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Effect of diets containing essential fatty acids-rich oil calcium soaps on functional lipid components of lamb tissues

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DANIEL MIERLIȚĂ
University of Oradea, Department of Animal Science, Oradea 410087, Bihor, Romania.
Address correspondence: University of Oradea, Department of Animal Science, 26, Gen. Magheru, 410087, Oradea, Romania.
Tel.: +40259321385; Email: dadi.mierlita@yahoo.com

Abstract

The present study examined the effects of feeding-protected lipid supplements rich in linoleic acid or linolenic acid on the lipid composition of muscle and adipose tissues of lambs. Thirty, 10-week-old Tsigai breed ram lambs were assigned to one of three experimental diets: no oil Ca soap (C-Control), with 4% sunflower oil Ca soaps (SO-high in 18:2n-6), with 4% camelina oil Ca soaps (CO-high in 18:3n-3). The diet high in α-linolenic acid produced the highest levels of n-3 FAs: 18:3n-3 (ALA-1.83%), 20:5n-3 (EPA-0.44%) and 22:6n-3 (DHA-1.03%) intramuscular fat. In addition, CO intramuscular fat has the lowest n-6/n-3 ratio (2.91), aterogenic index (0.61) and thrombogenic index (1.15). In the intramuscular fat of the diet high in linoleic acid (LA), conjugated linoleic acid (CLA) isomers and vaccenic acid (VA) reached their highest concentrations. Intramuscular depots clearly showed a greater content of PUFA n-3 and CLA, and a lower n-6/n-3 ratio than adipose tissues. The high levels of C18:1 trans-11 and C18:2 trans-10 cis-12 obtained from the SO and CO diets would suggest that Ca soaps only confer partial protection in the rumen. The results of this study indicated that linoleic acid was more effective in enhancing contents of VA and CLA in muscle and adipose tissue than linolenic acid, which contributes to the enrichment of FAs n-3 lamb meat.

Keywords: Oil Ca soaps, Omega-3 fatty acid, CLA, Atherogenicitiy index, Lamb meat.

1. Introduction

Consumers are becoming more aware of the relationships between diet and health and this has increased consumer interest in the nutritional value of foods. Nutritionist advisers recommended a higher intake of beneficial fatty acids, especially n-3 PUFA (polyunsaturated fatty acids) and conjugated linoleic acid (CLA) at the expense of n-6 PUFA. Thus, reducing SFA content and the n-6/n-3 ratio is of major importance in meat research. Many human dietary studies have reported that higher intakes of n-3 FA, CLA and 18:1-*trans* 11 have the potential to protect cells from diseases such as cancer, heart disease and arthritis (SIMOPOULOS [1]).

Several factors influence the functional lipid components (n-3 FA and CLA) content of lamb meat, such as breed, sex, seasonal variation, type of muscle, production practices; however, diet plays the most important role. Enhancing the beneficial effects of animal products is achieved through diet manipulation, such as the use of diets supplemented with plant oils with high purities of linoleic (C18:2n-6), linolenic (C18:3n-3) or oleic (C18:1n-9) fatty acids to improve the concentration of PUFA n-3 and CLA in lamb tissues (MANSO & al. [2]). These dietary practices can increase n-3 FA and CLA concentrations up to 3 fold (WOOD & al. [3]). Moreover, *trans*-11 18:1 (vaccenic acid, VA) is the precursor of *cis*-9, *trans*-11 18:2 (rumenic acid, RA) is the major CLA isomer in animal and humans and,

therefore, it might be considered as a fatty acid with beneficial properties (GARCIA & al. [4]).

The increase in 18-3n-3, as a percentage of total FA in muscle, will generally be higher when animals are fed a α-linolenic acid-rich source (fish oil, linseed) (RIBEIRO & al. [5]). Camelina oil is one of the few plant sources providing ample amounts of α-linolenic acid (32.8%; MIERLITA [6]) and could be an important source of 18:3n-3 for animal nutrition and also increase 18:3n-3 and its fatty acids metabolites (C20:5 - EPA and C22:6 - DHA) in meats.

Fat supplementation of the diet can lead to a reduction in fiber digestion in the rumen. In order to counter affect these undesirable effects, dietary supplementation of fat as a calcium soaps of fatty acids is a good alternative. Because the calcium salts at pH = 6.5-7 is neutral will not be affected in the rumen (pH=6.5-6.8), and finally in abomasum and duodenum with pH = 3.5 are fully opened and the fatty acids are available for absorption (ASGARI & al. [7]). Moreover, prevention of bio hydrogenation of polyunsaturated essential fatty acids in the rumen subsequently increases their absorption from the small intestine potentially increasing the supply of polyunsaturated fatty acids to the intramuscular and subcutaneous fat.

The aim of this study was to determine the effects of feeding-protected lipid supplements rich in unsaturated FAs (linoleic acid or α-linolenic acid) on the lipid composition of muscle and adipose tissues. The hypothesis to be tested in the current experiment was to improve fatty acid profile by increasing the level of functional lipid components (long chain n-3 FA, CLA and VA) in lamb meat and to reduce SFA content and the n-6/n-3 ratio.

2. Materials and Methods

Thirty 10-week-old Tsigai breed ram lambs were randomly allocated to one of three experimental diets (forage/concentrate ratio 40:60): no oil Ca soap (C-control), with 4% sunflower oil Ca soaps (SO-high in 18:2n-6), with 4% camelina oil Ca soaps (CO-high in 18:3n-3). Calcium soaps (Cs) were prepared in our laboratory, using sodium hydroxide and 20% calcium chloride solution via the precipitation method as described by ALEXANDER & al. [8]. The calcium soap formed was washed with tap water and was air dried in a dark room and stored at subzero temperature until used for feeding through inclusion in concentrated mixture. Diets were isonitrogenous. The energy value of the rations was 10.8 ME (MJ/kg DM) in the control group and 11.5 ME (MJ/kg DM) in experimental groups (Table 1). Animals were housed individually with free access to diet and water. Diet refusals were weighed and recorded daily. Animal weights and feed samples were obtained at 10 day intervals. The experiment lasted for 110 days, between May and August.

Samples of the diet were stored at 4°C. The basic chemical composition of feeds was determined using standard methods. Feed samples were vacuum packed and frozen at –20°C until analysis of the fatty acid composition. The fatty acid composition of fats from feeds was determined according to procedures developed by KRAMER & al. [9].

From each carcass, 150-200 g samples were taken from loin muscle (m. *longissimus dorsi*) and subcutaneous fat, between the 9^{th} – 10^{th} ribs (SZUMACHER-STRABEL & al. [10]). Adipose tissue samples from subcutaneous and intramuscular depot were packed well and frozen at -20°C until analysis for fatty acid composition. The samples were homogenized in chloroform/methanol (2:1, v/v) solution according to the procedure of FOLCH & al. [11] to extract the fat. Methylation of the lipids was conducted by following the method of LEPAGE & al. [12] prior to injecting into the gas chromatograph (Varian CP-3380, Inc.

Scientific Instruments, Palo Alto, CA, USA) equipped with a flame ionization detector and Chrompac CP-Sil 88 column (100 m, 0.25 mm, 0.2 μm film thickness, Varian, Inc. Scientific Instruments, Palo Alto, CA, USA). The injector and detector temperature was maintained at 250°C. The initial column temperature was 175°C (held for 30 min), and then increased by 15°C/min to 220°C (held for 40 min). Helium was used as the carrier gas at a constant flow of 1.0 mL/min. The identification of fatty acid peaks was made by comparison of retention times with the ones obtained for fatty acid methyl ester (FAME) standard mixtures acquired from Un-Check-Prep Inc. (Elysian, MN, USA) and from Supelco Inc. (Bellefonte, PA, USA). Additional standards of individual CLA isomers (C18:2 *cis*-9, *trans*-11, C18:2 *trans*-10, *cis*-12) were purchased from Matreya Inc. USA. Fatty acid composition was expressed as percentage of total FAMEs on basis of total mass.

The atherogenic index (AI) was calculated according to CHILLIARD & al. [13] as follows: AI = (C12:0 + 4 x C14:0 + C16:0)/(MUFA + PUFA);

whereas the thrombogenic index (TI) was calculated in accordance with ULBRICHT & al. [14] using the formula:

TI = (C14:0+C16:0+C18:0)/(0.5 x MUFA + 0.5 x n-6 PUFA + 3 x n-3 PUFA + n-3/n-6 PUFA).

The obtained data were subjected to variance analysis by using the General Linear Models procedure of SAS, Version 9.1.3 (SAS Institute, Cary, NC, USA) [15]. Multiple comparisons among means were done with the Duncan test. The level of significance was established at $P < 0.05$.

3. Results and Discussion

The diet compositions are summarized in Table 1. The crude protein content met the lambs' requirements. Calcium soap (Cs) is a rumen protected form of plant oils, so the rate of biohydrogenation of unsaturated fatty acids is lower and thus a major part of them can be incorporated into the muscle and subcutaneous fat. Linoleic acid (LA, C18:2n-6) was the most abundant fatty acid in the diets (46.19 - 67.20% of total fatty acids), followed by oleic acid (C18:1n-9), palmitic acid (C16:0) and linolenic acid (ALA, C18:3n-3). The LA in the SO diets was higher than the C and CO diets. The feed of the CO group contained approximately 2 times higher linolenic acid (C18:3n-3) than control and SO diet (table 1). Similar aspects of the fatty acid composition in diets were observed by EBRAHIMI & al. [16], when the kids' diet was supplemented with flax and sunflower oils.

The initial weight, final weight, daily TMR (total mixed ration) intake and feed conversion ratio did not differ between control and SO or CO treatment groups (data not shown). Values observed in the current experiment for these parameters are within those recorded in the bibliography for lambs reared under similar conditions (MANSO & al. [2]; BOLES & al. [18]; JERONIMO & al. [19]).

Obtained results of fatty acid composition in loin muscle and subcutaneous fat are presented in table 2 and 3, respectively. The highest statistically significant decrease in sum of saturated fatty acids (SFA) concentration was determined in all tested adipose tissue depots in animals fed with the CO diet. The most substantial increase in polyunsaturated fatty acids n-3 concentration and decreased n-6/n-3 ratio was obtained in all fat depots in lambs fed CO diet. The most substantial increase in conjugated isomers of C18:2 (*c*9,*t*11 and *t*10,*c*12) and *trans*-11 18:1 (VA, vaccenic acid), was observed in loin muscle and subcutaneous fat in lambs fed SO diet.

VELASCO & al. [20] reported higher values of SFA (66.32-63.71%) for muscle tissue and subcutaneous fat of lambs compared to our results (48.25-52.19%). In the current

study, the addition of calcium soap of plant oils (sunflower oil or camelina oil) to the diets caused a significant decrease in the proportion of palmitic acid (C16:0) in both intramuscular and subcutaneous fat, which is probably due to its lower proportion in the SO and CO than in the control diet. This agrees with previous works suggesting that the content of this fatty acid reflects its dietary concentration (COOPER & al. [21]; MANSO & al. [22]).

Table 1. Ingredients and chemical composition of lambs diets.

Variable	**Tratament**[1]		
	C	**SO**	**CO**
Ingredients (% of DM)			
Alfalfa hay	40.0	40.0	40.0
Corn grain	29.0	23.5	23.5
Triticale grain	15.0	15.0	15.0
Soybean meal	14.5	16.0	16.0
Sunflower oil Ca soaps	-	4.0	-
Camelina oil Ca soaps	-	-	4.0
Mineral mixture	1.0	1.0	1.0
Vitamin supliment	0.5	0.5	0.5
Chemical composition (% of DM)			
CP (Crude protein)	16.6	16.4	16.4
NDF (Neutral Detergent Fiber)	33.17	34.02	34.02
ADF (Acid Detergent Fiber)	18.34	18.60	18.60
EE (Ether extract)	2.38	5.12	5.23
ME (MJ/kg DM)[2]	10.8	11.5	11.5
Fatty acid composition (% of FAME[3])			
C14:0, Myristic	0.31	0.10	0.14
C16:0, Palmitic	13.84	7.11	6.07
C18:0, Stearic	4.92	4.54	2.11
C18:1n-9c, Oleic	16.30	14.83	34.57
C18:2n-6, Linoleic	56.12	67.20	46.19
C18:3n-3, α-Linolenic	3.41	3.88	7.64

[1]C-control:diet without oil Ca soaps; SO-diet supplemented with sunflower oil Ca soaps (high in 18:2n-6); CO-diet supplemented with camelina oil Ca soaps (high in 18:3n-3).
[2]Metabolizable energy, calculated using NRC (National Research Council [17]).
[3]Fatty acid methyl esters.

The highest values of total MUFA were found in the CO diet. The values of total MUFA in our study were similar to those reported by DIAZ & al. [23] on the *longissimus dorsi* muscle fatty acid contents of Spain and Uruguayan lambs fed concentrate (38.37-41.17%).

The dietary treatment did not significantly increase the proportion of polyunsaturated fatty acids group (PUFA total), but rather it modified its composition. The SO diet contained more linoleic acid (C18:2n-6) than the CO and control diets, due to the high level of C18:2n-6 in the calcium soap of sunflower oil which was added to the diet. This difference did not reflect in either the C18:2n-6 (8.92, 8.31 and 9.07 in the C, SO and CO samples, respectively) nor in its metabolite (C20:4n-6) level of intramuscular and subcutaneous fat. This result is in agreement with other authors, who observed that incorporation of C18:2n-6 into the muscle could not be influenced by higher plant oils (MANSO & al. [22]; ZSÉDELY & al. [24]). Other fat sources (unprotected rapeseed, fish oil, palm oil, sunflower oil), with the exception of the protected form of canola seed, could not affect it (ARANA & al. [25]; PENG & al. [26]). This suggests that biohydrogenation of linoleic acid may be very effective in the rumen (ZSÉDELY & al. [24]).

Table 2**.** Effect of diets containing linoleic acid- or linolenic acid-rich oil Ca soaps on the fatty acid composition (% of FAME) of intramuscular fat in lambs (mean ± SD)

Intramuscular fat	**Tratament[1]**		
	C	**SO**	**CO**
C12:0, Lauric	0.47 ± 0.04	0.45 ± 0.03	0.46 ± 0.06
C14:0, Myristic	3.43 ± 0.39	3.06 ± 0.22	3.07 ± 0.44
C16:0, Palmitic	24.62 ± 3.37^{c}	23.47 ± 2.18^{b}	21.48 ± 1.76^{a}
C18:0, Stearic	17.57 ± 1.52	17.69 ± 1.86	16.86 ± 1.51
C18:1n-9, Oleic	35.80 ± 2.34^{ab}	34.46 ± 3.29^{a}	37.43 ± 2.72^{b}
C18:1 *trans*-11, Vaccenic (VA)	2.57 ± 0.18^{a}	5.12 ± 0.24^{c}	3.74 ± 0.17^{b}
C18:2n-6, Linoleic (LA)	8.92 ± 0.34	8.31 ± 0.47	9.07 ± 0.29
CLA *cis*-9, *trans*-11, Rumenic (RA)	1.21 ± 0.13^{a}	1.96 ± 0.09^{b}	1.64 ± 0.11^{ab}
CLA *cis*-12, *trans*-10	0.09 ± 0.03^{a}	0.18 ± 0.07^{c}	0.14 ± 0.11^{b}
Total CLA	1.30 ± 0.16^{a}	2.14 ± 0.19^{b}	1.78 ± 0.13^{ab}
C18:3n-3, α-Linolenic (ALA)	0.67 ± 0.12^{a}	0.84 ± 0.17^{a}	1.83 ± 0.23^{b}
C20:4n-6, Arachidonic	1.14 ± 0.13	1.12 ± 0.10	0.87 ± 0.24
C20:5n-3, Eicosapentaenoic (EPA)	0.33 ± 0.04^{a}	0.39 ± 0.04^{ab}	0.44 ± 0.03^{b}
C22:5n-3, Docosapentaenoic (DPA)	0.09 ± 0.05	0.11 ± 0.04	0.11 ± 0.04
C22:6n-3, Docosahexaenoic (DHA)	0.70 ± 0.09^{a}	0.86 ± 0.13^{ab}	1.03 ± 0.10^{b}
Others	2.39 ± 0.16	2.08 ± 0.21	1.83 ± 0.17
SFA	46.09 ± 2.57^{b}	44.67 ± 1.85^{ab}	41.87 ± 2.61^{a}
MUFA	38.37 ± 1.02^{a}	39.48 ± 0.71^{ab}	41.17 ± 0.59^{b}
PUFA n-3[2]	1.79 ± 0.17^{a}	2.20 ± 0.10^{b}	3.41 ± 0.14^{c}
PUFA n-6[3]	10.06 ± 1.18	9.43 ± 1.63	9.94 ± 1.06
Total PUFA	13.15 ± 2.04^{a}	13.77 ± 1.58^{a}	15.13 ± 1.73^{b}
PUFA:SFA ratio	0.28 ± 0.03^{a}	0.31 ± 0.02^{ab}	0.36 ± 0.02^{b}
n-6/n-3 FA	5.62 ± 1.46^{c}	4.28 ± 1.09^{b}	2.91 ± 1.51^{a}
HFA[4]	28.52 ± 0.73^{b}	26.98 ± 0.91^{b}	25.01 ± 049^{a}
Atherogenic index (AI)	0.75 ± 0.04^{b}	0.68 ± 0.06^{ab}	0.61 ± 0.04^{a}
Thombogenic index (TI)	1.53 ± 0.12^{b}	1.41 ± 0.11^{b}	1.15 ± 0.09^{a}

[1]C-control:diet without oil Ca soaps; SO-diet supplemented with sunflower oil Ca soaps (high in 18:2n-6); CO-diet supplemented with camelina oil Ca soaps (high in 18:3n-3).
[2][C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3]; [3][C18:2n-6 + C20:4n-6];
[4]HFA (hypercholesterolaemic fatty acids) = C12:0 + C14:0 + C16:0.
FAME-fatty acid methyl esters; SD-standard deviation; CLA-conjugated linoleic acids. SFA-saturated fatty acid; MUFA-monounsaturated fatty acid; PUFA-polyunsaturated acid; FA-fatty acid.
[a,b,c] – Means within the same row with different letters differ significantly according to Duncan's tests ($p < 0.05$).

As was expected, lambs fed camelina oil Cs had a greater proportion ($P < 0.001$) of α-linolenic acid (C18:3n3). The lamb muscle and subcutaneous fat from the animals fed protected ALA (α-linolenic acid) supplement diet contained 1.18 - 1.26 times as much C18:3n-3 as the samples from animals fed the LA (linoleic acid) diet. MANSO & al. [22] reported a decrease in C18:3n-3 in response to the addition of sunflower oil in the diet; this could be related to the biohydrogenation process within the rumen. In the current experiment, the supplementation of diet with SO (calcium soap of sunflower oil) led to an increase in C18:3n-3 proportions of meat fat, which signifies that the treatment of sunflower oil with calcium salts has inhibited the biohydrogenation processes of fatty acids in the rumen.

The camelina oil Cs in the diet improved total n-3 FA content from 1.79% to 3.41% in intramuscular fat, and 0.64% to 1.90% from subcutaneous fat, due to a significant increase in muscle and subcutaneous fat 18:3n-3 (ALA), 20:5n-3 (EPA), 22:5n-3 (DPA) and 22:6n-3 (DHA) fatty acids (table 2 and 3). As noted in other studies, some of the dietary ALA escapes hydrogenation in the rumen and is subsequently metabolized to eicosapentanoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), which are found in ruminant tissues (cell membranes) (PONNAMPALAM & al. [27]).

The dietary replacement of sunflower oil Cs with camelina oil Cs has not increased the EPA and DHA in lamb meats significantly. The synthesis of EPA and DHA from dietary ALA seems to be limited, and thus the plant oils supplementation - rich in 18-3n-3 - is not be the best FA source when the objective is to increase EPA and DHA in ruminant meat (RIBEIRO & al. [5]). The addition of fish oil to ruminant diets has been shown to be more effective to elevate the EPA and DHA in the meat rather than flaxseed oil (COOPER & al. [21]).

Table 3**.** Effect of diets containing linoleic acid- or linolenic acid-rich oil Ca soaps on the fatty acid composition (% of FAME) of subcutaneous fat in lambs (mean ± SD).

Subcutaneous fat	**Tratament[1]**		
	C	**SO**	**CO**
C12:0, Lauric	0.73 ± 0.15	0.71 ± 0.17	0.77 ± 0.12
C14:0, Myristic	4.01 ± 0.73	3.66 ± 0.54	3.51 ± 0.47
C16:0, Palmitic	25.39 ± 1.71^{b}	24.18 ± 1.57^{ab}	23.07 ± 1.19^{a}
C18:0, Stearic	22.06 ± 1.40	21.61 ± 1.31	20.90 ± 1.98
C18:1n-9, Oleic	32.74 ± 1.75^{ab}	31.06 ± 1.49^{a}	33.54 ± 1.33^{b}
C18:1 *trans*-11, Vaccenic (VA)	4.83 ± 0.39^{a}	7.59 ± 0.40^{c}	6.08 ± 0.53^{b}
C18:2n-6, Linoleic (LA)	6.12 ± 1.04	6.29 ± 1.34	5.98 ± 0.82
CLA *cis*-9, *trans*-11, Rumenic (RA)	0.82 ± 0.20^{a}	1.36 ± 0.22^{b}	1.18 ± 0.21^{ab}
CLA *cis*-12, *trans*-10	0.06 ± 0.07	0.11 ± 0.09	0.09 ± 0.08
Total CLA	0.88 ± 0.35^{a}	1.47 ± 0.27^{b}	1.27 ± 0.23^{ab}
C18:3n-3, α-Linolenic (ALA)	0.51 ± 0.18^{a}	0.70 ± 0.11^{b}	1.58 ± 0.24^{c}
C20:4n-6, Arachidonic	0.46 ± 0.09	0.51 ± 0.14	0.41 ± 0.14
C20:5n-3, Eicosapentaenoic (EPA)	0.04 ± 0.01^{a}	0.05 ± 0.02^{a}	0.07 ± 0.02^{b}
C22:5n-3, Docosapentaenoic (DPA)	0.03 ± 0.07^{a}	0.03 ± 0.03^{a}	0.05 ± 0.03^{b}
C22:6n-3, Docosahexaenoic (DHA)	0.06 ± 0.05^{a}	0.10 ± 0.08^{b}	0.19 ± 0.12^{c}
Others	2.14 ± 0.08	2.03 ± 0.06	2.57 ± 0.11
SFA	52.19 ± 2.16^{b}	50.16 ± 3.32^{ab}	48.25 ± 2.22^{a}
MUFA	37.57 ± 1.29	38.65 ± 2.31	39.62 ± 1.07
PUFA n-3[2]	0.64 ± 0.10^{a}	0.89 ± 0.10^{b}	1.90 ± 0.12^{c}
PUFA n-6[3]	6.58 ± 1.29	6.80 ± 1.11	6.39 ± 1.40
Total PUFA	8.10 ± 1.52^{a}	9.16 ± 1.46^{b}	9.56 ± 1.10^{b}
PUFA:SFA ratio	0.15 ± 0.02^{a}	0.18 ± 0.02^{b}	0.20 ± 0.03^{c}
n-6/n-3 FA	10.28 ± 1.77^{c}	7.64 ± 1.39^{b}	3.36 ± 1.10^{a}
HFA[4]	30.13 ± 1.63^{b}	28.55 ± 1.26^{a}	27.35 ± 1.81^{a}
Atherogenic index (AI)	0.92 ± 0.05^{c}	0.83 ± 0.03^{ab}	0.77 ± 0.03^{a}
Thombogenic index (TI)	2.14 ± 0.15^{c}	1.94 ± 0.08^{b}	1.64 ± 0.09^{a}

[1]C-control:diet without oil Ca soaps; SO-diet supplemented with sunflower oil Ca soaps (high in 18:2n-6); CO-diet supplemented with camelina oil Ca soaps (high in 18:3n-3).
[2][C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3]; [3][C18:2n-6 + C20:4n-6];
[4]HFA (hypercholesterolaemic fatty acids) [C12:0 + C14:0 + C16:0].
FAME-fatty acid methyl esters; SD-standard deviation; CLA-conjugated linoleic acids. SFA-saturated fatty acid; MUFA-monounsaturated fatty acid; PUFA-polyunsaturated acid; FA-fatty acid.
^{a,b,c} – Means within the same row with different letters differ significantly according to Duncan's tests ($p < 0.05$).

EBRAHIMI & al. [16] shows that through the supplementation of kid diet with linoleic acid via flaxseed, a decrease in the concentration of C20:4n-6 in intramuscular and subcutaneous fat can be obtained. Our study showed no significant differences between treatments, regarding the C20:4n-6 found in intramuscular and subcutaneous fat, probably due to the lower quantity of linolenic acid with which the lambs' diet was supplemented.

Feeding SO increased the concentration of *cis*-9, *trans*-11 CLA. The *c*9,*t*11 CLA content in fat was higher in SO, and CO was higher than the control. The 18:1 *trans*-11 (VA) concentration was higher in the SO diet, followed by CO, and control diets. The results of this study indicated that linoleic acid (SO diet) was more effective in enhancing contents of

VA and CLA in intramuscular and subcutaneous fat than linolenic acid (CO diet). Supplementation of linoleic acid-rich sources, such as sunflower, in the diet of ruminants was the most effective way to increase CLA concentration in meat (WOOD & al. [3]). Supplementation with 3% and 6% of safflower oil increased the CLA percentage in lamb muscle by 134% (MIR & al. [28] and 306% (BOLES & al. [18]). Also, 8% to 10% addition of soybean oil to the diet promoted a 181% to a 331% increase in CLA of lamb muscle (BESSA & al. [29]). Similarly, significantly higher levels of *c*9,*t*11 CLA were also found in beef from cattle fed sunflower seed compared to flaxseed (MAPIYE & al. [30]).

Our present data confirmed an earlier observation (JERONIMO & al. [31]), who reported increased contents of 18:3n-3 and total n-3 long chain PUFA and decreased concentrations of C18:1 *t*11 and CLA *c*9,*t*11 in the intramuscular fat of the lambs, when sunflower oil was replaced by linseed oil as the fat supplement in the diet. The synthesis of VA and RA from C18:2n-6 may have been more efficient than that from C18:3n-3. The C18:2n-6 ruminal biohydrogenation occurs with an initial isomerization, with the formation of CLA *c*9,*t*11 and its reduction of C18:1 *t*11. The biohydrogenation of C18:3n-3 results in more diverse products including C18:3n-3, C18:1 *t*11, but not CLA *c*9,*t*11 (EBRAHIMI & al. [16]). Therefore, sunflower oil supplementation would produce more rumen-derived CLA *c*9,*t*11 than camelina oil. NOCI & al. [32] observed a higher CLA *c*9,*t*11 content in the intramuscular fat of heifers supplemented with sunflower oil than with flaxseed oil. This can be explained by the fact that most of the CLA *c*9,*t*11 present in tissues is derived from endogenous desaturation of C18:1 *t*11, which originates during ruminal biohydrogenation of C18:2n-6 (EBRAHIMI & al. [16]). However, the CLA *c*9,*t*11 is also synthesized by direct isomerization of C18:2n-6 in the rumen.

The other important aspect of nutritional value of food is the n-6/n-3 ratio, which should be at least 4:1 according to nutritionists (SIMOPOULOS [1]). In our trial the n-3 percentage increased due to the higher α-linolenic acid content and additionally the n-6 group was not influenced by calcium soap of plant oils in SO and CO groups. These changes resulted that the n-6/n-3 ratio being significantly narrower in CO samples (3.36-2.91), followed by SO (7.64-4.28), than that of the control lambs (10.28-5.62). In these studies, the n-6/n-3 ratio was more beneficial in intramuscular fat of lambs compared of subcutaneous fat. It can be considered a beneficial property for human nutrition. However, this value is higher for control and SO samples than the recommendation of nutritionists (SIMOPOULOS [1]).

In the present study, a significant decrease in the atherogenicity (AI) and trombogenic index (TI) was found in response to camelina oil Cs supplementation in intramuscular and subcutaneous fat. Intramuscular fat for SO diet showed only a tendency to have lower atherogenicity and trombogenic index, which could probably be related to the numeric changes in saturated and monounsaturated fatty acids, which did not reach the required significance level to be statistically different. On the other hand, subcutaneous fat seems to be more responsive to changes in the dietary fatty acid supply or changes in rumen metabolism than intramuscular fat, thus AI and TI registered significantly lower values in the SO diet compared to the control group; however, these values were higher than in the CO diet.

4. Conclusions

Supplementation with FA sources high in 18:3n-3 (camelina oil Cs), increases n-3 FA and narrows n-6/n-3 ratio in meat, while supplementation with FA sources high in 18:2n-6 (sunflower oil Cs), increases CLA and VA (18:1 *trans*-11) levels in meat, and decreases the n-6/n-3 ratio. Therefore, from a nutritional point of view, a mixture of sources may be an

alternative to increase the levels of health beneficial fats and to narrow the n-6/n-3 ratio in lamb meat.

References

1. A.P. SIMOPOULOS. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: Nutritional implications for chronic diseases. *Biomed. Pharmacother*, 60: 502 (2006).
2. T. MANSO, T. CASTRO, A.R. MANTECÓN, V. JIMENO. Effects of palm oil and calcium soaps of palm oil fatty acids in fattening diets on digestibility, performance and chemical body composition of lambs. *Anim. Feed Sci. Tech.*, 127: 175 (2006).
3. J.D. WOOD, M. ENSER, A.V. FISHER, G.R. NUTE, P.R. SHEARD, R.I. RICHARDSON, S.I. HUGHES, F.M. WHITTINGTON. Fat deposition, fatty acid composition and meat quality: A review. *Meat Sci.*, 78(4): 343 (2008).
4. P.T. GARCIA, J.J. CASAL. Effect of dietary plant lipids on conjugated linoleic acid (CLA) concentrations in beef and lamb meats. http://dx.doi.org/10.5772/52608 (2013).
5. C. RIBEIRO, D. OLIVEIRA, S. JUCHEM, T. SILVA, E. ÉLEN SILVEIRA NALÉRIO. Fatty acid profile of meat and milk from small ruminants: a review. *Rev. Bras. Zootecn.*, 40: 121 (2011).
6. D. MIERLITA. Effects of part-time grazing and feeding management on the fatty acid composition and antioxidant capacity in the milk of Turcana ewes. *J. Food Agric. Environ.*, 13(2): 130 (2015).
7. S. ASGARI, K. DAGHIGH. Response of lactating dairy ruminant to different profiles of dietary calcium soaps of fatty acids. *Int. J. Adv. Biol. Biom. Res.*, 1(4): 467 (2013).
8. G. ALEXANDER, Z.P. RAO, J.R. PRASAD. Effect of supplementing sheep with sunflower acid oil or its calcium soap on nutrient utilization. *Asian Austral. J. Anim.*, 15: 1288 (2002).
9. J.K. KRAMER, V. FELLNER, M.E. DUGAN, F.D. SAUER, M.M. MOSSOBA, M.P. YURAWECZ. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total *trans* fatty acids. *Lipids*, 32(11): 1219 (1997).
10. M. SZUMACHER-STRABEL, A. CIESLAK, A. NOWAKOWSKA, A. POTKANSKI. Feeding plant and fish oils to improve polyunsaturated fat concentrations in intramuscular, perirenal and subcutaneous lambs' fat. *Züchtungskunde*, 81(2): 133 (2009).
11. J. FOLCH, M. LEES, G.H.S. STANLEY. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.*, 226: 497 (1957).
12. G. LEPAGE, C.C. ROY. Direct transesterification of all classes of lipid in a one-step reaction. *J. Lipid Res.*, 27: 114 (1986).
13. Y. CHILLIARD, A. FERLAY, J. ROUEL, G. LAMBERET. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. J. Dairy Sci., 86(5): 1751 (2003).
14. T.L. ULBRICHT, D.A. SOUTHGATE. Coronary heart disease: Seven dietary factors. *Lancet*, 338: 985 (1991).
15. SAS. Statistical analysis software, version 9.1.3. SAS Institute Inc., Cary, NC, USA (2006).
16. M. EBRAHIMI, M.A. RAJION, Y.M. GOH. Effects of oils rich in linoleic and α-linolenic acids on fatty acid profile and gene expression in goat meat. *Nutrients*, 6: 3913 (2014).
17. National Research Council. Nutrient requirements of small ruminants. National Research Council, Ottawa, Canada (2007).
18. J.A. BOLES, R.W. KOTT, P.G. HATFIELD, J.W. BERGMAN, C.R. FLYNN. Supplemental safflower oil affects the fatty acid profile, including conjugated linoleic acid, of lamb. *J. Anim. Sci.*, 83: 2175 (2005).
19. E. JERONIMO, S.P. ALVES, S.V. MARTINS, J.A.M. PRATES, R.J.B. BESSA, J. SANTOS-SILVA. Effect of sodium bentonite and vegetable oil blend supplementation on growth, carcass quality and intramuscular fatty acid composition of lambs. *Anim. Feed Sci. Tech.*, 158(3-4): 136 (2010).
20. S. VELASCO, V. CANEQUE, S. LAUZURICA, C. PEREZ, F. HUIDOBRO. Effect of different feeds on meat quality and fatty acid composition of lambs fattened at pasture. *Meat Sci.*, 62: 457 (2004).
21. S.L. COOPER, L.A. SINCLAIR, R.G. WILKINSON, K.G. HALLETT, M. ENSER, D. WOOD. Manipulation of the n-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. *J. Anim. Sci.*, 82: 1461 (2004).
22. T. MANSO, R. BODAS, T. CASTRO, V. JIMENO, A.R. MANTECÓN. Animal performance and fatty acid composition of lambs fed with different vegetable oils. *Meat Sci.*, 83(3): 511 (2009).

23. M.T. DIAZ, I. ALVAREZ, J. DE LA FUENTE, C. SANUDO, M.M. CAMPO, M.A. OLIVER, M. FONT, I. FURNOLS, F. MONTOSSI, R. SAN JULIAN, G.R. NUTE, V. CANEQUE. Fatty acid composition of meat from typical lamb production systems of Spain, United Kingdom, Germany and Uruguay. *Meat Sci.*, 71: 256 (2005).
24. E. ZSÉDELY, A. KIRÁLY, Cs. SZABÓ, K. NÉMETH, O. DÓKA, J. SCHMIDT. Effect of dietary linseed oil soap on lamb meat. *World Acad. Sci. Eng. Technol.*, 6: 229 (2012).
25. A. ARANA, J.A. MENDIZABAL, M. ALZÓN, P. EGUINOA, M.J. BERIAIN, A. PURROY. Effect of feeding lambs oleic acid calcium soaps on growth, adipose tissue development and composition. *Small Rumin. Res.*, 63: 75 (2006).
26. Y.S. PENG, M.A. BROWN, J.P. WU, Z. LIU. Different oilseed supplements alter fatty acid composition of different adipose tissues of adult ewes. *Meat Sci.*, 68: 542 (2010).
27. E.N. PONNAMPALAM, N.J. MANN, A.J. SINCLAIR. Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid and *trans* fatty acids in Australian beef cuts: potential impact on human health. *Asia Pac. J. Clin. Nutr.*, 15(1): 21 (2006).
28. Z. MIR, M.L. RUSHFELDT, P.S. MIR, L.J. PATERSON, R.J. WESELAKE. Effect of dietary supplementation with either conjugated linoleic acid (CLA) or linoleic acid rich oil on the CLA content of lamb tissues. *Small Rumin. Res.*, 36: 25 (2000).
29. R.J.B. BESSA, P.V. PORTUGAL, I.A. MENDES, J. SANTOS-SILVA. Effect of lipid supplementation on growth performance, carcass and meat quality and fatty acid composition of intramuscular lipids of lambs fed dehydrated lucerne or concentrate. *Livest. Prod. Sci.*, 96: 185 (2005).
30. C. MAPIYE, J.L. ALHUS, T.D. TURNER, D.C. ROLLAND, J.A. BASARAB, T.A. MCALLISTER, H.C. BLOCK, B. UTTARO, Ó. LÓPEZ-CAMPOS, S.D. PROCTOR, M.E.R. DUGAN. Effects of feeding flaxseed or sunflower-seed in high-forage diets on beef production, quality and fatty acid composition. *Meat Sci.*, 95: 98 (2013).
31. E. JERÓNIMO, S. ALVES, J. PRATES, J. SANTOS-SILVA, R. BESSA. Effect of dietary replacement of sunflower oil with linseed oil on intramuscular fatty acids of lamb meat. *Meat Sci.*, 83: 499 (2009).
32. F. NOCI, P. FRENCH, F.J. MONAHAN, A.P. MOLONEY. The fatty acid composition of muscle fat and subcutaneous adipose tissue of grazing heifers supplemented with plant oil-enriched concentrates. *J. Anim. Sci.*, 85: 1062 (2007).