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Centesimal composition and fatty acids of meat from lambs fed diets containing soybean hulls[1]

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ABSTRACT - The objective of this study was to evaluate the centesimal composition and fatty acids in meat from animals fed diets containing levels of soybean hulls. The experiment was conducted at UESB in Itapetinga-BA, Brazil. Twenty-five confined Santa Inês rams were used. Treatments consisted of different levels of substitution of corn by soybean hulls (0, 25, 50, 75 and 100%) and elephant grass silage as forage. The design was completely randomized. Samples of concentrate and silage were collected to evaluate the fatty acid composition. The experimental period lasted 110 days. After this period, the animals were slaughtered and samples of the *longissimus* muscle were collected and vacuum-packed for further analysis. The levels of total lipid and protein showed quadratic effect with the increase of soybean hulls in the diet, as the moisture increased linearly with inclusion. There was no effect of treatments on the percentage of ash. The composition of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA and omega 6 (n-6) did not change, but increases in n-3 and reduction in the n-6:n-3 ratio were observed when the the amount of soybean hulls in the diet increased. The content of fatty acid conjugated linoleic acid (CLA) ranged from 3.0 to 4.0 g/kg at levels of substitution. The inclusion of soybean hulls in the diet increased CLA, the n-3 and decreased the n-6:n-3 ratio.

Key Words: CLA, meat quality, small ruminants

Introduction

In recent decades many research studies have sought alternatives to strengthen the internal market of sheep meat, because of the increasing consumption of this meat in some areas of Brazil (Marques et al., 2008). Among these strategies are offering quality products, best price, and thus, better acceptability.

Because of this this, studies have been developed in order to obtain improvements in sheep diet quality, so as to produce meat with adequate levels of fat and improve the fatty acid composition, reducing the levels of saturated fatty acids (Costa et al., 2008). Concomitantly, levels of unsaturated and polyunsaturated fatty acids would increase, ensuring greater consumer satisfaction, since the population is increasingly attentive to health and has sought to consume foods that may bring some benefit to it.

However, the conventional confined sheep feeding should be usually based on grain, hay or silage of good quality. However, these inputs can raise feed costs and, consequently, the economic viability of sheep breeding in relation to meat production. Thus, alternative feeds such as soybean hulls, which are obtained in the processing of this grain and have great prominence on the national scene, due to the high Brazilian soybean production (Restle et al., 2004), can be a resource in maintaining meat production, without compromising product quality.

Within this context, the objective of this study was to evaluate the centesimal composition and fatty acid of meat from lambs fed diets containing different levels of soybean hulls.

Material and Methods

Twenty-five Santa Inês lambs, average age of six months and weighting 22.0±2.26 kg were distributed in individual cemented pens of 1.10 × 1.0 m, with feed trough and drinking fountain. The animals were divided into five treatment groups using a randomized design. The treatments were soybean hulls replacing corn in the proportions of 0 (T1), 25 (T2), 50 (T3), 75 (T4) and 100 (T5) % (Table 1).

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The concentrate was formulated according to NRC (2007) for a daily gain of 200 g in an isoprotein diet formulated with corn, soybean meal, urea, mineral mixture and soybean hulls (Table 1). Elephant grass silage was used as forage and the forage:concentrate ratio was 60:40 (Table 2).

The experimental period lasted 110 days, with 14 days for adaptation to the plants and experimental diets. At the beginning of the adaptation period, pens were numbered, the animals were identified by earrings tags and dewormed against ecto and endo parasites.

Diets were provided daily at 06:00 a.m. and 03:00 p.m., with water *ad libitum*. The amounts of feed were provided in accordance with the feed intake of the animals, so as to provide leftovers of 5 to 10%.

The animals were weighted at baseline and every 21 days, always at the same time, before the first meal, after fasting from solid diet for approximately 12 hours.

Samples of the diets were collected throughout the experiment and stored in plastic bags previously identified and frozen at -10 °C to determine the composition of fatty acids. At the beginning of the laboratory analysis, samples were thawed at room temperature and oven-dried (65 °C, 72 hours) and processed in Willey type mills, with 1 mm mesh sieve.

At the end of the confinement the animals were sent to the Baby Bode slaughterhouse in the city of Feira de Santana - Bahia, Brazil, where they were kept at rest with liquid diet for 16 hours, according to the animal welfare standards. Subsequently, animals were anesthetized by means of electronarcosis, followed by bleeding, skinning and gutting. The carcasses were then sent to cold storage (0-4 °C) and after 24 hours of cooling, samples were taken from the *longissimus dorsi* muscle and frozen (-24 °C) until the beginning of testing, when they were thawed at room temperature, crushed, homogenized and analyzed in triplicate.

Moisture and ash analysis were performed according to the AOAC (Cuniff, 1998). The crude protein content of the sample was based on semi-micro process of Kjedahl according to the AOAC (Cuniff, 1998). The lipid fraction was extracted with a mixture of chloroform, methanol and water, respectively (2:2:1.8 v/v/v), according to Bligh & Dyer (1959).

In the lipid extraction step by Bligh & Dyer (1959), the moisture content was corrected to 80% for extraction of fatty matter from the concentrates and silage, to determine the fatty acid profile (Table 3).

Table 1 - Proportion (g/kg) of ingredients in the concentrates

Ingredients	Level of soybean hull in diet				
	0%	25%	50%	75%	100%
Corn	520	390	260	130	0
Soy meal	426	428	430	432	433
Soybean hull	0	130	260	390	520
Urea	24	22	20	18	17
Mineral mixture[1]	30	30	30	30	30
Total	1000	1000	1000	1000	1000

[1] Guaranteed levels (nutrients/kg): calcium - 170 g; sulphur - 19 g; phosphorus - 85 g; magnesium - 13 g; sodium - 113 g; copper - 600 mg; cobalt - 45 mg; chrome - 20 mg; iron - 1,850 mg; maximum fluoride - 850 mg; iodine - 80 mg; manganese - 1,350 mg; selenium - 16 mg; zinc - 4,000 mg.

Table 2 - Chemical composition of experimental diets

Components (g/kg)	Level of soybean hull in diet				
	0%	25%	50%	75%	100%
Dry matter	508	509	509	513	512
Crude protein[1]	185	170	160	18.7	17.6
Ether extract[1]	28	30	28	29	26
Ash[1]	70	75	75	76	77
NDF[1]	603	629	635	648	656
ADF[1]	315	328	350	359	368
TDN[1,2]	567.4	560	547.7	542.6	537.5

Source: Cappelle et al. (2001).
[1] g/kg of dry matter; NDF - neutral detergent fiber; ADF - acid detergent fiber; TDN - total digestible nutrients.
[2] TDN (%) = 74.49-0.5635*ADF (%).

Table 3 - Composition of fatty acids in experimental diets (g/kg)

Fatty acids	Level of soybean hull in diet				
	0%	25%	50%	75%	100%
C10:0	7.5	7.5	7.5	7.5	7.5
C12:0	78.7	78.7	78.7	78.7	78.7
C14:0	36.5	35.3	35.7	36.7	35.7
C15:0	3.4	3.3	3.4	3.5	3.5
C16:0	213.4	212.6	217.5	224.1	225.1
C16:1	9.1	8.9	9.7	9.7	9.4
C17:0	2.5	2.6	2.6	2.8	2.8
C17:1	0.4	0.4	0.4	0.3	0.4
C18:0	31.9	33.3	34.2	36.1	37.0
C18:1n-9t	3.0	2.9	2.8	2.9	2.7
C18:1n-9c	162.2	163.7	140.9	124.7	111.1
C18:1n-7	9.6	9.2	8.8	11	10.2
C18:2n-6	294.3	291.5	304.0	305.5	314.8
C18:3n-6	7.0	7.3	7.1	6.9	6.9
C18:3n-3	112.7	114.4	118.1	120.9	125.3
C20:1	4.5	5.0	4.6	4.6	4.5
C20:2	6.2	6.2	6.2	6.2	6.2
C22:1n-9	10.5	10.6	11.0	11.3	11.5
C22:4n-6	2.0	2.0	2.0	2.0	2.0
SFA	373.9	373.3	379.6	389.4	390.4
MUFA	203.8	205.2	182.8	168.9	76.8
PUFA	422.3	421.5	437.5	441.6	221.7
PUFA:SFA	15.9	15.9	15.8	14.7	15.2
n-6	303.4	300.9	313.2	314.5	323.7
n-3	112.7	114.4	118.1	120.9	125.3
n-6:n-3	76.5	66.1	55.8	48.8	42.5

SFA, MUFA and PUFA - saturated, mono and polyunsaturated fatty acids, respectively.

The transesterification of triacylglycerol was performed according to ISO 5509 (1978). The methyl esters were analyzed by gas chromatograph (Thermo-Finnigan), equipped with flame ionization detector and fused silica capillary column BPX-70 (12 0 m, 0.25 mm i.d.). The conditions of chromatographic analysis were the same used by Costa et al. (In press). The injection volume was 1.5 µL. Peak areas were determined by the method of normalization, using the software ChromQuest 4.1. The quantification of the fatty acids was performed after normalization of areas. The peaks were identified by comparing retention times of standard methyl esters of fatty acids (189-19, Sigma, USA) and after checking the equivalent chain length.

The results were subjected to analysis of variance (ANOVA) and regression at 0.05 probability using the statistical package SAEG (Sistema para Análises Estatísticas, version 9.1).

Results and Discussion

According to regression analysis, quadratic effect (P<0.05) was observed for the levels of soybean hull inclusion in sheep meat for total lipids content (Table 4).

The proportion of total lipids ranged from 10.16 to 17.00 g/kg, which is considered low, probably because the animals were uncastrated (Cezar & Souza, 2007) and could maintain a lower fat content, even with higher weights (Sainz, 2000). Madruga et al. (1999a) also stated that the age influences fat content because young animals have less fat in their muscle composition, since the ingested nutrients are converted into structure (bones and tissues). This lipid content concentration can be considered a plus before the search of consumers for foods with low fat.

The ash content in the meat has important biological functions, since it is a constituent of hormones and enzymes, among others (Pardi et al., 2006). The average found in meat (10.46 g/kg) was not influenced by treatments (P>0.05). In meat tissues this value is around 10 g/kg (Prado, 2004) with little variation, regardless of the treatment.

According to the data obtained, the increase in the level of soybean hull in the diet represented a decrease (P<0.05) of about 0.01613% in moisture content. Water represents 75% of the composition of the meat, and its content is inversely proportional to the fat content (Hedrick et al., 1994).

The protein showed quadratic effect (P<0.05) and increase in treatments containing up to 50% soybean hulls and a decrease in levels of 75 and 100%. Araújo et al. (2008), in a study with sheep, found that the presence of this ingredient in the diet reduced the digestibility of protein. Sarwar et al. (1991) stated that high levels of soybean hulls in the diets could reduce digestibility of nutritional components of food. Probably due to increased passage rate (Nakamura & Owen, 1989). Proteins are the second largest component of meat; they integrate the muscle and connective tissues. Its availability in essential amino acids and highly favorable digestibility characteristics provide it with high biological value (Pardi et al., 2001).

Ash, moisture and protein values were close to those presented in literature (Dhanda et al., 1999, 2003; Madruga et al., 1999b, 2001; Beserra et al., 2000, 2004; Silva, 2005) and vary from 0.79 to 1.68%, from 70.80 to 80.25% and from 18.50 to 23.39%, respectively.

Hashimoto et al. (2007) found that the centesimal composition of the *longissimus dorsi* muscle of sheep was not affected by the substitution of corn by soybean hulls, which was not observed in this study. Averages of 2.98% for total lipids, 0.97% for ash, 75.06% for moisture and 20.49% for protein were recorded.

Regarding the composition of fatty acids, no differences were found (P>0.05) for most of the acids found in the meat from lambs according to the levels of soybean hulls used in the diet (Table 5).

Table 4 - Centesimal composition of the *longissimus dorsi* muscle of Santa Ines lambs fed diets containing increasing levels of soybean hulls

	Replacement levels						P value[1]		
Variables (g/kg)	0%	25%	50%	75%	100%	CV (%)	L	Q	C
Total lipids[2]	10.16	1.492	15.76	17.00	15.00	19.95	0.0099	0.0133	0.9988
Ashes	11.05	9.00	10.60	10.87	10.78	10.75	0.8504	0.2627	0.059
Moisture[3]	752.77	752.69	748.32	741.26	738.31	12.9	0.0074	0.8893	0.8167
Protein[4]	220.89	229.74	227.71	217.42	212.41	44.3	0.0478	0.0407	0.2583

[1] L, Q and C - linear, quadratic and cubic order effects for the inclusion of soybean hulls in the diet.
[2] $\hat{Y} = 1.03361+0.0197627x-0.0001508x^2$ ($r^2 = 0.96$).
[3] $\hat{Y} = 75.4739-0.01613538x$ ($r^2 = 0.93$).
[4] $\hat{Y} = 22.2355+0.0293859x-0.000411x^2$ ($r^2 = 0.86$).

Table 5 - Fatty acid composition of the *longissimus dorsi* muscle (g/kg) of Santa Ines lambs fed diets containing increasing levels of soybean hulls

Fatty Acids	Replacement levels					CV (%)	P value[1]		
	0%	25%	50%	75%	100%		L	Q	C
				Saturated fatty acids					
C 10:0[2]	6.89	8.47	5.42	1.09	1.04	29.14	0.0142	0.6645	0.9598
C 14:0	16.19	17.80	14.16	18.09	19.20	19.85	0.2077	0.4638	0.9042
C 15:0	2.74	2.60	2.60	4.10	3.50	47.39	0.1674	0.9990	0.2992
C 16:0	261.44	259.72	243.58	248.71	251.38	7.45	0.2577	0.5832	0.9347
C 17:0[3]	8.52	8.37	10.37	10.67	10.95	12.72	0.0006	0.9854	0.2320
C 18:0[4]	184.22	184.22	213.79	185.67	179.03	9.45	0.9712	0.0488	0.9801
C 22:0	22.52	23.78	19.63	15.65	20.51	23.85	0.0945	0.5448	0.0528
				Monounsaturated fatty acids					
C 14:1	2.01	1.63	1.39	1.98	2.14	28.68	0.6273	0.0561	0.6787
C 16:1[5]	14.43	14.53	11.10	19.78	20.94	26.87	0.0084	0.1056	0.7999
C 17:1[6]	6.99	7.37	8.00	8.58	9.33	11.97	0.0004	0.9465	0.9999
C 18:1 n-9t	13.68	10.53	12.76	17.33	15.91	37.11	0.1447	0.8173	0.1413
C 18:1 n-9c	405.43	411.81	408.15	420.37	408.93	6.60	0.9535	0.9210	0.9712
				Polyunsaturated fatty acids					
C 18:2	52.81	44.41	46.19	37.26	45.84	24.01	0.1861	0.1963	0.9199
C 18: 3 n-3[7]	2.29	2.89	3.18	3.17	3.98	27.36	0.0068	0.9997	0.4916
CLA[8]	3.19	3.00	3.27	3.60	4.00	16.60	0.0117	0.2111	0.9151
C 20:3 n-6	1.64	1.71	1.90	2.53	1.73	55.36	0.7662	0.6814	0.3114
C 22:2 n-6	2.01	2.25	1.83	2.60	2.19	49.93	0.9313	0.9992	0.9764

[1] L, Q and C - linear, quadratic and cubic order effects for the inclusion of soybean hulls in the diet.
[2] $\hat{Y} = 0.0664927+0.0003807x$ ($r^2 = 0.46$).
[3] $\hat{Y} = 0.832125+0.00286597x$ ($r^2 = 0.85$).
[4] $\hat{Y} = 18.1308+0.0703175x-0.000738927x^2$ ($r^2 = 0.45$).
[5] $\hat{Y} =1.27098+0.00731632x$ ($r^2 = 0.54$).
[6] $\hat{Y} = 0.687843+0.00236184x$ ($r^2 = 0.99$).
[7] $\hat{Y} = 0.237034+0.00145721x$ ($r^2 = 0.90$).
[8] $\hat{Y} = 0.297132+0.000894937x$ ($r^2 = 0.80$).

Capric acid (C:10) was influenced by the substitution of corn (P<0.05), increasing linearly. This acid is transformed into the body monocaprin, a compound with antiviral and antimicrobial properties, important for human health.

The concentration of saturated fatty acids C14:0 (myristic acid), C16:0 (palmitic acid), considered hypercholesterolemic (Dietschy, 1998) was not affected (P>0.05) by replacement levels of corn in the diet, with values of 17.01 and 252.97 g/kg, respectively. However, palmitic acid (16:0) showed high levels in all treatments. The stearic acid (18:0), which showed a quadratic effect (P<0.05), although not having effect on blood cholesterol levels, despite having the second highest concentration in the meat, is considered neutral.

Meat from lambs fed diets containing soybean hulls presented linearly increased concentrations of C17:0 (margaric acid) (P<0.05). Rowe et al. (1999) and Diaz et al. (2002) found that meat from feedlot lambs had a higher content of C17:0, assuming a diet rich in energy, resulting in an increased concentration of acid. The microbial flora present in the rumen can synthesize their own lipids, starting from small precursors: acetate, propionate (Sauvant & Bas, 2001), especially the odd-chain fatty acids such as C17:0, which are synthesized by bacteria with utilization of propionate and are present in microbial lipids (Mansbridge & Blake, 1997).

Regarding the monounsaturated fatty acids (MUFA) there was a linear increasing effect (P<0.05) on replacement levels for C16:1 (palmitoleic acid) and C17:1 (heptadecanoic acid). The diet containing 100% of soybean hull presented a higher amount of C16:1 (20.94 g/kg) in relation to the treatment without this ingredient (14.43 g/kg); as for C17:1 (heptadecanoic acid), the diets that differed from treatment with higher values for this acid were the ones with levels 75 and 100% of soybean hull.

Monounsaturated fatty acids can be acquired through diet; however, some fatty acids are desaturated in the body - whose precursors are palmitic and stearic fatty acids, which produce, respectively, the palmitoleic (C16:1n-7) and oleic (C18:1n-9) fatty acids, by introducing a cis double bond between carbon 9 and 10 per an oxidative reaction catalyzed by acyl-CoA desaturase (Visentainer et al., 2003).

Of the total identified fatty acids, the oleic (C18:1 n-9) was the fatty acid that showed the highest values (410.93 g/kg). According to Sañudo et al. (2000), ruminants have high concentrations of oleic acid in the composition of intramuscular fat.

Working with feedlot lambs, Yamamoto et al. (2007) reported that the fatty acids found in highest concentration in the *longissimus muscle* were: oleic (C18:1n9c) with

414.6 g/kg; palmitic (C16:0) with 259.3 g/kg; stearic (C18:0) with 197.5 g/kg; linoleic (C18:2n-6) with 29.6 g/kg; and myristic (C14:0) acid, with 28.1 g/kg. In this study, there was similarity between the fatty acids found in highest concentration in the meat, but with different concentrations for C18:0 (189.3), C18:2n6 (45.3) and C14:0 (17.0 g/kg).

Madruga et al. (2005) also reported that among the total fatty acids identified, six fatty acids (C18:1, C18:0, C16:0, C18:2, C16:1 and C18:2) constituted over 90% of the total areas of the chromatograms. The most prevalent is C18:1, followed by C16:0 and C18:0.

In relation to the polyunsaturated fatty acids (PUFA), the linoleic acid (C18:2 n-6) with levels of 43.40%, and increase in ($P<0.05$) the α-linolenic acid (C18:3 n-3), were identified in meat. Wood et al. (2008) reported that only a small amount of C18:2 (about 10%) is available for incorporation into tissues, while Doreau & Ferlay (1994) found that from 85 to 100% of the acids C18:3 is biohidrogenated in the rumen. Thus, very little is available for incorporation into the tissues. These acids are considered essential and important because they are precursors of acids of n-6 and n-3 series family, respectively. Animals do not insert double bonds beyond carbon 9 and 10; therefore they are incapable of endogenously producing fatty acids of n-6 and n-3 families which can not be synthesized by animals, only by plants.

Conjugated linoleic acid responded linearly ($P<0.05$) to the levels of corn replacement. This suggests that there was an incomplete biohydrogenation of linoleic acid (C18:2) in the rumen microbiota, originating the conjugated linoleic acid, which is then absorbed and deposited in the muscle. This result is desirable, as CLA is an animal fatty acid with anticarcinogenic properties, and is related to the reduction of incidence of cardiovascular diseases, prevention and treatment of tumors (Tapiero et al., 2002).

According to regression analysis, the composition of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA/SFA ratio and omega 6 (n-6) were not influenced ($P>0.05$) by diets (Table 6).

Sheep meat is rich in saturated and monounsaturated fatty acids, with small amounts of polyunsaturated fatty acids. Saturated and monounsaturated fatty acids constituted, respectively, 491.7 and 456.2 g/kg.

In this experiment, the PUFA:SFA ratio found (1.1 g/kg) was lower than the recommended for a healthy diet, which must exceed 4 g/kg (Wood et al., 2003). However, data in the literature show that this ratio in meat is usually low, at around 1.0 (Scollan et. al., 2001). In ruminants, the polyunsaturated:saturated fatty acids ratio is lower due to the biohydrogenation of dietary unsaturated fatty acids by rumen microorganisms (Banskalieva et al., 2000). In lambs fed concentrate or forage, polyunsaturated fatty acids in the diet are biohydrogenated in the rumen, resulting in the predominant absorption of saturated fatty acids by the intestine. This is one of the reasons why lamb meat is characterized by high concentrations of saturated fatty acids and low ratio of PUFA:SFA (Cooper et al., 2004).

The fatty acid n-3 increased linearly with the levels used ($P<0.05$), which can be explained by the composition of experimental diets (Table 3), showing an increase of this fatty acid with the inclusion of soybean hulls.

The n-6:n-3 ratio changed ($P<0.05$), decreasing from 251.85 g/kg to 126.82 g/kg, the measure that increased levels of soybean hulls in the diet. Nutritionists have been stressing the importance of maintaining an optimal ratio of n-6:n-3 at levels below 40 g/kg, for reducing the risk of developing cancer or coronary complications, especially the formation of blood clots leading to heart attacks (Enser, 2001). However, most food in the human diet has higher levels.

Table 6 - Sums and ratios of major fatty acids present in the *longissimus dorsi* muscle (g/kg) of Santa Ines lambs fed diets containing increasing levels of soybean hulls

Fatty acids	Replacement levels					CV (%)	P value[1]		
	0%	25%	50%	75%	100%		L	Q	C
SFA	496.33	497.35	500.03	483.99	485.62	4.91	0.3219	0.9579	0.9245
MUFA	442.54	445.87	453.82	468.04	457.28	6.09	0.2008	0.9848	0.6821
PUFA	61.55	52.71	50.99	49.18	57.75	22.72	0.6848	0.1834	0.9764
PUFA/SFA	1.24	1.09	1.02	1.02	1.19	23.77	0.9308	0.1679	0.9956
n-6	56.07	48.38	44.76	42.41	49.77	24.26	0.2621	0.1753	0.9818
n-3[2]	2.29	2.89	2.83	3.17	3.98	26.79	0.0054	0.9574	0.4596
n-6:n-3[3]	251.85	166.28	176.67	134.04	126.82	31.89	0.00156	0.2688	0.6319

SFA, MUFA and PUFA - saturated, mono and polyunsaturated fatty acids, respectively; n-6 - omega 6 fatty acids; n-3 - omega 3 fatty acids.
[1] L, Q and C - linear, quadratic and cubic order effects for the inclusion of soybean hulls in the diet.
[2] $\hat{Y} = 0.233247+0.00145721x$ ($r^2=0.90$).
[3] $\hat{Y} = 22.9576-0.114691x$ ($r^2=0.80$).

Conclusions

The centesimal composition and the amount of fatty acids in the meat show little variation according to the substitution levels used, but the inclusion of soybean hulls in the diet increases the total lipid content, conjugated linoleic acid and n-3 (omega 3) and decreases the n-6:n-3 ratio.

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