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Research article
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Effect of genotype on adipose tissue fatty acids profile of two autochthonous sheep breed

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ABSTRACT: This research aimed to investigate the influence of different sheep genotype on the fatty acid profile of fatty tissue. Three months old lambs of Somborska cigaja (SC) breed and Čokanska cigaja (ČC) breed, the two different genotypes of autochthonous sheep, were used in the experiment. The fatty acid composition was determined in lambs adipose tissue samples. A total of sixty 24-week-old lambs were reared under standard productive conditions fed with compound concentrate feed and dried grass 5 weeks. Fatty acid profile analysis of lambs adipose tissue was performed on capillary gas chromatography with an FI detector. Total values of saturated fatty acids in the lambs adipose tissue ranged from 53.70% (SC) to 54.87% (ČC) with a statistically significant difference ($P < 0.05$). In our research C18:1 fatty acid with *cis*-Δ^9 configuration show significant differences ($P < 0.05$) between these two genotypes of lambs. Results of total PUFAs in our study indicate the significant influence of genotype ($P < 0.05$) adipose fatty acid profile of investigated two autochthonous sheep breeds. The recorded concentration of total PUFAs in SC amounted to 6.15%, while in ČC that amount was 4.69% with a significant difference, respectively. The obtained ratio of total n-6/n-3 fatty acids of 1.79 (SC), is highly lower compared to 21.33 obtained from ČC breed. According to obtain results, from the healthier aspect of consumer life, and decrease the incidence of possible inflammatory processes and disease, we would be recommended meat from Sobmorska cigaja as meat with better fatty acids profile.

Key words: Fatty acids, sheep, lambs, genotype, adipose tissue

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INTRODUCTION

The consumption of saturated fatty acids in human diets has been recommended to be reduced while the consumption of polyunsaturated fatty acids (PUFAs) to be increased (Simopoulos, 2002). Moreover, the regular consumption of n-6 PUFAs (C18:2n-6) should not be changed, while the consumption of n-3 fatty acids as an α-linoleic acid (C18:3n-3), eicosapentaenoic acid (C20:5n-3), and docosahexaenoic acid (C22:6n-3) should be increased roughly for twice of their existing level (Ebrahimi et al., 2018; Puvača et al., 2019). This is caused by the connection between long-chain n-3 nutritional fatty acids and a reduced thrombotic inclination to the blood and the risk of coronary heart disease (Edwards and O'Flaherty, 2008). Ruminant meat and meat products have made a significant contribution to providing total and saturated fatty acids (SFAs) in the diets of humans (Arsenos et al., 2006). The SFAs and PUFAs ratio of sheep meat is outlined as low, but the ratio C18:2n-6/C18:3n-3 and total n-6/n-3 ratio in lamb muscle is more favourable at 1.9 and 1.3, respectively. Nevertheless, a large issue presents the high proportion of SFAs in lamb meat which led to negative preference of customers for high concentration of saturated fats in meat (Cifuni et al., 1999). Having those facts in mind, methods for a fatty acid profile modification and its ratios balancing of lamb meat should be found. Although a great deal of work has been carried out with regard to nutritional methods to improve the PUFAs of lamb meat, there was little focus on the effect of the lamb genotype on fatty acid profiles (Arsenos et al., 2006). It has been confirmed that switching the lamb breed offers the ability to manipulate the composition of the meat fatty acids profile as well. Many scientists have noted that the levels of n-3 and n-6 PUFAs of Soay lambs breed in semimembranosus muscles were greater relative to the Suffolk breed of lambs (Laborde et al., 2001). This impact may be due to leaner lamb carcasses as ruminants were shown to have PUFAs preferentially deposited in phospholipids instead of in neutral storage lipids. Meat quality and the fatty acid structure of carcass fat in meat producing animals has been described among others as one of the most significant features of meat quality, besides the proximate, technological and sensory properties which play a very important role in the attractiveness of meat to customers (Schiavon et al., 2017). In the past, significant research has been carried out regarding the structure of fatty acids in lamb fat deposits (Enser et al., 1996). Designed research on the fatty acid structure of meat and carcass lipids in small ruminants has focused on several elements of feeding, breeding, and other management methods.

As already mentioned, there has been relatively little work done to assess the effect of lambs breed on the n-3 fatty acid composition of meat and adipose tissue (Hoffman et al., 2003). A lower ratio of n-6 to n-3 fatty acids is desirable for reducing the risk of many chronic diseases that have a high prevalence in developing countries (da Costa et al., 2013). For cardiovascular diseases, a ratio of 4/1 was associated with a 70% decrease in total mortality (Johnson, 2019). A reduced risk was connected to the reduced n-6/n-3 fatty acids ratio in females with breast cancer. A 2-3/1-ratio of rheumatoid arthritis suppressed inflammation, and a 5/1 ratio had a beneficial impact on asthma while a 10/1 ratio had negative effects. Studies of Simopoulos (2002) indicates that the optimal ratio of fatty acids may vary within the diseases. This is consistent with the fact that chronic diseases are multigenic and multifactorial. The possibility is that the therapeutic dose of n-3 fatty acids will depend on the degree of severity of disease resulting from the genetic predisposition. A lower ratio of n-6/n-3 fatty acids is more desirable in reducing the risk of many chronic diseases.

The study aimed to investigate the influence of sheep breed on the fatty acid profile of adipose tissue and to give possible suggestions for potential consumers of lamb meat in their daily nutrition.

MATERIALS AND METHODS

Animals, nutrition and experimental design

All experimental procedures with lambs have been approved by the competent Veterinary Authority according to the National legislation (Presidential Decree 56/2013 on harmonization of the Directive 2010/63/EU on the protection of animals used for scientific purposes).

A total of 60 lambs were introduced in the study at the age of 24-weeks. Lambs belonged to two autochthonous breeds of sheep, which were chosen as representatives of the most common breeds of sheep in Europe, north part of Serbia. Somborska Cigaja (SC) and Čokanska Cigaja (ČC) breed with an equal number of males and females were used, respectively. Lambs were reared under standard productive conditions fed with compound concentrate feed and dried grass 5 weeks. At the end of 29 weeks of age, six lambs of each breed as a statistically appropriate sample was

randomly selected and slaughtered, while their adipose tissue was stored for further analyses. Feedstuffs, the proximate and fatty acid composition of used compound feed in lamb nutrition is shown in Table 1.

Table 1. Feedstuffs, the proximate and fatty acid composition used in lambs nutrition

Feedstuffs, g/100g		Fatty acid, g/100g DM	
Dried grass	74.0	Lauric acid, C12:0	0.05
Wheat trop	10.5	Myristic acid, C14:0	0.05
Calcium soap of palm oil	4.4	Palmitic acid, C16:0	2.20
Soybean meal	5.6	Palmitoleic acid, C16:1	0.02
Molasses	2.5	Stearic acid, C18:0	0.30
Salt	0.5	Oleic acid, C18:1	1.33
Ammonium chloride	0.5	Linoleic acid, C18:2	1.14
Premix	2.0	α-linolenic acid, C18:3	0.70
Proximate composition, g/100g DM		Eicosapentaenoic acid, C20:5	0.002
DM, g/100g	89.3	Docosahexaenoic acid, C22:6	0.001
Organic matter	87.8		
Crude protein	13.4	Total	5.79
NDF	45.9		

Sample of adipose tissue collections

After 5 weeks on the adaptation diet, six lambs within each breed were transported to the slaughterhouse, according to EU regulations (Council Regulation, EEC No 1/2005). After 12 h of fasting, the animals were electrically stunned and slaughtered according to standard commercial procedures. Lambs carcasses subsequently were cooled at 4 °C for 24 h. Adipose tissue samples (full thickness, 50 × 50 mm) were dissected from the loin of the cold carcass, after which samples were vacuumed and stored at -18 °C, for the further fatty acids analysis.

Adipose tissue fatty acid profile analysis

Briefly, the fatty acids in the lambs loin were determined following the extraction of total lipids employing accelerated solvent extraction (ASE) on Dionex ASE 200. The mixture of n-hexane and isopropanol (60:40, v/v) was used for lipid extraction at 100 °C and a nitrogen pressure of 10.3 MPa in two static cycles lasting in total 10 minutes. Fatty acid methyl esters were separated on a polar cyanopropyl aril column HP-88 (column length 100 m, diameter 0.25 mm, film thickness 0.20 μm; Agilent, Santa Clara, USA), in a programmed temperature range, on capillary gas chromatography (Shimadzu 2010; Shimadzu, Kyoto, Japan), with an FID. The temperature of the injector was 250 °C and the detector temperature was 280 °C. The carrier gas was nitrogen, of flow rate 1.33 ml min and split ratio 1:50. The injected volume was 1 μl, and the duration of analysis 50 min 30 seconds. The identification of fatty acid methyl esters was based on their retention times compared with the standard, Supelco 37 Component FAME Mix (Supelco, Bellefonte, USA). The content of each fatty acid was expressed as the percentage of the total.

Statistical analyses

Before one-way ANOVA analysis, the data were checked for normal distribution and homogeneity of variances. The data expressed as percentages were transformed with the arcsine transformation. The mean values were compared by the Tukey HSD post hoc test. The results were presented as means ± SD, where the significance level of $P < 0.05$ was used. For statistical analysis statistical software Statistica 13 (TIBCO Software Inc., USA) was used.

RESULTS AND DISCUSSION

The obtained values of SFAs of both genotypes lambs adipose tissue are shown in Table 2. Total values of saturated fatty acids in the lambs tissue ranged from 53.70% (SC) to 54.87% (ČC) with a statistically significant difference ($P < 0.05$). The highest share of C16:0 (Palmitic acid) was recorded for both lambs breed (22.40 and 23.67%) with significant differences ($P < 0.05$). From the presented results it can be noticed that the higher share of SFAs is present in adipose tissue of Čokanska cigaja (ČC) breed of lambs. Significant differences between both lambs breed were not recorded ($P > 0.05$) regarding the only two SFAs C18:0 (Stearic acid) and C20:0 (Arachidic acid), while all other detected fatty acid showed significant differences between each other. Wachira et al. (2002) have shown similar results in their investigation which had the aim to investigate the influence of

nutrition with different fatty acids sources and breed. Their results show a significant influence of breed in C18:0 SFA which have ranged from 14.67% for Friesland breed to 15.07% for Soay breed, while nutrition didn't show any significant influence. The long-chain C20 fatty acids were present at very low levels in the subcutaneous adipose tissue of lambs in our research. This could be due to the small percentage of adipose tissue phospholipids as well as the small incorporation in ruminants of long-chain fatty acids into the triacylglycerol portion (Enser et al., 1996). As far as the fatty acid composition in lamb fat is concerned, our findings indicate that breed is very significant. The lambs breed affected most of the fatty acids profiles studied throughout the years in other experiments as well (Arsenos et al., 2006).

Table 2. Adipose tissue saturated fatty acids profile of lambs, %

Fatty acids	SC	ČC	*p*-value
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	
C8:0	4.68^{a} ± 0.632	3.48^{b} ± 0.705	0.004
C10:0	0.24^{b} ± 0.062	0.43^{a} ± 0.176	0.000
C14:0	3.61^{b} ± 1.393	4.94^{a} ± 1.380	0.001
C15:0	0.79^{b} ± 0.151	0.96^{a} ± 0.054	0.010
C16:0	22.40^{b} ± 0.801	23.67^{a} ± 0.404	0.001
C17:0	1.12^{a} ± 0.086	0.99^{b} ± 0.024	0.002
C18:0	20.60^{a} ± 1.780	19.87^{a} ± 1.53	0.332
C20:0	0.17^{a} ± 0.050	0.19^{a} ± 0.028	0.186
Total	53.70^{b} ± 0.610	54.87^{a} ± 0.530	0.009
Pooled SE	0.572	0.776	

Means in the same column with a common superscript letter are not significantly different ($P < 0.05$)
$\bar{x}$ - mean value of six replicates; SD – standard deviation; CV – coefficient of variation; SE – standard error

Besides our findings with two autochthonous breeds of lambs, the significant effect of breed on fatty acids composition in fat depots of suckled lambs from two dairy breeds was also reported (Cifuni et al., 1999; Hoffman et al., 2003). Also, it has been highlighted that sheep breed effects on fatty acid composition should be assessed at the same degree of maturity and fatness (da Costa et al., 2013; Hanuš et al., 2018; Laborde et al., 2001). Research of Arsenos et al. (2006) has shown that there are no significant differences between male and female lambs in the fatty acid composition of their carcass fat, except for C18:0 and C18:2 (Linoleic acid) fatty acids, what led to a conclusion that the effect of sex is generally very small and neglectable.

The results of monounsaturated fatty acids (MUFAs) obtain in our research have been shown in Table 3. Results of *cis* configuration (Δ^{9}, Δ^{13}, $\Delta^{5,8,11,14}$) of MUFAs with the two hydrogen atoms adjacent to the double bond stick out on the same side of the chain, show statistically significant ($P < 0.05$) differences in total MUFAs identified in our research. Significantly higher ($P < 0.05$) concentration of C16:1 (Palmitoleic acid) in SC lambs breed (1.68%) was recorded compared to ČC lambs breed (1.43%). The same significant tendency in C18:1 (Oleic acid) was maintained between investigated adipose tissue fatty acids in the experiment. On the other hand, a significant difference was not present ($P > 0.05$) considering the detected concentrations of C22:1 (Erucic acid) and C20:4 (Arachidonic acid) between investigated lambs (1.32 and 1.16%).

Table 3. Adipose tissue monounsaturated fatty acids profile of lambs, %

Fatty acid	SC	ČC	*p*-value
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	
C16:1n-7	1.68^{a} ± 0.294	1.43^{b} ± 0.060	0.037
C18:1n-9	33.10^{a} ± 2.472	29.46^{b} ± 1.4771	0.001
C22:1n-9 C20:4n-6	1.32^{a} ± 0.384	1.16^{a} ± 0.276	0.386
Total	36.15^{a} ± 0.788	32.05^{b} ± 0.600	0.004
Pooled SE	0.022	0.051	

Means in the same column with a common superscript letter are not significantly different ($P < 0.05$)
$\bar{x}$ - mean value of six replicates; SD – standard deviation; CV – coefficient of variation; SE – standard error

In research of Wachira et al. (2002) total muscle fatty acid concentration ranged from 3.66 to 2.90% with no significant effect of lambs breed, which is contrary to the results obtained from our research with adipose tissue of lambs. Further, in the dietary trial Wachira et al. (2002), have confirmed that lambs on the control diet, compared to lambs on the diet with flaxseed addition had higher concentrations of C18:1 fatty acid. Puvača et al. (2014) have shown that with the use of regression models is possible to predict deposition, e.g., concentrations of fatty acids in edible tissue of animals. In our research C18:1 fatty acid with *cis*-Δ^9 configuration show significant differences ($P < 0.05$) between two breeds, while in the research of Wachira et al. (2002), C18:1 fatty acid with *trans*-Δ^9 configuration (Elaidic acid) didn't show significant differences of this fatty acid in muscle tissue between different lamb breeds. Arsenos et al. (2006) showed no significant differences in fatty acids composition of different lambs breed fed with low and high concentrations of concentrate in the diet. Concentrations of C16:1 for low diet were in the range of 3.5 and 3.4% for Boutsko, Serres and Karagouniko indigenous Greeks breed, while the concentration of C18:1 in the high and low diet was higher and uniform for all mentioned breeds (41.9; 41.0 and 39.7%), respectively. Similarly to lambs, in other animal species such as fish, the same tendency was observed when fish fed with lower diets (Ljubojević et al., 2015). Gravador et al. (2018) have shown a significant difference in fatty acids profile in lambs of two different breeds after castration. In castrated lambs of Scottish Blackface and Texel × Scottish Blackface lambs breed significant increase in MUFAs and decrease in PUFAs was recorded.

Results given in Table 4 show a significant difference ($P < 0.05$) and the influence of sheep genotype on PUFAs profile of both investigated Somborska cigaja breed and Čokanska cigaja breed. The concentration of C18:2 (Linoleic acid) didn't show any significant differences ($P > 0.05$) between investigated breeds of lambs, with the equal presented amount of 3.83 and 3.84%, respectively. Opposite to C18:2 concentration in adipose tissue, the concentration of C18:3 PUFA (α-linolenic acid) showed significant ($P < 0.05$) influence of lambs breed with the recorded amount of 1.27% (SC) and 0.49% (ČC), respectively. The same tendency as for the C18:2 was observed for concentrations of C20:5 (Eicosapentaenoic acid) without the presence of significant differences ($P > 0.05$). The interesting results obtained in this study is a significant increase and difference ($P < 0.05$) in C22:5 (Docosapentaenoic acid). Adipose tissue of Somborska cigaja breed has recorded a significantly higher concentration of docosapentaenoic fatty acid (0.66%) compared to the concentration of the same fatty acid (0.17%) in adipose tissue of Čokanska cigaja breed ($P < 0.05$). Docosapentaenoic acid is an n-3 fatty acid that is structurally similar to eicosapentaenoic acid with the same number of double bonds, but two more carbon chain units (Yazdi, 2013). Docosapentaenoic acid designates any straight-chain C22:5 fatty acid, which is primarily used to designate two isomers, already known as n-6 and n-3. These designations describe the position of the double bond is 6 or 3 carbons closest to the carbon at the methyl end of the molecule and is based on the biologically important difference that n-6 and n-3 PUFAs are separate PUFAs classes, the n-6 fatty acids, and n-3 fatty acids, respectively (Edwards and O'Flaherty, 2008). Mammals, including humans, cannot interconvert these two classes and therefore must obtain dietary essential PUFAs from both classes to maintain normal health (Spector and Kim, 2015).

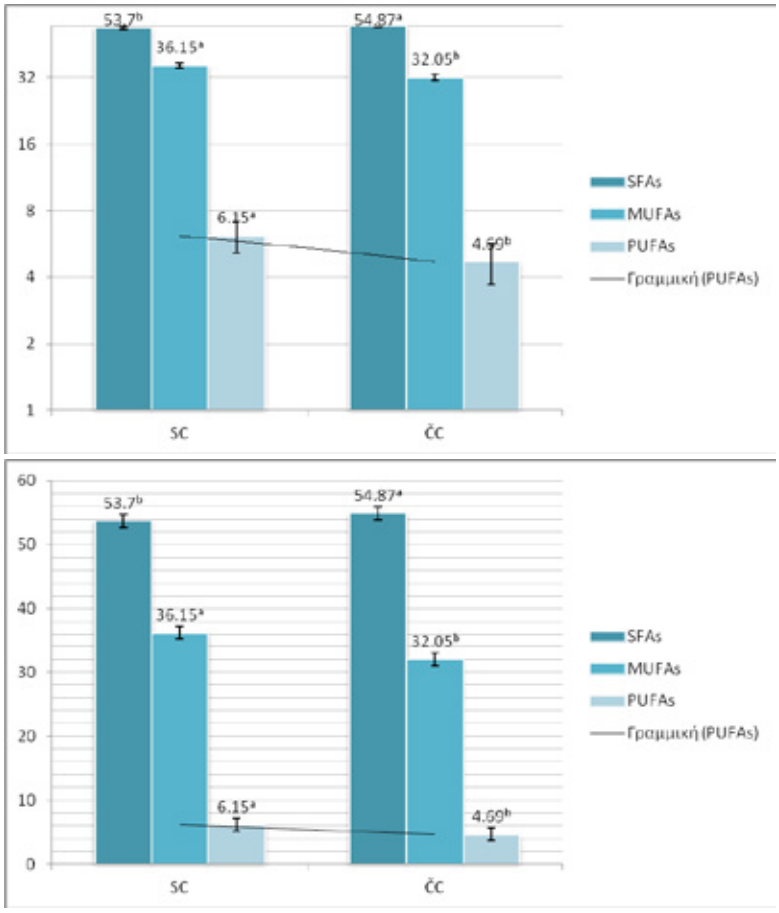
Table 4. Adipose tissue polyunsaturated fatty acids profile of lambs, %

Fatty acid	**SC**	**ČC**	*p*-value
	$\bar{x}$ ± SD	$\bar{x}$ ± SD	
C18:2n-6	3.83^a ± 0.579	3.84^a ± 1.071	0.996
C18:3n-3	1.27^a ± 0.157	0.49^b ± 0.138	0.000
C20:5n-3	0.20^a ± 0.058	0.15^a ± 0.039	0.426
C22:5n-3	0.66^a ± 0.139	0.17^b ± 0.065	0.000
Total	6.15^a ± 0.150	4.69^b ± 0.328	0.040
Pooled SE	0.048	0.092	

Means in the same column with a common superscript letter are not significantly different ($P < 0.05$)
$\bar{x}$ - mean value of six replicates; SD – standard deviation; CV – coefficient of variation; SE – standard error

Results of total PUFAs in our study indicate the significant influence of genotype ($P < 0.05$) adipose fatty acid profile of investigated two autochthonous sheep breeds. The recorded concentration of total PUFAs in SC amounted to 6.15%, while in ČC that amount was 4.69% with a significant difference, respectively. Similar results were obtained in the research of Ebrahimi et al. (2018) with the different goats genotypes as well in the research of Obućinski et al. (2019) with different cows breeds. Schiavon et al. (2017) in their research with dietary rumen-protected conjugated linoleic acid didn't record any significant influence of PUFAs changes in adipose and liver tissues, but the influence of breed could not be investigated in these research having in mind that experiment was conducted on one lamb genotype. Contrary to our results for Somborska cigaja and Čokanska cigaja, the research of Wachira et al. (2002) showed a similar proportion of the longer-chain n-3 PUFAs in all three investigated breeds; Suffolk, Soay, and Friesland. These results could be explained as the genotype difference between all five investigated breeds. Besides the genotype influence on adipose fatty acid profiles of sheep, investigations were conducted in a way of investigation the sheep milk quality as well (Skoufos et al., 2018). Wachira et al. (2002) indicated the presence of significant interaction between lambs breed and feed for the total content of fatty acids in their research. The same research revealed that Friesland lambs breed were observed to have the greatest content of fatty acids in the adipose followed by Soay lambs breed, while the Suffolk lambs breed had the greatest content when fed regular daily diet.

From Figure 1 it can be easily seen the share of total SFAs, MUFAs, and PUFAs in adipose tissue of lambs obtain in our study. Highest share takes the SFAa, then MUFAs, and at the end PUFAs. The linear trendline shows that lambs of genotype Somborska cigaja have recorded significantly ($P < 0.05$) higher share of total PUFAs (6.15%), compared to total PUFAs obtained from adipose tissue of Čokanska cigaja genotype (4.69%).



Means in the same column with a common superscript letter are not significantly different ($P < 0.05$)

Figure 1. Share of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) in adipose tissue of lambs, %

The ratio of total n-6 to total n-3 is not very useful (Ljubojević et al., 2015). Based on the gain results in our study the ratio of total n-6/n-3 fatty acids in Somborska cigaja breed is 1.79, which is highly lower compared to a ratio of these fatty acids obtained from breed Čokanska cigaja which is 21.33 (Table 5). The ratio of linoleic acid to α-linolenic acid is of important value, since these compete for processing by delta 6 desaturase, and should be consumed in a balanced way. Again the ratio of arachidonic acid to eicosapentaenoic acid is important as too high a ratio could promote different inflammation processes in the body (Ljubojević et al., 2015; Puvača et al., 2014). Regarding the ratio of C18:2n-6 and C18:3n-3 fatty acids in our study, the tendency is similar to the previous ratio, but with the values of 3.07/7.84 (SC/ČC).

Table 5. The ratio of selected fatty acids from adipose tissue of lambs

Fatty acid/Ratio	**SC**	**ČC**
	$\bar{x}$	$\bar{x}$
Total n-6	3.83	3.84
Total n-3	2.13	0.18
n-6/n-3	1.79	21.33
C18:2n-6	3.83	3.84
C18:3n-3	1.27	0.49
C18:2/C18:3	3.07	7.84
C20:4n-6	1.32	1.16
C20:5n-3	0.20	0.15
C20:4/C20:5	6.60	7.73

The recorded ratio between PUFAs C20:4n-6 (Arachidonic acid) and C20:5n-3 (Eicosapentaenoic acid) in our study was 6.60/7.73 (SC/ČC). On the other hand, according to Simopoulos (2002), human beings evolved on a diet with a ratio of n-6 to n-3 essential fatty acids of approximately 1 whereas in Western diets the ratio is 15/1-16.7/1. Diets which are deficient in n-3 fatty acids, with increased amounts of n-6 fatty acids, e.g., a very high n-6/n-3 ratio, is found to promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of n-3 PUFAs exert suppressive effects (Simopoulos, 2002).

CONCLUSIONS

Based on our findings, it can be concluded with certainty that the genotype of investigated sheep breeds Somborska cigaja and Čokanska cigaja have a significant influence on the fatty acid composition of adipose tissue. Obtained results indicate that Somborska cigaja compared to Čokanska cigaja have a higher share of MUFAs and PUFAs in adipose tissue, as well a much better ratio of total n-6/n-3; C18:2/C18:3 and C20:4/C20:5 fatty acids. According to obtain results, from the healthier aspect of consumer life, and decrease the incidence of possible inflammatory processes and disease, we would be recommended meat from Sobmorska cigaja as meat with better fatty acids profile. Nevertheless, the further investigation related to fatty acids profile of lambs influenced by breed, sex, and nutrition is more than necessary.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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