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Effect of the diet on performance characteristics and quality of meat fat of lamb by principal component analysis

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ABSTRACT. The objective of this study was to evaluate the effect of use of agroindustrial co-products in the diet of lambs on the performance and nutritional quality of meat fat. Twenty lambs were distributed into four experimental groups: control diet (C) or diets containing cottonseed (CS), sunflower meal (SFM), and castor cake (CC). During the experimental period, data on dry matter consumption (DMC) and total weight gain (TWG) were collected. After slaughter, samples of *Logissimus dorsi* (LD) muscle were taken for fatty acids and cholesterol analysis. Diets C and CS provided similar characteristics of fat quality and animal performance. However, SFM and CC diets tend to form isolated groups, with different fat and performance characteristics. The SFM diet results in meat with a higher saturated fatty acids and cholesterol content. The CC diet is related to improved performance characteristics and lower cholesterol content. In conclusion, the inclusion of CC in the experimental diets provided, in the proportions used in this experiment, better characteristics of animal performance and meat fat quality.

Keywords: castor cake; cholesterol; cottonseed; fatty acids; principal component analysis; sunflower meal.

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Introduction

The quantity and quality of fat in products of animal origin have been widely questioned from the point of view of human health. Ruminant meat, specially sheep, is basically made up of 45% to 54% saturated fatty acids (SFA), 39% to 47% monounsaturated fatty acids (MUFA) and 5% to 11% polyunsaturated fatty acids (PUFA) (Carneiro et al., 2016; Liu et al., 2015; Alizadeh, Shahneh, Yousefi, Omran, & Campbel, 2013; D'Alessandro, Maiorano, Kowaliszyn, Loiudice, & Martemucci, 2012).

Of these, SFA are related to an increase in blood cholesterol (Noto et al., 2016), while n-6 PUFA, which represents about 87% of all PUFA in sheep meat (Paim et al., 2014), have pro-inflammatory action. Reducing the concentration of these harmful health acids, cholesterol, as well as increasing the participation of n-3 PUFA, with anti-inflammatory effect (Grosso et al., 2014; Patterson, Wall, Fitzgerald, Ross, & Stanton, 2012; Simopulos, 2010), it is important to improve the fatty fraction of the meat.

Some strategies have been used seeking to obtain a meat fat with better nutritional quality, such as the manipulation of the animal diet. Today, there is a variety of foods with the potential to modify the tissue lipid profile and that can be used in the feeding of sheep, such as, for example, the co-products of oilseed processing: castor cake (Furtado et al., 2020; Alves et al., 2016; Pompeu et al., 2012) and sunflower meal (Haro, Gonzalez, Evan, Fuente, & Carro, 2019), and those of the textile industry, such as cottonseed (Paim et al., 2019; Paim et al., 2014; Dayani, Dadvar, & Afsharmanesh, 2011). However, the process of inserting alternative products in animal feed must seek, as well as the improvement of meat quality, maintenance or gain in terms of animal performance. Thus, the study of the relationship between field variables and meat quality data is extremely important to consider the best diet to be used in animal feed.

An important statistical tool capable of simultaneously evaluating more than one measure in individuals or objects under investigation is the Principal Component Analysis (PCA) multivariate technique. This

analysis allows the description of a large number of original variables from a smaller number of hypothetical variables (principal components), without significant loss of the original information (Pacheco, Casado, & Porras, 2013; Hair, Black, Babin, & Anderson, 2010).

Therefore, the hypotheses in the present study were: the use of agroindustrial by-products, in partial replacement to conventional concentrates, promoting the improvement of the quality of meat fat and animal performance.

The objective of this study was to evaluate the effect of the use of agroindustrial co-products in the diet of lambs on the performance and nutritional quality of meat fat by Principal Component Analysis.

Material and methods

All experimental procedures involving animals were conducted in accordance with the Committee of Animal Ethics (case No. 24/2013) of the State University of Southwest Bahia.

The study was conducted in Itapetinga, Bahia State, Brazil (15°14′56" S latitude, 40°12′10" W longitude, and 270 m elevation). Twenty Dorper x Santa Inês lambs, neutered males, approximately 90 days old, and with an average body weight of 20.1 ± 2.5 kg were used. The experiment was conducted in an completely randomized design, with the animals distributed in four experimental groups. Lambs were kept in individual stalls (1.5 x 1.0 m), equipped with a trough and drinking fountain in a covered stable.

Experimental diets (Table 1) were formulated according to the Nutrient Requirements of Small Ruminants (National Research Council [NRC], 2007) 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 [$Ca(OH)_2$ 1:10 (w/v)]. 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.).

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

	Diets[1]			
	C	CS	SFM	CC
Ingredient (g kg^{-1} Dry matter)				
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[2]	5.0	5.0	5.0	5.0
Chemical composition (g kg^{-1} Dry matter)				
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

[1]C: control; CS: cottonseed; SFM: sunflower meal; CC: castor cake. [2]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, 1,000 mg; iodine, 175 mg; manganese, 1,400 mg; selenium, 27 mg; zinc, 6,000 mg; nickel, 42 mg; fluorine, 650 mg.

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 am and 3:00 pm, admitting 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. The dry matter consumption and performance data are presented in Table 2.

Table 2. Dry matter intake and performance parameters of Dorper x Santa Inês lambs submitted to experimental diets with agroindustrial co-products.

Parameters	Diets				Mean ± MSE
	C	CS	SFM	CC	
ILW (kg)	19.58	19.59	21.02	19.20	20.07±0.55
FLW (kg)	34.27	35.67	35.89	38.51	36.08±0.57
ADG (kg day^{-1})	0.16	0.17	0.15	0.21	16.05±0.69
TWG (kg)	14.86	16.08	13.97	19.31	0.17±0.01
DMI					
(kg day^{-1})	1.08	1.07	1.09	1.26	1.13±0.04
(%BW)	3.92	3.65	3.88	4.45	3.98±0.09
g (kg^{-1})$^{-0.75}$	89.60	84.74	89.50	102.47	91.58±1.90

Mean (n = 5) ± Mean standard error; C: control; CS: cottonseed; SFM: sunflower meal; CC: castor cake; ILW = Initial Live Weight; FLW = Final Live Weight; ADG = Average Daily Gain; TWG = Total Weight Gain; DMI = dry matter intake; BW = body weight.

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 (Brasil, 1997). 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 24h, longitudinally sectioned, and samples were taken from the *Longissimus dorsi* (LD) of the right half of each carcass without removal of subcutaneous or intermuscular fat, for physical and chemical analyses.

The lipid fraction of LD was determined using the Bligh and Dyer (1959) method. The lipid fraction of experimental diets was determined using the method proposed by Folch, Lees, and Stanley (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^{-1} (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 1h 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 in three heating ramps as follows: 120°C (10 min.), 190°C (3°C min.$^{-1}$, 5 min.), 210°C (1°C min.$^{-1}$), and 240°C (10°C min.$^{-1}$, 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 (FA) from LD and experimental diets (Table 3) in g 100 g^{-1} 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. Calculations of the concentration of fatty acids contained in the samples were performed according to the equation proposed by Visentainer and Franco (2006):

$$\text{FA}\left(\text{g } 100\text{ g}^{-1}\right) = \frac{A_x.M_{13:0}.F_{RT}}{A_{13:0}.M_A.F_{CT}}$$

where:

A_x = Area of fatty acid methyl esters;

$A_{13:0}$ = Internal standard área;

$M_{13:0}$ = Mass of the internal standard added to the sample (mg);

M_A = Sample mass (g);

F_{RT} = Theoretical response factor of fatty acid methyl esters;

F_{CT} = Conversion factor to express the results in mg de FA g^{-1} de total lipids.

Table 3. Average content (g 100 g^{-1}) of fatty acids of the four experimental concentrates.

Fatty acids (g 100 g^{-1})	C	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

C: control; CS: cottonseed; SFM: sunflower meal; CC: castor cake.

Fatty acids, once identified and quantified, were used to determine the total amount (g 100 g^{-1}) of saturated fatty acids (∑SFA), monounsaturated (∑MUFA), polyunsaturated (∑PUFA), ∑n-3 PUFA and ∑n-6 PUFA. Some indices of nutritional importance were also calculated, including the ratio between ∑n-6 PUFA and ∑n-3 PUFA (n-6/n-3) and the ratio between PUFA and SFA (P/S), according to equation proposed by Enser, Hewitt, Fursey, and Wood (1996). The equations are shown below:

$$\text{n-6/n-3} = \frac{\sum \text{n-6 PUFA}}{\sum \text{n-3 PUFA}}$$

$$\text{P/S} = \frac{\text{C18:2n6c + C18:3n3}}{\text{C14:0 + C16:0 + C18:0}}$$

where: C18:2n6c = linoleic acid (g 100 g^{-1}), C18:3n3 = linolenic acid (g 100 g^{-1}), C14:0 = myristic acid (g 100 g^{-1}), C16:0 = palmitic acid (g 100 g^{-1}), C18:0 = stearic acid (g 100 g^{-1}).

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, Sawaya, Eberlin, and Bragagnolo (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 22h 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 1h 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 $min.^{-1}$. 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^{-1}) 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.

The Kaiser-Meyer-Olkin (KMO) and Bartlett's Sphericity tests were performed in the IBM SPSS statistical package, in order to verify the fit of the data to the Principal Component Analysis (PCA). The KMO value

found was 0.52. According to Faria, Burnquist, and Pestana (2011), KMO values below 0.5 indicate that the use of factor analysis is inappropriate. Bartlett's sphericity test was significant at 1% ($p < 0.000$), rejecting the null hypothesis that the correlation matrix is an identity matrix. Thus, these results meet the assumptions of using Principal Component Analysis.

ACP was performed by correlation matrix using the Statistica 6.0 program (StatSoft, Inc., Tulsa, OK, USA).

Results and discussion

The principal components with their respective eigenvalues and accumulated variance are shown in Table 4. Only the first four principal components were maintained in the experiment, as they have eigenvalues greater than 1, according to the criteria established by Kaiser for a correlation matrix (Braeken & van Assen, 2017). Together, these principal components kept 86.98% of the information contained in the original data, thus promoting a structural simplification of the data, while preserving the original information.

Table 4. Eigenvalues and percentage of explained and cumulative variance over the Principal Components (PC).

PC	Eigenvalues	Variance Explained (%)	Cumulative Variance (%)
1	3.88	38.82	38.82
2	2.28	22.80	61.62
3	1.46	14.63	76.25
4	1.07	10.74	86.98
5	0.59	5.86	92.84
6	0.49	4.86	97.70
7	0.18	1.82	99.52
8	0.04	0.39	99.91
9	0.01	0.08	99.99
10	0.00	0.01	100.00

The first principal component (PC1), which is equivalent to 38.82% of the total variation of the data (Table 4), is mainly represented by the original variables of higher weights: n-6 (-0.94) and PUFA (- 0.93) (Table 5); the grouping of these variables in a given PC was already expected, in view of the significant correlation ($p < 0.05$) between them (data not shown). Thus, PC1 fundamentally reflects polyunsaturated fatty acids, particularly n-6 PUFA, with a pro-inflammatory action (Grosso et al., 2014; Patterson et al., 2012); n-6 PUFA contributed approximately 80% of all PUFA (data not shown).

PC2, with 22.80% of the total variation of the data (Table 4), presented cholesterol (-0.82) and SFA (0.80) as predominant variables (Table 5). Such variables have a significant and directly proportional correlation (data not shown). Thus, PC2 can be interpreted as a negative index of meat fat quality, considering that the increase in blood cholesterol has been related to the consumption of saturated fatty acids (SFA) (Noto et al., 2016).

PC3, representing 14.63% of the original variations (Table 4), is basically a comparison between the group of n-3 PUFA (-0.85), with anti-inflammatory action, and the n-6/n-3 ratio (0.81) (Table 5), which, in high values, is associated with an increased risk of chronic inflammatory diseases (Grosso et al., 2014; Patterson et al., 2012).

Table 5. Weights of the variables under study in the first four principal components (PC).

Variables	PC1	PC2	PC3	PC4
SFA	-0.04	-0.80	0.12	0.47
MUFA	0.35	-0.68	0.04	0.11
PUFA	-0.93	-0.13	-0.13	0.31
P/S	-0.87	0.40	-0.03	-0.16
n-3	-0.49	-0.14	-0.85	0.07
n-6	-0.94	-0.07	-0.04	0.29
n-6/n-3	-0.52	0.10	0.81	0.24
Chol	0.11	-0.82	-0.03	-0.23
TWG	0.68	0.39	-0.17	0.43
DMI	0.51	0.36	-0.14	0.57

SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; n-3=∑n-3 PUFA; n-6=∑n-6 PUFA; P/S=(C18:2n6+C18:3n3)/(C14:0+C16:0+C18:0); n6/n3=∑n-6 PUFA/∑n-3 PUFA; Chol = cholesterol; TWG = Total Weight Gain; DMI = dry matter intake; BW = body weight.

Finally, PC4 can be interpreted as an index of consumption, since the variable with the highest weight was DMI (0.57) (Table 5);. According to Alves et al. (2016), Pompeu et al. (2012) and Sniffen, Beverly, Mooney, Roe, and Skidmore (1993), the consumption of dry matter can be decisive for the supply of nutrients necessary to meet the requirements of maintenance and weight gain of the animals.

When analyzing the graph of dispersion of experimental diets between the first two principal components (Figure 1a), it is observed that diets C and CS tend to form a single group, with similar characteristics of fat quality and animal performance. However, the SFM and CC diets form isolated groups, with different fat and performance characteristics.

When relating, along the abscissa axis (PC1), Figure 1(a and b), it is observed that diets C and CS tend to provide mainly fats with a higher rate of pro-inflammatory fatty acids (n-6 PUFA) , compared to the SFM and CC diets. Such result can be justified by the greater supply of linoleic acid (C18:2n6) by diets C and CS, in relation to diets SFM and CC (Table 3). C18:2n6 is considered an essential fatty acid and precursor to n-6 PUFA (Syadati, Mirzaei-Aghsaghali, Fathi, & Davuodi, 2012).

When the analysis is performed by the ordinate axis (PC2), it appears that the SFM diet leads to the production of a more saturated fat with a higher cholesterol content, compared to the C, CS, and CC diets. Of these, the CC diet has, basically, all its points on the positive PC2 scale, indicating the lowest contribution to the elevation of cholesterol.

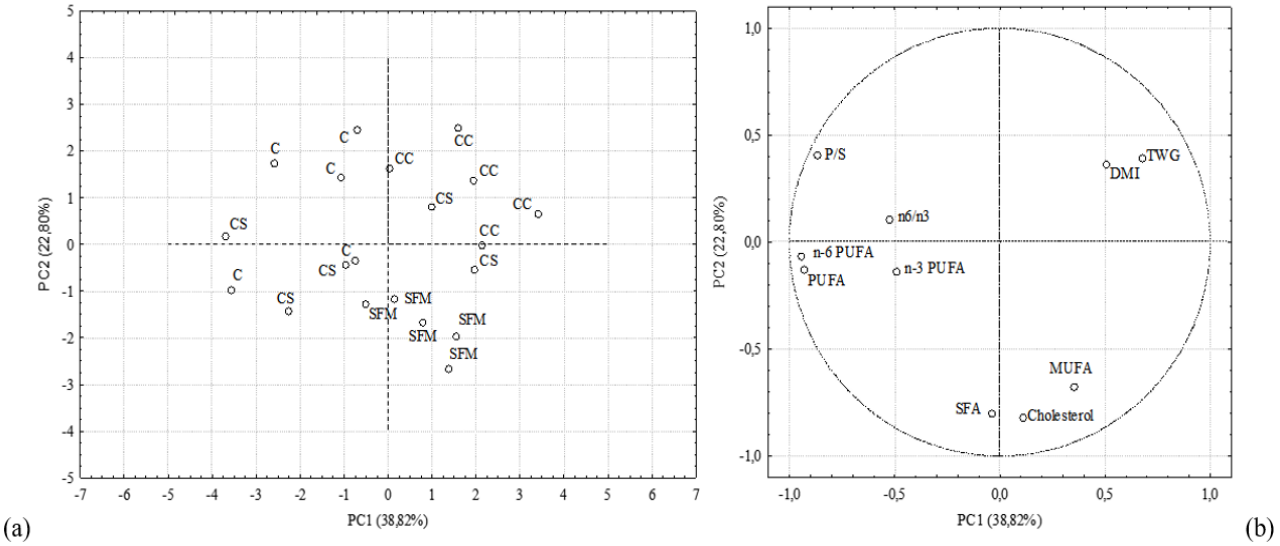

Figure 1. Distribution of experimental diets (a) and weights of the variables under study (b) between the first two principal components.

According to Słomińska-Wojewódzka and Sandvig (2013), Sandvig and van Deurs (2002) and Grimmer, Iversen, Van Deurs, and Sandvig (2000), the cholesterol concentration in the plasma membrane is closely related to the intracellular transport of ricin. Grimmer et al. (2000) report that the removal of cholesterol from the plasma membrane, in addition to reducing the endocytosis of ricin - which, once inside the cell, has a toxic effect - increases its degradation by 40%. Thus, a possible residual presence of ricin in the castor cake, even after treatment for detoxification, may have promoted changes in cholesterol tissue synthesis, in order to reduce endocytosis and increase the degradation of ricin.

Furthermore, analyzing by quadrants, it is noted that the CC diet is related to higher values for DMI and TWG, and is therefore associated with better characteristics of animal performance. Increased DMI with the inclusion of CC in the sheep diet may be associated with improved palatability of the diet (Gowda et al., 2009). According to Alves et al. (2016) detoxified castor cake is recommended as an alternative protein source to soybean meal in the lambs diet.

Conclusion

The inclusion of CC in the experimental diets provided better characteristics of animal performance and meat fat quality, in the proportions used in this experiment.

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