and 7th year
- There is a significant correlation between age of animal and parameters of trophy score, where changes in age contribute to almost one third of changes in trophy score parameters.

Promena vrednosti mernih elemenata ocene parogova i ukupne ocene trofeja u odnosu na uzrast srndaća (*Capreolus capreolus* L.)

Zoran Popović, Vesna Davidović, Ivana Božičković, Bojan Stojanović, Bojan Ivanović, Mirjana Bojanić Rašović

Rezime

Cilj rada je bio da se utvrdi promena vrednosti mernih elemenata ocene parogova i ukupne ocene trofeja u odnosu na uzrast srndaća (*Capreolus capreolus* L.). Istraživanje je obavljeno na 228 trofeja srndaća iz lovišta "Barajevska reka" u Srbiji, uzrasta od jedne do sedam godina. Od druge do četvrte godine rast rogovlja je bio najintenzivniji, a utvrđene razlike su bile signifikantne (P<0,001) u odnosu na jednogodišnje jedinke. Najveća prosečna dužina grana parogova je zabeležena u periodu oko šeste godine (22,4±2,05 cm), pri čemu su utvrđene razlike bile signifikantne (P<0,001) u odnosu na mlađe jedinke, osim u odnosu na jedinke u starosti od tri godine (P>0,05). Od druge do pete godine uočen je postepeni porast mase rogovlja (od 192,2±30,41 g do 221,9±61,86 g) i zapremine rogovlja (od 74 cm^3 do 90 cm^3), dok su vrednosti mase rogovlja (291,8±58,43 g i 319,1±98,89 g) i zapremine rogovlja (123,8 cm^3 i 121,2 cm^3) kulminirale kod jedinki u starosti šest i sedam godina. Ukupne ocene trofeja su rasle od prve do sedme godine (42,6±7,86 prema 97,4±27,40), pri čemu su razlike u ocenama trofeja kod jedinki uzrasta šest i sedam godina bile signifikantno više u odnosu na jedinke starosti od jedne do pet godina (P<0,001). Najkvalitetnije rogovlje imaju jedinke uzrasta šest i sedam godina, kada bi trebalo i vršiti odstrel.

Ključne reči: *Capreolus capreolus* L., parogovi, starost, trofej

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DEVELOPMENT AND EFFECT OF A *LACTOBACILLUS PLANTARUM* INOCULANT ON QUALITY OF MAIZE GRAIN SILAGE

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Abstract: The main aim of these studies was the characterisation and identification of lactic acid (LAB) bacteria isolated from untreated silage, and the effect of selected bacteria (inoculant was called Silko for maize) on ensiling of maize high-moisture grain. Four isolates of LAB (L1, L2, L3 and L4) were characterised by the use of phenotypic assays and identified by phylogenetic analysis of 16S rRNA as *L. plantarum*. The fresh maize high-moisture grain was ensiled with a Silko for maize inoculant, inoculant available in the market (positive control) and no additive (untreated; negative control). After 60 days of ensiling, the results showed that the chemical composition and fermentation characteristics were better in treated silages with inoculants compared to the negative control. The contents of ash, fat and lactic acid (LA) were significantly higher in the silages treated with inoculants than in negative control. In comparison, the contents of cellulose, acid detergent fibre (ADF), neutral detergent fibre (NDF), NH_3-N/total nitrogen and butyric acids (BA) were considerably lower in silage treated with Silko for maize compared to the positive control. The Silko for maize improve nutritional value and fermentation of maize grain silage and is a competitive product on the market.

Keywords: maize, silage, inoculant, *Lactobacillus plantarum*, chemical composition, fermentation parameters

Introduction

Preparation of quality silage is crucial for the profitability of livestock farms because it is a source of food for the periods of the year when animal nutrition is inadequate in terms of quantity and quality. Maize is a vital crop for world farmers. Maize grain is used to satisfy the energy requirement of livestock. It can be ensiled and used as an animal feed ingredient. Ensiling of moist maize

grains has several advantages, such as savings on drying costs, the high nutritional value of silage, easy use. In the case of low maize grain prices on the market, the valorisation of maize is possible through animal products, and planning of the expected profit improves.

On the other hand, the preparation and storage of silage on farms is a significant problem, since it is necessary to maintain high-quality silage and achieve the maximum profitable production of milk and meat *(Aragón, 2012)*. Silage quality directly affects feed intake and utilisation of nutrients in ruminants, as well as on milk production *(Huhtanen et al., 2003)*. A decrease in pH prevented the loss of nutrients in silage due to higher lactic acid production *(Saarisalo et al., 2007)*. However, when silages are exposed to the air during the opening, it leads to increase the activity of undesirable microorganisms, and that causes the decrease of dry matter and quality *(Borreani et al., 2018)*. Also, during the ensiling process is very important that the forage mass more compacted and that the less oxygen remains in the silage. Accordingly, the inoculants are used to increase the level of lactic acid and the aerobic stability of silage over a more extended period after the opening of the silage. The use of silage inoculants (starter cultures) during silage provides a reduction in pH and the growth of undesired aerobic microorganisms *(Zielińska and Fabiszevska, 2018)*.

The biological additives that are used for conservation of silage include saprophytic, safe bacteria within the *Lactobacillus* sp, which use as consortium as multiple strains (more strains inside same species), or mixed strains including different species *(Jalč et al., 2009)*. In the world market, there are various silage bacteria inoculants for maize, among which are the frequently *Lactobacillus* sp. They are classified into two metabolic categories: homofermentative and heterofermentative bacteria *(Contreras-Gouveia and Muck, 2006)*. Homofermentative bacteria produce about 90% of lactic acid and belonging to various generations of *Lactobacillus* including the most well-known strains of *L. plantarum*. These bacteria dominate inoculants products in the world. One of the reason, because it is highly competitive with epiphytic lactic acid bacteria in silage, produces large amounts of lactic acid, reduces pH and nutritional losses *(Lynch et al., 2012; Đorđević et al., 2017)*. Also, it reduces ammonia nitrogen *(Queiroz et al., 2013)*. In general, silage treated *L. plantarum* has a better fermentation quality than in untreated silage *(Liu et al., 2016)*. However, according to *Muck (2013),* silage inoculants produced in cold regions may or may not be effective when used in hot regions.

The purpose of this study a display of development *L. plantarum* inoculant (Silko for maize) and to investigate the effect on the quality of maize grain silage. The first step in the experimental work was the isolation of a large number of bacteria from different silage samples, their phenotypic and genotypic identification at the level of the species, and prepares the bacterial consortium.

Materials and Methods

Procedure for isolation bacteria. For microbiological testing, each sample was taken, about 10 g silage in 300 ml of sterile Erlenmeyer bottle of 300 ml, and 90 ml of purified water was added and incubated with stirring at 120 rpm, 30 min *(Ekundayo, 2014)*. Subsequently, the serial decimal (from 10 times) dilution to 10^{-7} was prepared in sterile phosphate buffer, pH 7.2. After that, 100 µl of each dilution was smeared on the Man, Rogosa, Sharp (MRS) agar plate (Torlak, Serbia), and were cultivated at 37°C in the anaerobic jar (BioMerieux, France), during the 72 h. The separated colonies were inoculated on MRS agar. After cultivation, it was used for phenotypic and genotypic characterization and further checked for treatment silage. All bacterial isolates were stored at 5ºC±3ºC and subculture every two weeks. For long-term storage, stock from overnight cultures was prepared and frozen in cryoprotective agents 20% glycerol and stored at -80 °C.

Preliminary phenotypic characterization. Single, clearly separated colonies were used for morphological characterisation. Initially, preliminarily tested for Gram reaction by Gram staining and catalase enzyme. The following was done sporulation, the growth temperatures range (15°C, 30°C, 37°C and 45°C); in substrates with different osmotic pressure (2%, 4% and 6.5% (w/v) NaCl), growth in aerobic and anaerobic conditions in MRS broth, during the 72h. In the next step, selected isolates were tested by standard API 50CH test, according to the manufacturer's instructions (Bio-Merieux, Montalien-Vecien, France). All experiments were done in triplicate. The isolates were further checked for inoculant on ensiling of maize high-moisture grains.

Isolation of DNA and PCR identification. Total DNA isolation from *L. plantarum* was done with commercial kits according to the manufacturer's protocol (BIOLINE, United Kingdom). Isolated DNA from the samples was used to identify the bacteria. Using PCR, the 16s rRNA gene was amplified using universal primers 27f (AGAGTTTGATCMTGGCTCAG) and 1492 (TACGGYTACCTTGTTACGACTT). PCR reaction was carried out in a reaction mixture of 50 µl according to the manufacturer's protocol (Thermo Fisher Scientific, USA). The PCR reactions were carried out according to the following conditions:

1. One cycle of initial denaturation 95 ºC 5min,
2. 40 cycles of denaturation 95 ºC 30s, annealing 30s 53 ºC and elongation 72 ºC 1min,
3. One cycle of final extension 72 ºC 5min.

The PCR product was checked on 2% agarose gel using electrophoresis and then purified using a commercial kit (Zymo Research, Irvine, USA) and sent to

sequencing in 'MACROGEN' (Netherland) sequencing service. The sequence was bioinformatics processed using the Basic Local Alignment Search Tool (BLAST) on the NCBI.

Agar well diffusion methods. Agar-well diffusion method (AWD) was used *(Harris et al., 1989)* for detection of cross inhibition (antimicrobial activity) between alone strains of the genus *Lactobacillus*.

Preparation of silage. Maize hybrid ZP 684 (FAO 600 maturity group) was grown on a plot at the Research and Development Centre 'Agrounik' (44 ° 52 'N, 20 ° 05' E), Serbia during 2017. Preceding crop was winter wheat. Maize was sown on April 15. The sowing density was 60.000 plants ha^{-1}. The hybrid was harvested with a combine harvester in August when the grains had 26-32% moisture. The grains were ground in a mill to a 3-4 mm particle size. Approximately 100 kg was taken from the field and brought to the laboratory. Three treatments were used:

1. the untreated maize (negative control),
2. positive control (commercial inoculant added at 2 l t^{-1} of grain, contained the *L. plantarum* at total concentration 1 x 10^5 CFU g^{-1} of inoculant),
3. Silko for maize (number of colony-forming units in inoculant is 1 x 10^{10} CFU ml^{-1}; applied at a rate of 5 ml t^{-1} of grain). Maize was packed and compressed in polyethene containers volume 6l and covered with foil and a layer of sand.

Silage analysis. After 60 days of ensiling, about 450 g samples of the maize grain silage were taken from the containers for analysis. Standard methods according to *AOAC (2000)* were used to determine the contents of dry matter (DM), ash, crude fat (CF), crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF). Weende method was used to determine the content of cellulose, while method, according to *Licitra et al. (1996)* for the content of soluble nitrogen/total nitrogen. The content of NH_3-N/total nitrogen was determined using a Kjeltec System 1026. A gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) was used to determine the contents of lactic- (LA), acetic- (AA) and butyric acids (BA). The pH value was measured using an electronic digital pH meter (Hanna Instruments HI 83141 pH meter).

Statistical analysis. One-Way ANOVA was used in the analysis of experimental data, using Statistical software Statistica version 10 (StatSoft, Tulsa, Oklahoma, USA). The randomized complete block analysed the trial with three replicates. Tukey's test ($P \leq 0.05$) was used to compare the results.

Results

Preliminary phenotypic characterization. From 15 silage samples, 62 different colony morphologies were isolated. After the preliminary testing, 17 isolates of *Lactobacillus* and 2 *Pediococcus* were isolated. Four *Lactobacillus* isolates were denoted in laboratory collection bacteria as L1, L2, L3 and L4 and selected based on their high LA production. The morphological, cultural and physiological characteristics of the selected LAB performed in Table 1. According to its fermentative properties (carbohydrate substrate 49), all those which one tested *Lactobacillus* isolates showed the highest similarity to bacteria belonging to *L. planarum* / *L. pentosus*.

Table 1. Morphological, cultural and physiological characteristics selected LAB

Isolate	L1	L2	L3	L4
Gram staining	Gram-positive rods	Gram-positive rods	Gram-positive rods	Gram-positive rods
Colony morphology	White colonies convex, entire, opaque, diameter 3mm	White colonies convex, entire, opaque, diameter 3mm	White colonies convex, entire, opaque, diameter 2mm	White colonies convex, entire, opaque, diameter 3-4mm
Growth in anaerobic conditions	+	+	+	+
Growth in microaerophilic conditions	+	+	+	+
Production of gas from glucose	-	-	-	-
Catalase test	-	-	-	-
Growth temperature				
15 °C	+	+	+	+
30 °C	+	+	+	+
37 °C	+	+	+	+
40 °C	+	+	+	+
45 °C	-	-	-	-

Molecular identification. According to phenotypic and molecular characterisation (complete sequence16S rDNA isolate L1, L2, L3 and L4), the bacterial isolates were identified as *L. plantarum* and signed among our laboratory isolates as *L. plantarum* - L1, *L. plantarum* - L2, *L. plantarum* - L3 and *L. plantarum* - L4. Before forming a consortium of bacteria, we checked whether cross-inhibition occurs among the individual strains using the agar diffusion method. After 24 h cultivation bacteria, cross-inhibition between lactobacilli tested was not detected (Table 2).

Table 2. Testing of antagonism between selected strains *L. plantarum* by AWD methods

Indicator strain	Zone of inhibition growth (mm)			
	Test strain			
	L. p- L1	L. p- L2	L. p- L3	L. p- L4
L. p- L1	/	0	0	0
L. p- L2	0	/	0	0
L. p- L3	0	0	/	0
L. p- L4	0	0	0	/

Values are means of triplicate determinations with standard deviations; 0: do not zone inhibition growth; / do not test

Chemical composition of maize grain sample before ensiling. Chemical composition of maize grain sample before ensiling is shown in Table 3.

Table 3. Chemical composition of the maize sample before ensiling

Parameter	Control
Dry matter (DM) (g kg^{-1})	612.75
Ash (g kg^{-1} DM)	14.41
Crude fat (g kg^{-1} DM)	55.8
Crude protein (g kg^{-1} DM)	101.2
Cellulose (g kg^{-1} DM)	41.9
Acid detergent fibre (ADF) (g kg^{-1} DM)	39.1
Neutral detergent fibre (NDF) (g kg^{-1} DM)	240.0

Table 4. Chemical composition of maize by wet grain silage (untreated silage and silage treated with inoculants).

Parameter	Control	Positive control	Silko for maize	M	F test
Dry matter (DM) (g kg^{-1})	605.20	603.50	605.00	604.57	ns
Ash (g kg^{-1} DM)	9.33^{c}	10.29^{b}	11.29^{a}	10.30	**
Crude fat (g kg^{-1} DM)	36.39^{c}	38.96^{b}	41.08^{a}	38.81	**
Crude protein (g kg^{-1} DM)	86.44^{b}	91.51^{a}	89.53^{a}	89.16	*
Cellulose (g kg^{-1} DM)	40.96^{b}	40.34^{b}	39.38^{a}	40.23	*
Acid detergent fibre (ADF) (g kg^{-1} DM)	32.34^{c}	31.81^{b}	30.86^{a}	31.67	**
Neutral detergent fibre (NDF) (g kg^{-1} DM)	222.14^{b}	226.91^{b}	171.37^{a}	206.81	**

Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$); ** - significant at 1% level of probability, * - significant at 5% level of probability and ns - not significant.

Effect of the inoculants on maize grain silage quality. Results showed that the ash, crude fat and crude protein were significantly higher, while ADF was significantly lower in silages treated with bacteria inoculants than in control (Table

4). The cellulose and NDF were significantly lower in silage treated with Silko for maize compared to positive control and negative control. The dry matter did not differ among treatments.

Fermentation characteristics of silage are influenced by inoculants (Table 5). The values of pH, NH_3-N/total nitrogen, AC and BA were lower, while LA was higher in treatments with inoculants than in negative control. The pH and AA did not differ among positive control and Silko for maize.

Table 5. Fermentation characteristics of maize by wet grain silage (untreated silage and silage treated with inoculants).

Parameter	Control	Positive control	Silko for maize	M	F test
pH	4.27^{a}	3.77^{b}	3.83^{b}	3.96	**
NH_3 - N/TN (g kg^{-1} TN)	55.27^{a}	37.34^{b}	32.28^{c}	41.63	**
Lactic acid - LA (g kg^{-1} DM)	68.51^{c}	72.10^{b}	73.11^{a}	71.24	**
Acetic acid - AA (g kg^{-1} DM)	5.30^{b}	3.40^{a}	3.40^{a}	4.03	**
Butyric acid - BA (g kg^{-1} DM)	0.11^{c}	0.05^{b}	0.02^{a}	0.06	**

DM – dry matter; TN – total nitrogen; Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$), ** – significant at 1% level of probability.

Discussion

Based on LA production, four strains of *L. plantarum* have been selected. Due to the absence of cross-inhibition, they are selected as a consortium of bacteria. Also, strains of *Lactobacillus* belong to the GRAS (General Recognized as Safety). The use of bacterial inoculants in the initial phases of fermentation in grass silage, grass-clover, alfalfa and maize aims to decrease in pH to avoid the rapid growth of harmful microorganisms and losses of dry matter and increase aerobic silage stability *(Jatkauskas et al., 2013).* In generally, *L. plantarum* inoculants used in our study improve fermentation, promoting LA production, decrease pH, NH_3-N/total nitrogen, AA and BA acid contents in silage.

The higher ash and crude fat contents were recorded in silages treated with inoculants. The higher ash content is the result of the metabolism of inoculated strains of bacteria which using soluble components and thus increase the relative ash content *(Đorđević et al., 2017)*.

Crude protein is one of the most critical animal food quality parameters so it is crucial to maintain its high level in silage. Our results showed that the silages treated with *L. plantarum* inoculants have significantly higher crude protein content compared to control. According to *Abdul Rahman et al. (2017)*, addition of *L. plantarum* to silage increases crude protein content due to higher production of protein in the form of nitrogen content. The *L. plantarum* possess reductases that can reduce nitrates and nitrites to ammonia and other ammonia compounds *(Rooke and Hatfield, 2003)*. This contributes to the increase in total protein content,

although these compounds within total proteins mainly occur as non-protein nitrogen. Also, the low pH in treated silages inhibited protein degradation, as evidenced by research of *Vukmirović et al. (2011)*.

The lowest cellulose content was recorded in maize silage treated with Silko for maize. According to *Sadiya and Ibrahim (2015),* the *Lactobacillus* sp. produces enzymes for hydrolysis of cellulose. Therefore, we can assume that the strains of *L. plantarum* produce these enzymes, as indicated by the research of *Đorđević et al. (2017)*. Likewise, *Koc et al. (2009)* found the lowest cellulose content in sunflower silages treated with inoculant containing *L. plantarum* and *Enterococcus faecium*.

ADF and NDF were lowest in silage treated with Silko for maize. NDF did not differ between negative and positive controls. The Silko for maize increases digestibility and dry matter intake of silage and can be expected to will have a positive impact on animal performance because of the lower ADF and NDF levels in food increase animal productivity *(López et al., 2018)*.

The pH range 3.77-4.27 indicated of well-preserved silage. The inoculants promoted the silage acidification. The low pH preserves nutrients and promotes homofermentative lactic acid bacteria in silage *(Li et al., 2015)*. In essence, the low pH in silage reduced survival of yeasts, moulds and other undesirable silage microorganisms like Clostridia, prevent heating and spoilage silage and dry matter losses *(Ren et al., 2018)*.

The content of NH_3-N/total nitrogen was lowest in silage treated with Silko for maize. However, the NH_3-N content of treated and untreated silages was <100 g/kg N which suggests successful preservation. Therefore, proteins were degraded to a greater extent in untreated silage where the pH was higher than in silage treated with Silko for maize. Thus, we can be assumed that in silage treated with Silko for maize inoculant, is the higher protein content in an intact form which animals can be utilized directly. According to *Contreras-Govea et al. (2013)* silage treated with *L. plantarum* has more true protein than untreated silage, and has positive effects on milk production.

The studied silages have the higher content of LA and lower contents of AA and BA than reference values for high-quality silage (> 6.5%, < 3-4% and < 0.5%, respectively), indicating proper lactic acid fermentation. According to *Shaver (2003)*, good silage contains from 65 to 75% LA. In our research, all silages have satisfactory LA content. The highest LA and lower BA levels were recorded in silage treated with Silko for maize. The highest AA level was recorded in untreated silage. Generally, the increase in LA content in silage decreased pH and prevents secondary fermentation. The low pH inhibits clostridia growth, resulting in lower BA content in investigated silages. Consistent with our findings, *Muck (2013)* concluded that the homolactic bacteria have greater efficiency of glucose utilisation, produced higher LA in silage, reduced pH, and thus prevent undesirable microbes. According to *Kung and Shaver (2001)*, the ratio of LA to AA in the

silage should be of more than 3:1, indicating the excellent fermentation. In our case, the addition of inoculants increased this ratio compared to control silage and indicated very good fermentation.

Conclusions

Addition of Silko for maize inoculant increased LA concentration and decreased ADF and NDF, NH_3-N/total nitrogen and BA concentration compared to positive control and negative control at day 60 of fermentation. Accordingly, a consortium of bacteria belongs to *L. plantarum* to improve fermentation and preserve the nutritional value of maize grain silage.

Razvoj i uticaj *Lactobacillus plantarum* inokulanta na kvalitet silaže od zrna kukuruza

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Rezime

Cilj ovih istraživanja bio je karakterizacija i identifikacija bakterija mlečne kiseline (BMK) izolovanih iz netretirane silaže, kao i efekat odabranih bakterija (inokulant nazvan Silko za kukuruz) na siliranje vlažnog zrna kukuruza. Četiri izolata BMK (L1, L2, L3 i L4) su okarakterisani upotrebom fenotipskih testova i identifikovani filogenetskom analizom 16S rRNA kao *L. plantarum*. Vlažno zrno kukuruza silirano je sa Silkom za kukuruz, inokulantom koji je dostupan na tržištu (pozitivna kontrola) i bez primene inokulanta (netretirana; negativna kontrola). Nakon 60 dana od siliranja, rezultati su pokazali da su hemijski sastav i fermentacione karakteristike bolji u silažama tretiranim sa inokulantima u poređenju sa negativnom kontrolom. Sadržaj pepela, masti i mlečne kiseline bio je značajno veći u silažama tretiranim sa inokulantima nego u negativnoj kontroli. Sadržaj celuloze, kiselih (ADF) i neutralnih deterdžentskih vlakana (NDF), amonijačnog azota u ukupnom azotu i buterne kiseline (BA) bio je značajno niži u silaži tretiranoj sa Silkom za kukuruz nego u pozitivnoj kontroli. Silko za kukuruz poboljšava hranjivu vrednost i fermentaciju silaže od zrna kukuruza i predstavlja konkurentan proizvod na tržištu.

Ključne reči: kukuruz, silaža, inokulant, *Lactobacillus plantarum*, hemijski sastav, fermentacione karakteristike

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Milan M. Petrović[1], Stevica Aleksić[1], Milan P. Petrović[1], Milica Petrović[2], Vlada Pantelić[1], Željko Novaković[1], Dragana Ružić-Muslić[1]

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Corresponding author: Milan M.Petrović, **e-mail address**
Review paper

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EFFECTS OF REARING SYSTEM AND BODY WEIGHT OF REDBRO BROILERS ON THE FREQUENCY AND SEVERITY OF FOOTPAD DERMATITIS

Zdenka Škrbić, Zlatica Pavlovski, Miloš Lukić, Veselin Petričević

Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia
Corresponding author: Zdenka Škrbić, **e-mail address**
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