



## Fatty acid composition in muscles from lambs fed diets containing agroindustrial co-products

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**ABSTRACT** - The objective of this study was to evaluate the effects of supplementing diet of lambs with agroindustrial co-products on the nutritional quality of meat fat and study the variation of fat quality parameters based on the type of muscle. Twenty lambs were distributed into four experimental groups and fed a control diet or diets containing cottonseed, sunflower meal, and castor cake. After 106 experimental days, the animals were slaughtered. Samples of *Biceps femoris*, *Longissimus dorsi*, *Semimembranosus*, and *Triceps brachii* muscles were taken for fatty acid and cholesterol analyses. Castor cake and sunflower meal diets produced meats with lower levels of n-6 polyunsaturated fatty acids (PUFA) compared with the control diet. Castor cake diet contributed to tissue reduction of n-3 PUFA. Lower conjugated linoleic acid concentration and polyunsaturated:saturated fatty acid (P:S) ratio and higher saturated fatty acids content and Thrombogenicity Index were observed in *Longissimus dorsi* meat. Of the co-products studied, although castor cake diet reduced cholesterol levels in *Biceps femoris* muscle, sunflower meal was identified as the preferred choice for lamb feeding, since it reduced n-6 PUFA without, however, decreasing n-3 PUFA. *Longissimus dorsi* meat had the lowest fat quality indexes. The more oxidative muscles, such as *Biceps femoris*, tend to generate meats with higher cholesterol concentrations.

Key Words: castor cake, cholesterol, cottonseed, sunflower meal

### Introduction

The nutritional quality of animal fat is determined by its fatty acid composition. In the case of ruminants, numerous strategies have been used to promote beneficial changes in the fatty acid profile of meat, such as modification of animal diet.

Currently, a variety of oilseed co-products are used to feed sheep, including castor cake (Alves et al., 2016), sunflower meal (Majewska et al., 2016), and cottonseed (Paim et al., 2014). These co-products, besides being a good source of protein, have a high fat content, consisting of polyunsaturated fatty acids (PUFA). Thus, these co-products

have the potential to improve meat fat, with a direct consequence on its nutritional value and acceptability.

In general, the nutritional improvement of meat fat involves a reduction in the concentration of saturated fatty acids (SFA), which are associated with coronary diseases, and n-6 PUFA, which are pro-inflammatory. Meat quality can also be improved by increasing the concentration of n-3 PUFA and conjugated linoleic acid (CLA) isomers, associated with protection against cancer, diabetes, and cardiovascular diseases (Koba and Yanagita, 2014; Lands 2014; Zong et al., 2016).

Strategies for lipid meat modification are important because red meat consumption has often been related to the development of cardiovascular diseases (Gomide et al., 2013) due to the quantity and quality of its fat.

In this study, we aimed to evaluate the effects of supplementing the diet of lambs with agroindustrial co-products (cottonseed, sunflower meal, and castor cake) on the nutritional quality of meat fat and study the variation of fat quality parameters based on the type of muscle.

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## Material and Methods

All experimental procedures involving animals were conducted in accordance with the Committee of Animal Ethics (case no. 24/2013). The study was conducted in Itapetinga, BA, Brazil (15°14'56" S latitude, 40°12'10" W longitude, and 270 m elevation). Twenty Dorper × Santa Ines lambs, neutered males, approximately 90 days old, and with an average body weight of 20.1±2.5 kg were used. Animals were randomly distributed into four experimental groups. Lambs were kept in individual stalls, equipped with a trough and drinking fountain in a covered stable. Experimental diets were formulated according to the Nutrient Requirements of Small Ruminants (NRC, 2007, Washington, DC, USA) for an average daily gain of 200 g. A conventional concentrate (control diet) was used, based on ground corn, soybean meal, urea, and mineral supplement, with partial substitution of soybean meal and corn with one of three agroindustrial co-products: cottonseed, sunflower meal, or detoxified castor cake with aqueous solution of calcium hydroxide [Ca(OH)<sub>2</sub> 1:10 (w/v)] (Table 1). Diets were formulated to maintain protein and energy balance with a concentrate:voluminous ratio of 50:50 with hay made from Tifton-85 grass (*Cynodon spp*).

Diets were provided *ad libitum*, representing 4.5% of body weight as a complete blend. Animals were fed twice per day, in equal portions, at 7:00 and 15:00 h, admitting

Table 1 - Proportion of ingredients and chemical composition of experimental diets

	Diet <sup>1</sup>			
	Control	CS	SFM	CC
Ingredient (g kg <sup>-1</sup> DM)				
Tifton-85 hay	500.0	500.0	500.0	500.0
Ground corn	365.0	305.0	345.0	345.0
Soybean meal	120.0	80.0	60.0	60.0
Cottonseed	-	100.0	-	-
Sunflower meal	-	-	80.0	-
Castor cake	-	-	-	80.0
Urea	10.0	10.0	10.0	10.0
Mineral supplement <sup>2</sup>	5.0	5.0	5.0	5.0
Chemical composition (g kg <sup>-1</sup> DM)				
Dry matter	861.8	866.3	874.4	858.1
Organic matter	944.1	942.8	942.9	936.7
Crude protein	166.8	166.6	165.6	166.9
Ether extract	28.5	23.4	20.2	22.1
Mineral matter	55.9	57.2	57.1	63.3
Neutral detergent fiber	591.1	594.8	571.1	579.3
Non-fibrous carbohydrates	317.7	317.5	339.9	341.4
Total carbohydrates	748.8	752.8	757.1	747.7

<sup>1</sup> CS: cottonseed; SFM: sunflower meal; CC: castor cake.

<sup>2</sup> Composition (supplement per kg) = calcium, 155 g; phosphorus, 65 g; sodium, 155 g; sulfur, 12 g; magnesium, 6 g; copper, 100 mg; cobalt, 175 mg; iron, 1000 mg; iodine, 175 mg; manganese, 1400 mg; selenium, 27 mg; zinc, 6000 mg; nickel, 42 mg; fluorine, 650 mg.

Source: Silva (2015).

20% of leftovers. The total quantity of feed supplied was adjusted daily, based on the amount of leftovers from the previous day to provide voluntary intake. A trial period of 106 days was established, with 14 days of adaptation to the experimental conditions (diet and management). Animals were identified and dewormed during the adaptation period.

At the end of the experimental period, after a 16-h fasting period, animals were slaughtered using procedures according to Regulations of the Serviço de Inspeção Federal. Slaughter was performed using the method of cerebral concussion desensitization, followed by bleeding, which involved severing the jugular and carotid veins. Animals were skinned and eviscerated with subsequent removal of the paws, head, and testicles, obtaining the entire carcass. Whole carcasses were then chilled at 4 °C for 24 h, longitudinally sectioned, and samples were taken from the *Biceps femoris* (BF), *Longissimus dorsi* (LD), *Semimembranosus* (SM), and *Triceps brachii* (TB) of the right half of each carcass without removal of subcutaneous or intermuscular fat, for physical and chemical analyses; the samples to be analyzed (n = 20) presented a mean fat content of 4.48 g 100 g<sup>-1</sup> (BF), 4.94 g 100 g<sup>-1</sup> (LD), 3.96 g 100 g<sup>-1</sup> (SM), and 4.43 g 100 g<sup>-1</sup> (TB).

The lipid fraction of meat samples was determined using the Bligh and Dyer (1959) method. The lipid fraction of experimental diets was determined using the method proposed by Folch et al. (1957). Lipids were transesterified (method 5509; ISO, 1978) to obtain fatty acid methyl esters (FAME). Approximately 200 mg of fat extracted from meat samples and experimental diets were weighed in screw-cap glass tubes. The following reagents were then added: 2 mL of n-heptane, 1 mL of the methyl tridecanoate (C13:0) solution at 2 mg/mL (internal standard), and 2 mL of a 2 M solution of KOH in methanol. The tube was closed, shaken vigorously for 5 min, and left to rest for 1 h for phase separation. Finally, an aliquot of approximately 1.5 mL from the upper phase was transferred to a vial and stored at -10 °C until chromatographic analysis.

Fatty acid methyl esters were analyzed using a gas chromatograph (Varian CP-3800) equipped with a flame ionization detector and a CP-SIL 88 fused silica capillary column (100 m, 0.25 mm internal diameter and 0.2 µm film). The total chromatographic run time was 71.25 min, divided into three heating ramps as follows: 120 °C (10 min), 190 °C (3 °C min<sup>-1</sup>, 5 min), 210 °C (1 °C min<sup>-1</sup>), and 240 °C (10 °C min<sup>-1</sup>, 10 min). The injector and detector temperatures were 250 and 290 °C, respectively, using a 1:120 split ratio and 1.0 µL injection volume.

The identification of fatty acids was based on a comparison with the retention times of a standard mixture of 37 FAME (18919 Supelco, USA) and linoleic acid geometric isomers c9t11 and t10c12 (O5632 Sigma, USA).

The quantification of fatty acids from meat samples and experimental diets (Table 2) in g 100 g<sup>-1</sup> of total lipids was performed based on Sigma's internal methyl tridecanoate (C13:0) standard, added to the samples at the start of the transesterification procedure.

Cholesterol analysis of meat samples was performed in two stages: sample preparation (direct saponification and extraction of the unsaponifiable fraction), according to the methodology proposed by Saldanha et al. (2006), with some modifications, followed by chromatographic quantification of cholesterol.

Two grams of meat, previously ground, was placed in a test tube with a screw-cap with 4 mL of 50% KOH solution and 6 mL of ethyl alcohol. The mixture was then vortexed for 1 min and kept in the dark for 22 h for the complete saponification reaction. Next, 5 mL of distilled water and 10 mL of hexane p.a. were added. The mixture was vortexed again for 5 min and kept in the dark for 1 h until phase separation. The upper hexane fraction was collected, transferred to a flat-bottomed flask, and evaporated in a rotary evaporator (water bath temperature 33-34 °C). The remaining residue was diluted with 2.5 mL of mobile phase (acetonitrile:isopropanol 85:15 v/v, chromatographic grade), passed through a 25-mm syringe micro-filter (Chromafil®) with a polytetrafluoroethylene (PTFE) filter membrane (pore size 0.45 µm), and analyzed

by high-performance liquid chromatography (HPLC) for quantification of cholesterol.

A liquid chromatograph (Shimadzu) was used, with an analytical column C18 (15 cm × 4.6 mm × 5 µm) coupled to a UV spectrophotometric detector at 202 nm. The oven temperature was adjusted to 40 °C and the analysis time to 10 min. The mobile phase used was acetonitrile:isopropanol 85:15 (v/v), with a constant flow rate of 2.0 mL min<sup>-1</sup>. Injections of 100 µL were performed in duplicate, and cholesterol peak areas were determined using LCSolution® software. Cholesterol was identified by comparing peak retention time of the samples with the cholesterol standard (Cholesterol, code C8667, Sigma-Aldrich®). Quantification (mg 100 g<sup>-1</sup>) was performed by applying the integrated areas of peaks obtained from the equation of the straight line produced on the calibration curve, fitted with the cholesterol standard.

A split-plot scheme in space was used, with the experimental diets (A<sub>i</sub>) completely randomized into plots, and the muscle (B<sub>j</sub>) and the interaction (AB)<sub>ij</sub> in the subplots, with 5 replicates, as follows:

$$Y_{ijk} = \mu + A_i + \delta_{ik} + B_j + (AB)_{ij} + e_{ijk}$$

Data were subjected to analysis of variance (ANOVA). The effect of the plot (A<sub>i</sub>) was evaluated with the experimental error associated with it (δ<sub>ik</sub>), and the effect of the subplot (B<sub>j</sub>) and the interaction (AB)<sub>ij</sub> were tested based on the experimental error of the subplot (e<sub>ijk</sub>). The interaction, when significant (P<0.05), was dismembered, and the following analyses were conducted: for comparison between levels of B at the same level of A, we used e<sub>ijk</sub>; the levels of A at each level of B were tested using a combined error, as proposed by Satterthwait (1946); the means of the factors, in the case of significant dismemberment, were compared using the Tukey test (P<0.05); for non-significant interactions, the means of the individual factors (A and B), when significantly different (P<0.05), were compared using the Tukey test (P<0.05). The GLM procedure from the SAS (Statistical Analysis System, University Edition) package was used for all analyses.

## Results

The use of agroindustrial co-products did not modify (P>0.05) the tissue levels of the main SFA found. Only behenic acid (C22:0) had its concentration reduced (P<0.05) by cottonseed and castor cake diets (Table 3). *Longissimus dorsi* meat had a higher (P<0.05) concentration of palmitic acid (C16:0) than BF meat. A higher (P<0.05) stearic acid (C18:0) concentration was detected in LD than in BF, SM, and TB meats (Table 3). Together, C16:0 and C18:0 represented

Table 2 - Average content (g 100 g<sup>-1</sup>) of fatty acids of the four experimental concentrates

Fatty acid (g 100 g <sup>-1</sup> )	Concentrate <sup>1</sup>			
	Control	CS	SFM	CC
c14:0 (myristic acid)	0.04	0.35	0.07	0.08
c16:0 (palmitic acid)	11.91	16.08	10.36	11.44
c16:1 (palmitoleic acid)	0.11	0.26	0.13	0.12
c17:0 (margaric acid)	0.07	0.09	0.08	0.09
c17:1 (heptadecenoic acid)	0.00	0.03	0.09	0.02
c18:0 (stearic acid)	1.95	2.10	1.98	1.97
c18:1 n9t (elaidic acid)	0.00	0.12	0.00	0.02
c18:1 n9c (oleic acid)	24.84	19.37	24.59	22.96
c18:2 n6c (linoleic acid)	34.90	39.35	30.34	28.58
c20:0 (arachidic acid)	0.49	0.32	0.43	0.46
c20:1 (gadoleic acid)	0.18	0.10	0.17	0.16
c18:3 n3 (linolenic acid)	1.18	0.60	0.79	0.79
c22:0 (behenic acid)	0.22	0.15	0.23	0.20
c20:3 n6 (dihomo-γ-linolenic acid)	0.02	0.00	0.00	0.00
c20:4 n6 (arachidonic acid)	0.00	0.00	0.01	0.00
c24:0 (lignoceric acid)	0.19	0.12	0.18	0.18
c22:6 n3 (docosahexaenoic acid)	0.04	0.01	0.04	0.06
Saturated fatty acids	14.88	19.21	13.34	14.49
Polyunsaturated fatty acids	36.14	39.96	31.18	29.43

<sup>1</sup> CS: cottonseed; SFM: sunflower meal; CC: castor cake.

Table 3 - Average content of saturated fatty acids and their largest grouping in muscles of Dorper × Santa Ines lambs subjected to diets with agroindustrial co-products

Fatty acid (g 100 g <sup>-1</sup> )	Muscle (M)	Diet <sup>1</sup> (D)				Mean±SEM	Pr>F		
		Control	CS	SFM	CC		D	M	D×M
C10:0 (capric acid)	BF	0.07	0.08	0.07	0.07	0.07±0.00	0.642	0.235	0.440
	LD	0.08	0.07	0.09	0.08	0.08±0.00			
	SM	0.07	0.08	0.09	0.07	0.08±0.00			
	TB	0.08	0.07	0.09	0.07	0.08±0.00			
	Mean±SEM	0.07±0.00	0.07±0.00	0.08±0.00	0.07±0.00				
C12:0 (lauric acid)	BF	0.08	0.12	0.07	0.09	0.09A±0.01	0.548	0.002	0.267
	LD	0.05	0.07	0.07	0.06	0.06B±0.01			
	SM	0.06	0.08	0.09	0.07	0.07AB±0.01			
	TB	0.06	0.09	0.09	0.07	0.08AB±0.01			
	Mean±SEM	0.06±0.00	0.09±0.01	0.08±0.01	0.07±0.01				
C14:0 (myristic acid)	BF	1.75	1.99	1.54	1.70	1.74±0.10	0.874	0.066	0.304
	LD	1.55	1.69	1.79	1.67	1.67±0.09			
	SM	1.76	1.96	2.03	1.63	1.84±0.10			
	TB	1.90	1.90	1.92	1.81	1.88±0.09			
	Mean±SEM	1.74±0.08	1.88±0.05	1.82±0.13	1.70±0.10				
C15:0 (pentadecylic acid)	BF	0.30	0.35	0.23	0.29	0.29A±0.02	0.368	0.001	0.427
	LD	0.22	0.25	0.23	0.22	0.23B±0.01			
	SM	0.28	0.35	0.29	0.27	0.30A±0.02			
	TB	0.26	0.30	0.27	0.27	0.28A±0.01			
	Mean±SEM	0.27±0.01	0.31±0.01	0.25±0.01	0.27±0.02				
C16:0 (palmitic acid)	BF	18.74	18.41	17.57	17.86	18.14B±0.50	0.645	0.021	0.967
	LD	22.45	19.84	20.84	19.93	20.76A±1.03			
	SM	19.85	17.52	19.96	17.51	18.71AB±0.66			
	TB	20.26	18.42	19.61	18.70	19.25AB±0.67			
	Mean±SEM	20.32±1.17	18.55±0.52	19.50±0.56	18.50±0.55				
C17:0 (margaric acid)	BF	0.84	0.90	0.73	0.87	0.84±0.03	0.631	0.358	0.390
	LD	0.70	0.80	0.76	0.75	0.78±0.03			
	SM	0.78	0.91	0.87	0.85	0.85±0.03			
	TB	0.76	0.80	0.84	1.02	0.85±0.05			
	Mean±SEM	0.79±0.03	0.85±0.03	0.80±0.03	0.87±0.05				
C18:0 (stearic acid)	BF	14.46	15.35	11.61	12.25	13.42B±0.65	0.094	<0.001	0.419
	LD	18.37	18.38	14.15	13.45	16.09A±1.13			
	SM	11.82	13.59	12.53	11.11	12.26B±0.42			
	TB	11.00	13.36	12.31	10.32	11.75B±0.49			
	Mean±SEM	13.91±1.22	15.17±0.61	12.65±0.47	11.78.0±0.60				
C20:0 (arachidic acid)	BF	0.09	0.08	0.06	0.08	0.08A±0.00	0.717	0.000	0.412
	LD	0.09	0.08	0.07	0.08	0.08A±0.00			
	SM	0.06	0.06	0.07	0.06	0.06B±0.00			
	TB	0.06	0.06	0.06	0.07	0.06B±0.00			
	Mean±SEM	0.07±0.00	0.07±0.00	0.07±0.00	0.07±0.00				
C22:0 (behenic acid)	BF	0.15	0.09	0.17	0.11	0.13A±0.01	0.001	0.023	0.277
	LD	0.14	0.08	0.10	0.09	0.10B±0.01			
	SM	0.16	0.11	0.11	0.10	0.12AB±0.01			
	TB	0.17	0.09	0.15	0.09	0.12AB±0.01			
	Mean±SEM	0.15a±0.01	0.09c±0.01	0.13ab±0.01	0.10bc±0.00				
Saturated fatty acids	BF	36.48	37.36	32.07	33.32	34.81B±1.10	0.529	0.003	0.822
	LD	43.72	41.27	38.11	36.34	39.86A±2.11			
	SM	34.85	34.65	36.04	31.66	34.30B±0.96			
	TB	34.55	35.10	35.35	32.42	34.35B±1.16			
	Mean±SEM	37.40±2.29	37.10±0.96	35.3.92±1.06	33.44±1.12				

BF - *Biceps femoris*; LD - *Longissimus dorsi*; SM - *Semimembranosus*; TB - *Triceps brachii*.

<sup>1</sup> CS: cottonseed; SFM: sunflower meal; CC: castor cake.

Mean (n=20) ± standard error of the mean.

Means followed by the same letter in the rows (a-c) or columns (A-C) do not differ (P>0.05) by the Tukey test.

about 91% of the SFA and 44% of all identified fatty acids. In general, higher ( $P<0.05$ ) SFA concentration was detected in LD than in BF, SM, and TB meats (Table 3).

The castor cake and sunflower meal diets promoted higher ( $P<0.05$ )  $\Delta$ -9 desaturase C18 activity compared with the cottonseed diet. Minor ( $P<0.05$ )  $\Delta$ -9 desaturase activity was verified in LD than in BF, SM, and TB meats (Table 4).

As for SFA, the agroindustrial co-products used did not modify ( $P<0.05$ ) the tissue levels of the main MUFA found. Only gadolenic acid (C20:1) had its concentration reduced with inclusion of cottonseed. We detected no significant differences ( $P>0.05$ ) in the total MUFA concentrations between the experimental diets or tested meats (Table 5). Oleic acid (C18:1n9c) represented about 91.31% of the MUFA and 44.51% of all identified fatty acids.

*Longissimus dorsi* meat had lower ( $P<0.05$ ) concentrations of myristoleic (C14:1) and palmitoleic (C16:1) acids compared with SM and TB, and a lower ( $P<0.05$ ) concentration of heptadecanoic acid (C17:1) than BF, SM, and TB (Table 5).

For PUFA, the control and cottonseed diets promoted higher ( $P<0.05$ ) concentrations of C18:2n6c and total PUFA compared with the castor cake diet. We detected no significant differences ( $P>0.05$ ) in the C18:3n3 concentrations among the experimental diets. Higher ( $P<0.05$ ) concentration of arachidonic acid (C20:4n6) was observed in the animals receiving the control diet. The castor cake and sunflower meal diets resulted in lower ( $P<0.05$ ) tissue concentrations of total n-6 PUFA than the

control diet. Lower ( $P<0.05$ ) tissue concentration of n-3 PUFA was observed in the animals receiving the castor cake diet compared with those that received the control diet (Table 6).

Meat of BF showed the highest ( $P<0.05$ ) concentration of linoleic (C18:2n6c) and linolenic (C18:3n3) acids than meat of LD, SM, and TB. Lower ( $P<0.05$ ) concentrations of CLA, represented by C18: 2n9c11t isomer, and total PUFA were observed in LD than in BF, SM, and TB meats. For the n-3 and n-6 PUFA, the LD meat had a lower ( $P<0.05$ ) concentration than the BF meat (Table 6).

We detected a larger ( $P<0.05$ ) n6:n3 ratio in TB than in SM meat. A lower ( $P<0.05$ ) polyunsaturated:saturated fatty acid (P:S) ratio was observed in LD than in BF, SM, and TB meats (Table 7).

An effect of an interaction ( $P<0.05$ ) was observed for the cholesterol concentration. With the cottonseed diet, BF muscle meat contained the highest cholesterol concentration. With the control and sunflower meal diets, BF muscle meat contained the highest cholesterol concentration than SM and TB. On the other hand, in the BF muscle, lower cholesterol concentration was verified with the castor cake diet compared with the other diets (Table 7).

## Discussion

Of the SFA, greater attention has been given to the C16:0 acid because of its potential for hypercholesterolemic action (Zock et al., 1994), and to C18:0, which reduces

Table 4 - Indexes of desaturase activities in muscles of Dorper  $\times$  Santa Ines lambs subjected to diets with agroindustrial co-products

	Muscle (M)	Diet <sup>1</sup> (D)				Mean $\pm$ SEM	Pr>F		
		Control	CS	SFM	CC		D	M	D $\times$ M
$\Delta$ -9 desaturase C14	BF	2.80	2.61	3.39	2.73	2.88B $\pm$ 0.16	0.148	<0.001	0.098
	LD	2.23	1.63	2.87	2.57	2.32C $\pm$ 0.13			
	SM	3.75	3.48	3.24	3.50	3.49A $\pm$ 0.16			
	TB	3.91	3.05	3.44	3.37	3.44A $\pm$ 0.14			
	Mean $\pm$ SEM	3.17 $\pm$ 0.21	2.69 $\pm$ 0.18	3.24 $\pm$ 0.15	3.04 $\pm$ 0.16				
$\Delta$ -9 desaturase C16	BF	7.57	6.70	7.64	7.54	7.36A $\pm$ 0.18	0.608	<0.001	0.087
	LD	5.64	5.41	6.58	6.62	6.06B $\pm$ 0.21			
	SM	8.04	8.43	7.67	7.70	7.96A $\pm$ 0.28			
	TB	8.81	7.37	7.33	7.14	7.66A $\pm$ 0.30			
	Mean $\pm$ SEM	7.51 $\pm$ 0.33	6.98 $\pm$ 0.37	7.30 $\pm$ 0.15	7.25 $\pm$ 0.28				
$\Delta$ -9 desaturase C18	BF	69.18	67.55	75.08	72.69	71.12B $\pm$ 1.23	0.002	<0.001	0.053
	LD	64.30	62.95	72.09	71.36	67.67C $\pm$ 1.23			
	SM	73.97	70.46	73.54	73.97	72.93AB $\pm$ 0.61			
	TB	75.72	69.82	73.26	76.71	73.67A $\pm$ 0.78			
	Mean $\pm$ SEM	70.79ab $\pm$ 1.31	67.69b $\pm$ 1.11	73.49a $\pm$ 7.56	73.68a $\pm$ 0.81				

BF - *Biceps femoris*; LD - *Longissimus dorsi*; SM - *Semimembranosus*; TB - *Triceps brachii*.

<sup>1</sup> CS: cottonseed; SFM: sunflower meal; CC: castor cake.

Mean (n=20)  $\pm$  standard error of the mean.

Means followed by the same letter in the rows (a-c) or columns (A-C) do not differ ( $P>0.05$ ) by the Tukey test.

$\Delta$ -9 desaturase C14 = 100 [C14:1 / (C14:1 + C14:0)];  $\Delta$ -9 desaturase C16 = 100 [C16:1 / (C16:1 + C16:0)];  $\Delta$ -9 desaturase C18 = 100 [C18:1n9c / (C18:1n9c + 18:0)].

HDL cholesterol levels (Schwab et al., 1996). Variation in the tissue concentration of C16:0 is mainly due to the endogenous synthesis of fatty acids, known as new synthesis or lipogenesis, which, in non-lactating ruminants, occurs mainly in adipose tissue. For this process, 50-80% of all required NADPH is derived from glucose oxidation via the pentose phosphate pathway (Nafikov and Beitz, 2007). Thus, predominantly oxidative muscles such as BF tend to provide lower C16:0 levels, because they present a lower concentration of glycogen compared with muscles of intermediate to glycolytic metabolic activity, such as LD (Briand et al., 1981; Gomide et al., 2013; Johnsen et al., 2013). The higher tissue concentration of C18:0 in LD meat is related to the lower

indexes of  $\Delta$ -9 desaturase (Table 4), which is responsible for C18:0 desaturation to C18:1n9c (Choi et al., 2013).

The lower concentrations of C14:1 and C16:1 in the LD than in SM and TB meats (Table 5) might be associated with its lower  $\Delta$ 9 desaturase C14 and C16 activities (Table 4). According to Smith et al. (2009), only  $\Delta$ -9 desaturase is able to act on SFA by converting them to MUFA.

The highest concentration of C18:2n6c and C18:3n3, which occurred in BF meat (Table 6), may be related to its muscle metabolism. In sheep, the BF muscle is characterized by its oxidative metabolism (Johnsen et al., 2013), with LD and SM considered to have intermediate to glycolytic metabolism (Briand et al., 1981; Ithurralde et al., 2015) and

Table 5 - Average content of monounsaturated fatty acids and their largest group in muscles of Dorper  $\times$  Santa Ines lambs subjected to diets with agroindustrial co-products

Fatty acid (g 100 g <sup>-1</sup> )	Muscle (M)	Diet <sup>1</sup> (D)				Mean $\pm$ SEM	Pr>F		
		Control	CS	SFM	CC		D	M	D $\times$ M
C14:1 (myristoleic acid)	BF	0.05	0.05	0.05	0.05	0.05B $\pm$ 0.00	0.742	<0.001	0.293
	LD	0.03	0.03	0.05	0.04	0.04B $\pm$ 0.00			
	SM	0.07	0.07	0.07	0.06	0.07A $\pm$ 0.00			
	TB	0.08	0.06	0.07	0.06	0.07A $\pm$ 0.00			
	Mean $\pm$ SEM	0.06 $\pm$ 0.00	0.05 $\pm$ 0.00	0.06 $\pm$ 0.00	0.05 $\pm$ 0.00				
C16:1 (palmitoleic acid)	BF	1.53	1.32	1.45	1.44	1.44AB $\pm$ 0.05	0.206	0.001	0.082
	LD	1.26	1.13	1.47	1.41	1.32B $\pm$ 0.05			
	SM	1.74	1.56	1.66	1.46	1.60A $\pm$ 0.05			
	TB	1.92	1.47	1.54	1.46	1.60A $\pm$ 0.08			
	Mean $\pm$ SEM	1.61 $\pm$ 0.08	1.37 $\pm$ 0.06	1.53 $\pm$ 0.05	1.44 $\pm$ 0.06				
C17:1 (heptadecenoic acid)	BF	0.42	0.43	0.48	0.45	0.46A $\pm$ 0.02	0.645	<0.001	0.920
	LD	0.30	0.31	0.37	0.39	0.34B $\pm$ 0.01			
	SM	0.48	0.51	0.51	0.53	0.51A $\pm$ 0.02			
	TB	0.48	0.44	0.47	0.50	0.47A $\pm$ 0.02			
	Mean $\pm$ SEM	0.42 $\pm$ 0.02	0.42 $\pm$ 0.03	0.46 $\pm$ 0.02	0.47 $\pm$ 0.02				
C18:1n9t (elaidic acid)	BF	1.43	1.56	0.84	1.05	1.22 $\pm$ 0.13	0.087	0.253	0.351
	LD	1.02	1.38	0.91	0.97	1.07 $\pm$ 0.07			
	SM	1.10	1.32	0.97	1.00	1.10 $\pm$ 0.07			
	TB	1.02	1.35	1.07	0.95	1.10 $\pm$ 0.06			
	Mean $\pm$ SEM	1.14 $\pm$ 0.12	1.40 $\pm$ 0.05	0.95 $\pm$ 0.06	0.99 $\pm$ 0.06				
C18:1n9c (oleic acid)	BF	32.72	32.33	34.94	32.07	33.02 $\pm$ 0.94	0.665	0.997	0.640
	LD	31.39	31.60	36.28	33.63	33.22 $\pm$ 1.07			
	SM	33.83	32.55	34.77	31.55	33.18 $\pm$ 1.03			
	TB	34.24	31.14	33.66	33.63	33.17 $\pm$ 1.02			
	Mean $\pm$ SEM	33.05 $\pm$ 1.29	31.91 $\pm$ 1.10	34.91 $\pm$ 0.69	32.72 $\pm$ 0.73				
C20:1 (gadoleic acid)	BF	0.04	0.04	0.05	0.05	0.05 $\pm$ 0.00	0.039	0.206	0.929
	LD	0.04	0.03	0.05	0.05	0.04 $\pm$ 0.00			
	SM	0.04	0.03	0.06	0.03	0.04 $\pm$ 0.00			
	TB	0.04	0.03	0.05	0.04	0.04 $\pm$ 0.00			
	Mean $\pm$ SEM	0.04ab $\pm$ 0.00	0.03b $\pm$ 0.00	0.05a $\pm$ 0.00	0.04ab $\pm$ 0.00				
MUFA	BF	36.21	35.75	37.82	35.12	36.22 $\pm$ 0.97	0.721	0.970	0.610
	LD	34.05	34.49	39.15	36.50	36.04 $\pm$ 1.09			
	SM	37.26	36.04	38.04	34.63	36.49 $\pm$ 1.08			
	TB	37.78	34.49	36.86	36.64	36.44 $\pm$ 1.08			
	Mean $\pm$ SEM	36.32 $\pm$ 1.31	35.19 $\pm$ 1.15	37.97 $\pm$ 0.76	35.72 $\pm$ 0.79				

BF - *Biceps femoris*; LD - *Longissimus dorsi*; SM - *Semimembranosus*; TB - *Triceps brachii*; MUFA =  $\Sigma$  monounsaturated fatty acids.

<sup>1</sup> CS: cottonseed; SFM: sunflower meal; CC: castor cake.

Mean (n=20)  $\pm$  standard error of the mean.

Means followed by the same letter in the rows (a-c) or columns (A-C) do not differ (P>0.05) by the Tukey test.

Table 6 - Average content of polyunsaturated fatty acids and their groups in muscles of Dorper × Santa Ines lambs subjected to diets with agroindustrial co-products

Fatty acid (g 100 g <sup>-1</sup> )	Muscle (M)	Diet <sup>1</sup> (D)				Mean±SEM	Pr>F		
		Control	CS	SFM	CC		D	M	D×M
C18:2n6t (linolelaic acid)	BF	0.11	0.13	0.10	0.09	0.11A±0.00	0.013	0.005	0.081
	LD	0.09	0.10	0.09	0.10	0.09B±0.00			
	SM	0.12	0.13	0.10	0.09	0.11A±0.00			
	TB	0.12	0.12	0.09	0.10	0.11A±0.00			
	Mean±SEM	0.11ab±0.01	0.12a±0.00	0.09b±0.00	0.09b±0.00				
C18:2n6c (linoleic acid)	BF	1.70	1.70	1.82	1.30	1.63A±0.08	0.001	<0.001	0.063
	LD	1.34	1.44	1.19	1.11	1.27B±0.05			
	SM	1.69	1.63	1.14	1.16	1.41B±0.07			
	TB	1.69	1.57	1.32	1.16	1.44B±0.07			
	Mean±SEM	1.61a±0.06	1.59a±0.05	1.37ab±0.08	1.18b±0.05				
C18:3n6 (γ-linolenic acid)	BF	0.00	0.01	0.01	0.00	0.01±0.00	0.222	0.899	0.119
	LD	0.01	0.01	0.00	0.01	0.00±0.00			
	SM	0.00	0.01	0.00	0.01	0.01±0.00			
	TB	0.01	0.01	0.00	0.00	0.01±0.00			
	Mean±SEM	0.01±0.00	0.01±0.00	0.00±0.00	0.00±0.00				
C18:3n3 (linolenic acid)	BF	0.20	0.18	0.18	0.16	0.18A±0.01	0.356	<0.001	0.875
	LD	0.15	0.13	0.14	0.14	0.14B±0.00			
	SM	0.18	0.16	0.15	0.15	0.16B±0.01			
	TB	0.17	0.14	0.16	0.14	0.15B±0.01			
	Mean±SEM	0.17±0.01	0.15±0.01	0.16±0.01	0.15±0.01				
CLA (cis9-trans11) (ruminic acid)	BF	0.34	0.40	0.29	0.30	0.33A±0.02	0.327	<0.001	0.543
	LD	0.23	0.28	0.25	0.24	0.25B±0.01			
	SM	0.30	0.38	0.29	0.28	0.31A±0.02			
	TB	0.29	0.35	0.30	0.30	0.31A±0.01			
	Mean±SEM	0.29±0.02	0.35±0.01	0.28±0.02	0.28±0.01				
C20:3n6 (dihomo-γ-linolenic acid)	BF	0.03a	0.01ab	0.03aA	0.00b	0.02±0.00	0.015	0.041	0.041
	LD	0.02	0.01	0.01B	0.01	0.01±0.00			
	SM	0.04a	0.03ab	0.01bA	0.2b	0.03±0.00			
	TB	0.04a	0.02ab	0.03abA	0.01b	0.02±0.00			
	Mean±SEM	0.03±0.00	0.02±0.00	0.02±0.00	0.01±0.00				
C20:4n6 (arachidonic acid)	BF	0.37	0.20	0.32	0.19	0.27±0.03	0.001	0.081	0.512
	LD	0.28	0.19	0.18	0.17	0.21±0.02			
	SM	0.38	0.32	0.22	0.24	0.29±0.02			
	TB	0.46	0.22	0.35	0.18	0.30±0.04			
	Mean±SEM	0.37a±0.04	0.23b±0.00	0.27b±0.02	0.20b±0.01				
C20:5n3 (eicosapentaenoic acid - EPA)	BF	0.03	0.01	0.02	0.01	0.02AB±0.00	0.013	0.010	0.396
	LD	0.02	0.01	0.00	0.01	0.01B±0.00			
	SM	0.04	0.04	0.02	0.02	0.03A±0.00			
	TB	0.03	0.01	0.04	0.01	0.02AB±0.01			
	Mean±SEM	0.03a±0.00	0.02ab±0.00	0.02ab±0.00	0.01b±0.00				
C22:6n3 (docosahexaenoic acid - DHA)	BF	0.01	0.00	0.00	0.00	0.00±0.00	0.136	0.056	0.167
	LD	0.00	0.00	0.00	0.00	0.00±0.00			
	SM	0.01	0.02	0.01	0.00	0.01±0.00			
	TB	0.02	0.00	0.02	0.00	0.01±0.00			
	Mean±SEM	0.01±0.00	0.00±0.00	0.01±0.00	0.00±0.00				
PUFA	BF	2.79	2.64	2.78	2.07	2.57A±0.12	0.001	<0.001	0.147
	LD	2.15	2.18	1.86	1.79	1.99B±0.08			
	SM	2.78	2.72	1.94	1.97	2.35A±0.11			
	TB	2.83	2.44	2.32	1.92	2.38A±0.12			
	Mean±SEM	2.64a±0.12	2.50a±0.09	2.23ab±0.11	1.94b±0.07				
n-3 PUFA	BF	0.23	0.19	0.21	0.18	0.20A±0.01	0.031	0.003	0.406
	LD	0.18	0.14	0.14	0.15	0.15B±0.01			
	SM	0.24	0.22	0.18	0.17	0.20A±0.01			
	TB	0.22	0.15	0.22	0.15	0.18AB±0.01			
	Mean±SEM	0.22a±0.01	0.17ab±0.01	0.19ab±0.01	0.16b±0.01				

Continues...

Table 6 (Continued)

Fatty acid (g 100 g <sup>-1</sup> )	Muscle (M)	Diet <sup>1</sup> (D)				Mean±SEM	Pr>F		
		Control	CS	SFM	CC		D	M	D×M
n-6 PUFA	BF	2.21	2.05	2.29	1.59	2.04A±0.10	0.000	0.001	0.087
	LD	1.73	1.75	1.47	1.39	1.59B±0.07			
	SM	2.24	2.13	1.47	1.51	1.84AB±0.09			
	TB	2.33	1.94	1.80	1.46	1.88A±0.11			
	Mean±SEM	2.13a±0.10	1.97ab±0.07	1.76bc±0.10	1.50c±0.06				

BF - *Biceps femoris*; LD - *Longissimus dorsi*; SM - *Semimembranosus*; TB - *Triceps brachii*; PUFA =  $\Sigma$  polyunsaturated fatty acids; n-3 PUFA =  $\Sigma$  n-3 polyunsaturated fatty acids; n-6 PUFA =  $\Sigma$  n-6 polyunsaturated fatty acids.

<sup>1</sup> CS: cottonseed; SFM: sunflower meal; CC: castor cake.

Mean (n=20) ± standard error of the mean.

Means followed by the same letter in the rows (a-c) or columns (A-C) do not differ (P>0.05) by the Tukey test.

Table 7 - Quality index of fat and cholesterol content (mg 100 g<sup>-1</sup>) in Dorper × Santa Ines lambs subjected to diets with agroindustrial co-products

	Muscle (M)	Diet <sup>1</sup> (D)				Mean±SEM	Pr>F		
		Control	CS	SFM	CC		D	M	D×M
n-6:n-3	BF	9.69	11.35	10.98	9.64	10.42AB±0.51	0.101	0.026	0.113
	LD	9.68	12.58	10.42	9.44	10.53AB±0.49			
	SM	9.73	10.14	8.56	8.86	9.32B±0.35			
	TB	10.90	12.94	8.79	9.64	10.57A±0.53			
	Mean±SEM	10.00±0.33	11.75±0.56	9.69±0.43	9.39±0.42				
P:S	BF	0.05	0.05	0.07	0.05	0.05A±0.00	0.094	<0.001	0.065
	LD	0.04	0.04	0.04	0.03	0.04B±0.00			
	SM	0.06	0.05	0.04	0.04	0.05A±0.00			
	TB	0.06	0.05	0.04	0.04	0.05A±0.00			
	Mean±SEM	0.05±0.00	0.05±0.00	0.05±0.00	0.04±0.00				
Cholesterol	BF	47.01Aa	51.25Aa	50.99Aa	41.25b	47.63±1.05	0.003	<0.001	0.042
	LD	43.77AB	46.14B	48.06AB	42.82	45.20±0.79			
	SM	39.27B	41.81B	44.71B	41.56	41.84±0.92			
	TB	40.58B	45.10B	45.26B	41.85	43.20±0.88			
	Mean±SEM	42.66±1.01	46.07±0.92	47.26±0.96	41.87±0.70				

BF - *Biceps femoris*; LD - *Longissimus dorsi*; SM - *Semimembranosus*; TB - *Triceps brachii*; n-6:n-3 =  $\Sigma$ n-6 PUFA: $\Sigma$ n-3 PUFA; P:S = (C18:2n6c + C18:3n3):(C14:0 + C16:0 + C18:0).

<sup>1</sup> CS: cottonseed; SFM: sunflower meal; CC: castor cake.

Mean (n=20) ± standard error of the mean.

Means followed by the same letter in the rows (a-c) or columns (A-C) do not differ (P>0.05) by the Tukey test.

TB ranging from intermediate to oxidative (Ithurralde et al., 2015). According to Wood et al. (2003), most oxidative muscles present a higher proportion of PUFA, represented mainly by C18:2n6c and C18:3n3 acids, due to their higher phospholipidic fraction. C18:1n6c and C18:3n3 acids are precursors of n-6 and n-3 PUFA and, therefore, contribute to a higher proportion of n-6 and n-3 PUFA in the BF than in the LD (Table 6).

The lowest  $\Delta$ -9 desaturase C18 activity in LD meat (Table 4) may explain its lower concentration of CLA. At a tissue level, the synthesis of CLA occurs through the endogenous conversion of the vaccenic acid (C18:1t11) by the action of enzyme  $\Delta$ -9 desaturase (Koba and Yanagita, 2014).

Higher concentration of C18:2n6c in animals receiving control and cottonseed diets compared with castor cake diet (Table 6) resulted from the higher supply of C18:2n6c of

these diets (Table 2). In contrast to C18:2n6c, the higher content of C18:3n3 in the control diet concentrate (Table 2) did not modify (P>0.05) the percentage of this fatty acid in the tissues. This might be due to the fact that C18:2n6c is preferably incorporated into the phospholipidic fraction of muscle tissue relative to C18:3n3 (Wood et al., 2008). Thus, in diets with high C18:2n6c ratios, such as the control diet, there tends to be more difficulty incorporating C18:3n3.

Higher tissue concentration of C20:4n6 in the animals receiving the control diet was an expected result, considering that this diet provided the highest concentration of C18:2n6c (Table 2), a fatty acid precursor of n-6, generated by the  $\Delta$ -5 and  $\Delta$ -6 desaturases (Wood et al., 2008). However, the same effect was not observed with the cottonseed diet, which might be related to  $\Delta$ -5 and  $\Delta$ -6 desaturase enzymes, as well as  $\Delta$ -9 desaturase, having



reduced activity in the presence of cyclopropenoid fatty acids, such as malvalic and sterculic, which are present in cotton oil (Cao et al., 1993; Bichi et al., 2012).

Higher content of C18:2n6c in control and cottonseed diets (Table 2) provided a higher tissue concentration of C18:2n6c, as well as n-6 PUFA, compared with castor cake diet (Table 6). In addition, the control diet provided a higher tissue concentration of n-3 PUFA than did castor cake diet (Table 6), which can be explained by the greater supply of C18:3n3 by the control (Table 2).

Despite the variation between the muscles in terms of the n-6:n-3 and P:S ratios (Table 7), which act as indicators of the risk of chronic inflammatory diseases, all had values outside the standards established for human nutrition, namely less than 4 for n-6:n-3 and higher than 0.45 for P:S (Wood et al., 2008).

Cholesterol concentration in muscles is closely related to its metabolic activity. More oxidative muscles have a higher tissue cholesterol concentration due to their higher phospholipid ratio (Chizzolini et al., 1999). Of the muscles studied, BF has the highest oxidative metabolic characteristics. The *Longissimos dorsi* and SM are considered as intermediate metabolic muscles (they have high glycolytic and oxidative activity) (Briand et al., 1981). *Triceps brachii* is characterized, depending on the muscle fraction analyzed (*caput longum* or *laterale*), as having intermediate to oxidative metabolism (Ithurralde et al., 2015). Thus, the grouping tendency of LD, SM, and TB muscles in the same category of metabolic activity might account for the absence of variation in the cholesterol concentration among these muscles, and their differentiation relative to the BF, as evidenced with the cottonseed diet (Table 7).

The lower tissue cholesterol concentration promoted by the castor cake diet compared with other diets (Table 7) might not be directly related to its fatty acid profile. According to Majewska et al. (2016), dietary fatty acids in sheep have little or no relation to the tissue cholesterol concentration, but have a direct effect on the plasma. The cholesterol concentration in the plasma membrane is closely related to the intracellular transport of ricin. Grimmer et al. (2000) reported that the removal of cholesterol from the plasma membrane reduces the endocytosis of ricin (which, once inside the cell, shows a toxic effect), in addition to increasing its degradation by 40%.

Thus, the possible residual presence of ricin in castor cake, even after detoxification treatment, might have promoted modifications in tissue synthesis of cholesterol to reduce endocytosis and increase ricin degradation.

## Conclusions

Of the co-products investigated in this study, sunflower meal is the most suitable for replacing conventional concentrate, providing meat with lower concentrations of n-6 PUFA without reducing the levels of n-3 PUFA as observed in the castor cake diet. In addition, the meat from *Longissimus dorsi* muscle has the lowest fat quality indexes (e.g., conjugated linoleic acid and P:S ratio), as well as the highest saturated fatty acid levels. We found that the more oxidative muscles (e.g., *Biceps femoris* muscle) tend to generate cholesterol-rich meats.

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