THE EFFECTS OF BREED ON CONJUGATED LINOLEIC ACID (CLA) AND OTHER FUNCTIONAL LIPID COMPONENTS OF SHEEP MEAT AND ADIPOUSE TISSUE

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Abstract

This study was conducted in order to evaluate the influence of race on content of functional fatty acids (Omega-3, conjugated linoleic acid-CLA, trans-Vaccenic acid-VA) in lamb carcass fat, in similar feeding. In this regard, the research was carried out on three local breeds which are better represented in the northwest of the country: Turcana, Tigaie and Transylvanian Merino. In the case of Tigaie breed we monitored the impact of fat type (intramuscular vs. fat deposit), and the body part for harvesting purposes (longissimus dorsi vs. biceps femoris and subcutaneous fat vs. perirenal fat). Livestock were sacrificed at the end of the 60 days fattening period, ant it was set the fatty acid composition of the intramuscular fat in longissimus dorsi muscle. Significant differences were detected in the acid content of polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) of intramuscular fat in favor of Tigaie breed. Differences were noticed between breeds in terms of content in Omega-3 FA, and in the content of conjugated linoleic acid (CLA), with higher levels in Tigaie breed lambs as against the other two breeds studied. The share of vaccenic acid (C18:1 trans-11) in the intramuscular fat structure was not influenced by breed, this FA being an intermediate product of ruminal biohydrogenation of PUFA in food. In general, the findings showed that intramuscular fat in Tigaie breed lambs were healthier for human consumption due to low content in saturated fatty acids (SFA), and higher in functional fatty acids, i.e. FA n-3 and CLA which suggest that the Tigaie breed could be used to produce sanogeneous sheep meat.

Key words: Breed, type of tissue, Omega-3 FA, CLA, Atherogenicitiy index

INTRODUCTION

Conjugated Linoleic Acid (CLA) is a general term used to define more geometric and positional isomers of octadecadienoic acid containing a pair of double chains in a conjugated configuration. In experimental livestock models it has been shown that CLA, but especially the cis-9, trans-11 CLA (also called rumenic acid) has beneficial effects in the human body, reducing carcinogenesis, atherosclerosis, cholesterol, diabetes and the weight of body fat (Belury 2002; Ip et al., 1999; Lee et al., 1994; Parodi, 1997). The main source of CLA for human body and especially in rumen acid which represents 80-90% of the total CLA isomers is the food derived from ruminants (milk, dairy products and meat). The rumenic acid (RA, cis-9, trans-11 CLA) is formed as an intermediate product during ruminal biohydrogenation of linoleic acid in the food and its transformation into stearic acid (C18:0), or is formed by the endogenous synthesis in the body tissue, using as the vaccenic acid (VA, C18: 1 trans-11) as substrate, which

is also formed during the process of ruminal biohydrogenation of the fatty acids in the diet (Grinari and Bouman, 1999).

In human nutrition, along with CLA and VA, two other fatty acids are considered important for health, i.e. linoleic acid (C18:2n-6, LA) and α-linolenic acid (C18:3n-3, ALA). These essential FA have an important role in the prevention and control of cardiovascular diseases, the development and proper functioning of the nervous system, prevention of rheumatoid arthritis, but they also serve as precursors to a number of other bioactive compounds which are important to the health of consumers. Thus, the α-linolenic acid is a precursor for other fatty acids of the Omega-3 series (EPA-ecosapentaenoic acid, C20:5n-3 and DPA-docohexaenoic acid, C22:6n-3), with the participation of specific desaturases.

The share and proportion of fatty acids in animal fats are determined primarily by nutritional factors, but they may be influenced by a number of genetic (breed) or physiological factors (body weight and slaughter age, sex, type of fat: intramuscular or deposit region, body region, etc.) (Wood et al., 2008). Breed may influence the fatty acid profile of intramuscular and deposit fat in ruminants. This finding was not confirmed by Radzik-Rant et al. (2014) who found that breed (genotype) did influence the concentration of MUFA (monounsaturated fatty acids) in intramuscular fat of lambs and not that of PUFA, as expected. The authors have reached the conclusion that primitive breeds deposit less intramuscular fat and the fatty acid profile is more favourable to consumer health compared with improved genotypes which deposit more fat in the carcass, the latter being richer in MUFA and SFA.

The main purpose of this research was to investigate the relations between some internal animal dependent factors (i.e.breed) and the functional content of fatty acids (i.e. Omega-3, CLA and VA) of fat in sheep carcass. In this respect research was carried out on three local breeds which are better represented in the northwest of the country: Turcană, Tigaie and Transylvanian Merino. In the case of Tigaie breed one studied the influence of the fat type of (intramuscular vs. fat deposit), but also the harvesting regions (*longissimus dorsi* vs. *biceps femoris* and subcutaneous fat vs. perirenal adipouse tissue).

MATERIAL AND METHOD

In the case of sampling (muscle and adipouse tissue) for analysis purposes there were used 24 samples of young lambs from commercial farms, distributed as follows: four rams and four females for each of the three breeds studied (Tigaie - Ti, Turcană – Tu, and Transylvanian Merino-TM). 60 days prior to sampling for analysis and slaughter purposes, respectively, in the diet of animals, there were used fodder uniform structure

based on natural hay and combined fodder while observing the specific nutritional requirements. Hay was provided *ad libitum* and fodder combined were administered twice a day i.e. 400 g per capita. Thus it was eliminated the influence of food on the fatty acid profile of the carcass.

Slaughtering for control purposes was preceded by a 12 hours diet and sampling for analysis purposes from *Longissimus dorsi* muscle, *biceps femoris* and perirenal and subcutaneous adipouse tissue was made the next day of slaughtering after carcass drying (24 hours at 4°C). In order to establish the fatty acid profile of the carcass, sampling of individual muscle (*longissimus dorsi* - lower back and *biceps femoris*) and adipouse tissues (subcutaneous - lower back and perirenal) was performed. The samples were individually packed in plastic bags and stored at -20°C before analysis.

Extraction of lipids was carried out with a mixture of chloroform: methanol (2:1 vol./vol.), and the fatty acids methyl esters (FAME) were prepared using boron trifluoride (BF3) and methanol (14% w/w), as described by Watkins et. al. (1997). In order to quantify the isomer of conjugated linoleic acid (CLA), lipids extracted from tissue samples were methylated (sodium methoxide) based on the procedure described by Li and Watkins (1998). The lipids were dissolved in toluene (1ml) in a Teflon tube with a screw cap. FAME were identified using HP 5890 gas chromatograph equipped with a 30 m length DB23 column. Gas chromatograph was run at 140°C for 2 min., the temperature increasing by 1.5°C/min. up to 198°C and then held for 7 minutes. CLA isomers were further analyzed using a 100 m length SP2560 capillary GC column. For each operation with gas, the chromatography, injector and detector with flame ionization showed temperatures ranging between 225°C and 250°C. FAME were identified by comparing their retention time against the standard.

All laboratory test results were statistically processed and interpreted, using the SAS procedure (version 8.0; SAS Inst., Inc., Cary, NC) for repeated measurements and the 't' test. Differences were considered significant for $p < 0.05$.

RESULTS AND DISSCUSIONS

The effect of breed on fatty acid profile was set for intramuscular fat in *longissimus dorsi* muscle (LD). TM lambs breed meat had an intramuscular fat content almost doubled compared to that of Ti breed which recorded the lowest share of fat in LD. Similar results were obtained by Borys et al. (2005)in the case of primitive breeds compared with the improved breeds, but they were not confirmed by Martinez-Cereso et al. (2005) who found no differences between different genotypes in terms of LD muscle fat content. The amount of cholesterol per 100 g intramuscular

fat was not influenced by race, being highlighted, however, the lower share recorded in Ti breed (see Table 1 below).

The predominant fatty acids in the intramuscular fat all three breeds were as follows: palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1 cis).

Among saturated fatty acids (SFA), the largest concentration of palmitic acid and stearic acid in intramuscular fat was registered in TM breed lambs. The total content of SFA was lower in intramuscular fat in breeds with a low degree of improvement i.e. Ti and Tu, no significant differences between them being recorded.

Out of the total monounsaturated fatty acids (MUFA) in all three races, the oleic acid represented more than 90%, the highest concentration in intramuscular fat composition being recorded in the TM breed. However, among the three races there were no significant differences in the total MUFA content in intramuscular fat. In contrast to our results, Radzik-Rant et al. (2014) found that breed (genotype) influenced the concentration of MUFA (monounsaturated fatty acids) in intramuscular fat of lambs and did not affect the PUFA content, as expected.

The data showed in Table 1 show that, out of the three breeds studied (i.e. Transylvanian Merino-TM, Turcană-Tu, Tigaie-Ti), the highest share of fatty acids function (n-3 FA and CLA) in *longissimus dorsi* muscle was recorded in Ti breed while Turcana breed was placed the lowest in his ranking. In the case of Transylvanian Merino, n-3 PUFA and CLA values were very close to those found in Tigaie breed, but in the case of TM breed a higher content of saturated fatty acids (47.54% vs. 43.97% of FAME) was recorded. The content of fat in intramuscular trans-vaccenic acid (VA, C18:1 trans-11) was not influenced by the breed, this being a fatty acid generated during ruminal biohydrogenation of polyunsaturated fatty acids, in particular the linoleic (C18:2n-6) and linolenic (C18:3n-3) acids in the diet (Chilliard et al., 2003). The high concentration of C18:2n-6 and C18:3n-3 and the low concentration of palmitic acid (C16:0) in the composition of intramuscular fat in the Tsigai breed resulted in a PUFA/SFA ratio which is more favourable to the health of consumers.

The high content of n-6 PUFA in Tsigai breed is due largely to the high content of linoleic acid in intramuscular fat. Moreover, in the case of this breed, the high content of C18:2n-6 improved the synthesis of arachidonic acid (C20:4n-6) in the intramuscular fat. These findings on the content of n-6 PUFA in the intramuscular fat in the Tigaie breed are similar to those reported by Fisher et al. (2000) for the Soay breed lambs when compared to the Suffolk breed lambs showed higher concentrations of n-6 PUFA in the semimembranosus muscle.

Table 1

The influence of breed on fatty acid composition and sanogeneous lipid indices of intramuscular fat (*longissimus dorsi*)

	Transylvanian Merino-TM	Turcana-Tu	Tsigai-Ti
Total lipids g/100g	6,71[b]	5,17[ab]	3,76[a]
Cholesterol mg/100g	63,6	62,4	61,2
Fatty acids profile (% din FAME)			
C12:0	2,17	1,95	1,81
C 14:0	5,04	4,90	5,13
C 16:0	18,67[b]	16,64[a]	16,79[a]
C 18:0	23,83	22,91	22,05
C 16:1	4,59[b]	3,36[a]	3,22[a]
C 18:1 cis [1]	27,62[b]	25,41[a]	25,06[a]
C 18:1 trans-11 (VA)	3,33	3,68	3,69
C 18:2 n-6 trans	3,29[ab]	2,91[a]	3,74[b]
C 18:2 n-6 cis	4,80	4,28	4,95
C 18:2 c9, t11	0,89[a]	1,04[a]	1,94[b]
C 18:2 t11, t13	0,15	0,18	0,21
C 18:2 t10, c12	0,35	0,29	0,23
C 18:3 c9, c12, c15 n-3	2,88[a]	2,80[a]	3,79[b]
C 20:2 n-6	1,81[a]	2,27[b]	1,68[a]
C 20:4 n-6	2,92[b]	2,18[a]	3,04[b]
C 20:5 n-3 EPA	1,88[ab]	1,42[a]	2,01[b]
C 22:3 n-3	0,36	0,36	0,41
C 22:5 n-3 DPA	0,33	0,35	0,33
C 22:6 n-3 DHA	1,58[ab]	1,04[a]	1,90[b]
SFA	47.54[b]	44.45[a]	43.97[a]
MUFA	30.95[b]	29.09[ab]	28.75[a]
PUFA	19,85[b]	17,61[a]	22,25[b]
PUFA n-6	12,82[b]	11,64[a]	13,41[b]
PUFA n-3	7,03[b]	5,97[a]	8,84[b]
CLA – total	1,39[a]	1,51[a]	2,38[b]
Health lipid indices			
PUFA/SFA	0,42[a]	0,40[a]	0,51[b]
n-6/n-3 PUFA	1,82[b]	1,95[b]	1,52[a]
HFA	25,88[b]	23,49[a]	23,73[a]
h/H	1,96	1,99	2,15
AI	0,81[b]	0,82[b]	0,76[a]
TI	1,03[b]	1,07[b]	0,84[a]
DI (18:2 c9,t11)	21,08[a]	22,03[a]	34,47[b]

[1] C 18:1 cis = C 18:1 cis 9 + C 18:1 cis 11 + C 18:1 cis 12; AI: indexul aterogenic (AI = (C12:0+(C14:0x4)+C16:0)/UFA); TI: indexul trombogenic (12:0+16:0+18:0)/[(0.5×MUFA)+(0.5×n-6PUFA)+(3×n-3PUFA)+(n-3PUFA/n-6PUFA)]; VA: acidul trans-vaccenic (C18:1 t11); CLA: acidul linoleic conjugat (izomerul C18:2 c9,t11); h/H: raportul acizilor grasi hipocolesterolemianti/hipercolesterolemianti (C18:1+PUFA)/(C12:0+C14:0+C16:0; DI (18:2 c9,t11): indexul Δ^9-desaturaza (18: c9,t11)=100(18:2 c9,t11/(18:2 c9,t11 + 18:1 t11)).

This finding may result from the low concentration of total lipids in muscle and a higher proportion of phospholipids in total fat structure. Various amounts of Omega-3 intramuscular fat in the lambs of the three breeds studied indicate the presence of different amounts of phospholipids in the muscles and a different metabolism of linolenic acid (C18:3n-3) to produce the long-chain fatty acids of the n-3 FA series.

Intramuscular fat had a higher content of α-linolenic acid (C18: 3n-3) in the case of the Ti breed compared to the other two breeds (3.79 vs 2.80-2.88%). Similar findings were noticed on the content of long-chain fatty acids of the Omega-3 series (EPA - ecosapentaenoic acid, C20:5n-3 and DPA - docohexaenoic acid, C22: 6n-3), which recorded significantly higher values in the case of Ti breed (see Table 1 above). The lambs of the breeds Ti and TM showed higher proportions of Omega-6 fatty acids in the intramuscular fat as against those from the Tu breed where one recorded the lowest functional concentrations of lipid components.

The breed influenced significantly the content of intramuscular fat in CLA and, in particular, in rumen acid (c9, t11 CLA) and which were significantly higher in the case of lambs of the Ti breed (Ti > Tu > TM). Isomer t10, c12 CLA showed higher concentrations in the case of lambs of the TM breed, but the differences were not statistically consistent.

In conclusion, the breed has a significant influence on the FA profile, the intramuscular fat in the case of lambs of Tigaie breed shows the highest concentration of fatty acids (especially ALA, CLA, EPA and DHA), the lowest content of saturated fatty acids (SFA) and the best sanogeneous lipid indices (the ratio n-6/n-3 FA, AI and TI). Consequently, the Tigaie breed is a genetic resource that could be used effectively in the sustainable production systems to produce sanogeneous sheep meat.

The main lipid indices considered in evaluating the quality of sanogenetic quality of foods with high fat content are as follows: total fat content, PUFA/SFA ratio, n-6/n-3 ratio (Department of Health, 1994), atherogenic index (AI), thrombogenic index (TI) and fatty acid desaturation index (Ulbricht and Southgate, 1991). In our study, the best values of the sanogenic lipid indices of intramuscular fat of lambs were obtained from the Tigaie breed, the differences being statistically consistent. Thus, when compared to the lambs from the TM and Tu breeds, in the case of the lambs

of Ti breed the PUFA/SFA ratio was higher by 30%, while the n-6/n-3 ratio was lower by 22%, which suggests a more favourable effect on consumers' health. This is due to the higher proportion of fatty acids in the Omega-3 series in the structure of intramuscular fat of the Tigaie breed lambs. Generally, a PUFA/SFA ratio over 0.45 and a n-6/n-3 ratio below 4.0 are required in the human diet for combating lifestyle related diseases such as coronary heart disease and cancer (Simopoulos, 2002). Intramuscular fat of lambs in the Tigaie breed was within such parameters that define the sanogeneous fat quality, recording a value of 0.51 for the PUFA/SFA ratio and 1.52 respectively for the n-6/n-3 ratio. The lower concentration of hypercholesterolemia fatty acids (C12:0, C14:0 and C16:0) and the higher concentration in the case polyunsaturated fatty acids (in particular for n-3 PUFA series) in the case of the Tigaie breed lambs led to a significant decrease in the atherogenic index (AI) and thrombogenic index (TI) values. The improved AI and TI indices were positively correlated with a higher content of intramuscular fat in functional fatty acids (n-3 FA, CLA). Atherogenic index (AI) and thrombogenic index (TI) characterize food fats in terms of impact on consumer health, the fat with a higher value or atherogenic and thrombogenic index being harmful to human health.

In order to quantify the amount of CLA in intramuscular fat coming from endogenous synthesis (formed mainly at tissue level) by converting *trans*-vaccenic acid under the action of the enzyme Δ^9 - desaturase, we estimated the activity of this enzyme by means of the desaturation index (DI 18:2 c9, t11) using the equation proposed by Pilarczyk et al., (2015). The highest desaturation index value (DI 18 2 c9, t11) was found in the lambs of Tsigai breed, suggesting a high enzymatic activity level and therefore a larger amount of CLA c9, t11 of intramuscular fat was formed by enzymatic desaturation of vaccenic acid (C18:1 t11) in various tissues. Thus, these findings are confirmed by studies developed by Chilliard et al., (2003) which mention that the activity of desaturases has a breed nature with a strong genetic determinism. All these changes are consistent with the lower concentration of stearic acid (C18:0) in the intramuscular fat of Ti breed lambs; stearic acid is the final product of ruminal biohydrogenation of polyunsaturated fatty acids in the diet, and in particular, in the case of linoleic acid (Chilliard et al., 2007).

Since, in the case of Tigaie breed, we achieved the highest concentrations of functional fatty acids in intramuscular fat, we carried on our studies in this breed to determine whether the fatty acid profile is influenced by the type of tissue (intramuscular fat vs. fat deposit) or body region (*longissimus dorsi* muscle vs. *biceps femoris* muscle, subcutaneous adipouse tissue vs. perirenal adipouse tissue).

The fatty acid composition of the intramuscular fat, compared to the deposit fat is characterized by a lower content of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) and a higher proportion of polyunsaturated fatty acids (PUFA) (Table 2).

Table 2

Fatty acid profile of different body regions in young fattened sheep, Tsigai breed (g/100g FAME

	Intramuscular fat			Adipos fat		
	Longissimus dorsi	*Biceps femoris*	p	Perirenal fat	Subcut. fat	p
Total lipides (%)	6,25	11,21	***	94,9	93,6	NS
C 12:0	0,02	0,02	NS	0,03	0,03	NS
C 14:0	4,60	4,24	NS	2,99	2,45	*
C 16:0	18,87	20,39	*	23,62	17,58	***
C 18:0	12,29	11,77	NS	18,60	19,64	*
C 20:0	0,12	0,10	NS	0,40	0,36	NS
C 18:1 cis	25,16	27,88	*	35,62	43,95	***
C 18:1 trans-11	3,14	3,48	NS	3,56	3,29	NS
C 18:2 n-6	9,74	10,30	NS	4,29	2,78	**
C 18:3 n-3	3,71	5,91	***	2,68	4,55	***
CLA total	2,64	2,04	*	1,01	1,98	***
C 20:2 n-6	0,75	0,81	NS	0,30	0,38	NS
C 20:4 n-6	3,80	5,12	**	0,83	0,51	**
C 20:5 n-3 EPA	2,69	3,53	*	0,21	0,10	***
C 22:3 n-3	0,74	0,81	NS	0,04	0,05	NS
C 22:5 n-3 DPA	0,58	0,94	**	0,21	0,15	**
C 22:6 n-3 DHA	2,64	2,74	NS	0,12	0,07	*
SFA	35,90	36,52	NS	45,64	40,06	**
MUFA	28,30	31,36	*	39,18	47,24	***
PUFA	27,29	32,20	**	9,69	10,57	NS
PUFA n-3	10,36	13,93	**	3,26	4,92	***
PUFA n-6	14,29	16,23	*	5,42	3,67	***
DI (18:2 c9,t11)	45,67	36,95	*	22,10	37,57	**

In general, in intramuscular fat the amount of Omega-3 fatty acids was approx. 3 times higher and the CLA was approx. 2 times higher than in the case of at deposit. Long-chain polyunsaturated fatty acids (C20 - C22) had much lower concentrations in the adipouse tissue compared to the muscle tissue, even by 10-20 times. This can be explained both by the low proportion of phospholipids in the adipouse tissue and the low rate of incorporation of long-chain polyunsaturated fatty acids in the triacylglycerol fraction in ruminants (Ashes et al. 1992; Enser et al. 1996).

The most important differences in the fatty acid profile of the intramuscular fat were found in the content of ALA, CLA and EPA, in favour of BF muscle, which also allowed to achieve better sanogenic lipid indices (n-6/n-3 ratio, AI, TI) when compared to LD muscle related ones (fig. 1).

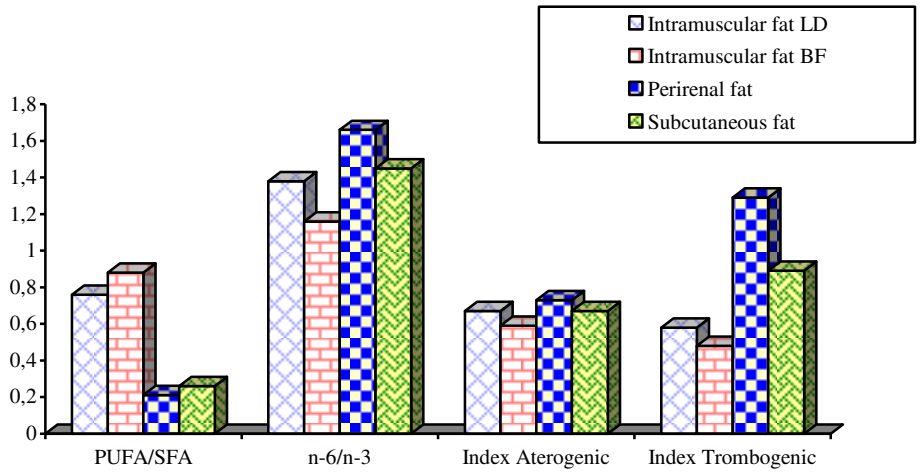

Fig. 1. The influence of physiological factors (type of tissue and anatomical region) on sanogenous lipid indices of carcass fat (LD-*Longissimus dorsi*; BF-*Biceps femoris*)

In the case of fat deposit, within the subcutaneous adipouse tissue one recorded a fatty acids profile which is more favourable to human health, and containing a lower amount of palmitic acid (C16:0), and a higher content of functional fatty acids (C18:3n-3, long-chain n-3 FA and CLA) as against the perirenal adipouse tissue.

In humans, trans fatty acids can have adverse effects, leading to higher levels of LDL ("bad cholesterol") levels and cardiovascular disease (Department of Health, 1994). Nevertheless, epidemiological studies (Willett et al., 1993) suggest that monounsaturated trans fatty acids resulting from the rumen fermentation (especially the trans-Vaccenic acid, C18:1 trans-11) are not risk factors for cardiovascular diseases. Elevated levels of vaccenic acid in foods derived from ruminant animals can have a positive contribution to the health of consumers because they turn into CLA at tissues level in the presence of specific desaturase (Knecht et al. 1996; Finnegan & Williams, 2001). In the study we conducted one did not find significant differences in terms of the content of vaccenic acid (18:1 trans-11) neither from intramuscular deposit fat, nor by anatomical region of body fat origin. Our results in lambs were confirmed by Maleki et al., (2015) but are inconsistent with those reported by Schena et al. (2007), who found significant differences in the concentration of C18:1 trans-11 in the intramuscular and subcutaneous fat in calves, depending on breed.

CONCLUSIONS

The highest proportion of functional fatty acids (cis-9, trans-11 CLA, C18:3n-3 and long-chain n-3 FA) in the intramuscular (LD) fat composition was recorded in lambs in the Tigaie breed and, especially, in rams fattened in intensive fattening system. While monitoring the distribution of fatty acids in various tissues of the carcass (*longissimus dorsi*-LD, *biceps femoris* - BF, *perirenal and subcutaneous adipouse tissue*) it was found that polyunsaturated fatty acids and the functional fatty acids are found in higher proportions in the muscle tissue and the saturated ones are found in the fat deposits and, in particular, in the perirenal adipouse tissue. Of the two muscles submitted for analysis, the highest amount of Omega-3 fatty acids was found in the BF intramuscular fat (leg) and the highest amount of CLA was found in LD. Sheep meat fat content in trans-vaccenic acid (VA, C18:1 trans-11) which is considered beneficial to human health because it is the precursor to CLA was not influenced by breed, type of tissue or body region.

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