CHEMICAL AND FATTY ACIDS COMPOSITION OF RUMP CAP FROM YOUNG BULLS FED PROTECTED OR UNPROTECTED OILS[1]

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ABSTRACT: Strategies to improve the nutritional aspects of beef, mainly the fatty acids composition, have become an important goal to the scientific community. The use of different oils sources could be an interesting device due its polyunsaturated fatty acids composition. The chemical and fatty acid composition of rump cap (*Biceps femoris*) from 35 Nellore young bulls finished at feedlot (96 days) were analyzed. These animals were fed a control diet with sugar cane and concentrate without oil or diets containing sugar cane and concentrate with different sources of oil (soybean or linseed), protected or not from ruminal degradation. A randomized block design was adopted with five treatments and seven replications. The means were compared using orthogonal contrasts at 0.05 significance level. Animals fed diets with oil showed higher levels ($P<0.05$) of protein and lower levels ($P<0.05$) of ash than control diet. Lower cholesterol ($P<0.05$) levels resulted from linseed oil added treatment compared to soybean oil (37.70 and 43.80 mg/100 g, respectively); on the other hand, cholesterol levels increased ($P<0.05$) for protected oils compared to non-protected (44.53 and 33.97 mg/100 g). Oil added diets resulted in higher ($P<0.05$) linolenic acid levels. Linseed oil increased ($P<0.05$) the levels of the fatty acids C14:1, C16:1 and C18:1 n9. Addition of linseed oil, whether protected or not, to the animal diets improves the fatty acid composition of the rump cap by increasing the amount of omega-3 fatty acids and improving the omega-6:omega-3 ratio.

Keywords: beef, cholesterol, fat, polyunsaturated fatty acids.

COMPOSIÇÃO QUÍMICA E ÁCIDOS GRAXOS DA PICANHA DE TOURINHOS ALIMENTADOS COM ÓLEOS PROTEGIDOS OU NÃO PROTEGIDOS

RESUMO: Estratégias para melhorar os aspectos nutricionais da carne, principalmente a composição em ácidos graxos, tornaram-se importante objetivo para a comunidade científica. A utilização de diferentes fontes de óleo poderia ser uma ferramenta interessante devido à sua composição em ácidos graxos poli-insaturados. A composição química e de ácidos graxos da picanha (*Biceps femoris*) de 35 tourinhos Nelore terminados em confinamento (96 dias) foram analisadas. Os animais foram alimentados com uma dieta controle contendo cana-de-açúcar e concentrado sem óleo ou dietas contendo cana-de-açúcar e concentrado com diferentes fontes de óleo (soja ou linhaça), protegido ou não de degradação ruminal. Utilizou-se delineamento em blocos casualizados, com cinco tratamentos e sete repetições. As médias foram comparadas usando contrastes ortogonais

em nível de 5% de significância. Animais alimentados com dietas contendo óleo apresentaram teores mais elevados (P<0,05) de proteína e menores teores de cinza (P<0,05) na carne em relação àqueles alimentados com dieta controle. O óleo de linhaça reduziu (P<0,05) a concentração de colesterol em relação ao óleo de soja (37,70 e 43,80 mg/100 g, respectivamente). Por outro lado, o teor de colesterol da carne aumentou (P<0,05) para os animais alimentados com óleos protegidos em comparação aos não-protegidos (44,53 e 33,97 mg/100 g). O óleo adicionado às dietas resultou em maior (P<0,05) concentração do ácido graxo linolênico. O óleo de linhaça aumentou (P<0,05) os teores dos ácidos graxos C14:1, C16:1 e C18:1 n9. A adição de óleo de linhaça, seja protegido ou não, em dieta para tourinhos Nelore melhora a composição de ácidos graxos da picanha, aumentando a quantidade de ácidos graxos ômega-3 e melhorando a relação omega-6:omega-3.

Palavras-chave: ácidos graxos poli-insaturados, carne, colesterol, gordura.

INTRODUCTION

Recently, the scientific community has been striving to develop foods that contribute to human health. Herdmann *et al*. (2010) stated that consumers are becoming more aware of animal well being and increasingly interested in consuming animal products with good nutritional quality such as improved ratio of the omega-6 and omega-3 fatty acids and lower cholesterol levels.

According to Shingfield *et al*. (2013), there is a need to the world´s population decrease the intake of total fat, saturated fatty acids (SFA) and trans fatty acids (TFA) and an increase in the consumption of the long-chain omega-3 polyunsaturated fatty acids (PUFA), 20:5 n-3 and 22:6 n-3. Along these lines, Wood *et al*. (2003) reported that nutritional strategies to manipulate the composition of beef fatty acids are being assessed in order to produce healthier cuts. The modification of fatty acid composition of beef represents one means to lower the intake of saturated fatty acids and increase monounsaturated (MUFA) and PUFA in the human diet without changes in consumer eating habits, while at the same time maintaining the potential benefits associated with the macro- and micronutrients in these foods (Shingfield *et al*., 2013).

According to Lange *et al.* (2014) the brain relies on both macro- and micro-nutrients for optimal development and function. PUFAs are known to play an important role in neuronal development and functioning of the central nervous system. The brain of mammals is particularly rich in long-chain PUFAs from omega-3 and omega-6 families, particularly docosahexaenoic acid (DHA, C22:6 n-3) and arachidonic acid (AA, C20:4 n-6). Makulska-Gertruda *et al*. (2014) assessed the effects of dietary omega-3 fatty acid supplementation on attention and impulsivity in an animal model and found improve. The omega-3 PUFA-enriched diet reduced impulsivity in spontaneously hypertensive rat compared with rats fed with the omega-3 PUFA-deficient diet.

A great diversity of meat cuts are being used in today´s world cuisine depending on the country and region. Studies assessing the characteristics of the *Biceps femoris* muscle (rump cap), a highly valued cut in the Brazilian market, are virtually non-existent. Therefore, this study aims to assess the chemical and lipid composition of rump cap and to establish the relationship between omega-3 or omega-6 rich diets to Nellore young bulls, with protected or unprotected oil from ruminal degradation.

MATERIAL AND METHODS

The experimental feedlot study was conducted at FCAV/Unesp/Jaboticabal, SP, Brazil (21°14'S and 48°17'W). Thirty five Nellore young bulls were confined in individual pens with an initial body weight of 402.69 ± 14.90 kg and 18 ± 2 months of age. The animals were grouped by weight, randomly assigned to different treatment groups and subsequently adapted to specific management conditions and diets for 28 days. During this period, their diet was composed of 50% of concentrate (corn, soybean meal, citrus pulp and a mineral mixture) and 50% of forage (sugarcane). The experimental period consisted of 96 days after the adaptation, over which the feed were offered in two meals: one at 8:00 h (40% of the total diet) and another at 14:00 h (60% of the total diet).

The experimental diets were formulated from different lipid sources (soybean oil, protected soybean oil, linseed oil and protected linseed oil) or a control treatment (without oil source added) using RLM® (ESALQ, USP, Piracicaba, São Paulo, Brazil) software, with nutritional demands estimated by

the CNCPS system (Fox *et al*., 1992) aiming maximum weight gain (Table 1). All treatments used as exclusive forage source sugarcane variety IAC 86 2480, which was harvested and chopped daily.

The vegetable oils were used with the objective of increasing energy concentration without increasing the concentrate portion. For the protected soybean oil treatment, the commercial product Megalac-E® was used. This product is rich in omega-6 fatty acids that is generated from soybean oil by calcium salt saponification, protecting the long-chain polyunsaturated fatty acids. Since protected linseed oil is not commercially available, a method was developed at Jaboticabal to obtain this product from regular linseed oil. The linseed oil was saponified with sodium hydroxide in 65% of ethanol using an unheated plastic drum. The mixture was stirred until glycerol and soap were produced. Once the saponification reaction was complete, a saturated solution of calcium chloride was added to precipitate the soap. The mixture of water and glycerol was then collected and the calcium soap was dried at room temperature. According to Oser (1965), fat saponification results in a product with high total fat composition, low content of free fatty acids and almost no oxidation. The unprotected oils (soybean or linseed) were added without processing.

At the end of the experimental period, the animals were transported to a slaughterhouse 200 km away from the feedlot, stunned and slaughtered. Mean slaughtering weight was 532.17 ± 30.2 kg, with 55.32% of carcass yield and mean fat cover thickness of 7.00 mm. Immediately after slaughter, carcasses were stored at 4 ºC for 24 h. From the left side, triangular standard cuts of *Biceps femoris* muscle of about 1.5 kg were removed and taken to the laboratory for subsequent analysis. All experimental procedures were approved by the Commission on Ethics and Animal Welfare (process no. 021167-07).

Samples of 10 g were taken from the core of *Biceps femoris* muscle to determine moisture, protein, ether extract and ash content according to the methodology described by AOAC (1995). Cholesterol content of the *Biceps femoris* muscle was determined using a spectrophotometer as previously described by Bragagnolo and Rodriguez-Amaya (1995). Total lipids were assessed by extraction from samples (10 g approximately) of *Biceps femoris* muscle in 200 mL of a chloroform-methanol mixture (2:1). From this extract, 5 mL of sample was dried using nitrogen gas, followed by addition of 10 mL of 12% KOH in 90% ethanol. The solution was then placed in a water bath at 80ºC and agitated for 15 minutes. At the end of this process, 5 mL of water was added, and after cooling, 10 mL of hexane was added and the solution was agitated by vortexing. After phase separation, a 10 mL sample was dried using nitrogen gas. Finally, 6 mL of acetic acid saturated with concentrated ferrous sulfate was added. Once cooled, the solution was analyzed using a spectrophotometer at 538 nm.

Cross section samples of the freeze-dried *Biceps femoris* muscle were collected and kept frozen until analysis were performed to determine the fatty acid composition of the fresh meat. Fat was extracted using a mixture of chloroform-methanol, as reported by Bligh and Dyer (1959), and fatty acid methyl esters were obtained by ISO (1978). Qualitative and quantitative fatty acid contents were determined using a gas chromatograph with a bus communication module with a flame ionization detector and fused silica capillary column (OMEGAWAX250). The 30 m column with 0.25 mm diameter and film thickness of 0.25 µm used helium as a carrier gas at a flow of 1ml/min. A 1 µl aliquot of the sample was injected into a "split" at 1/100 ratio and temperature 250ºC. The temperature of the oven was programmed to remain at 100ºC for two minutes and then increase to 220ºC at 4ºC/minute rate for 25 minutes, while the detector was at 280ºC. Fatty acid methyl esters present in the beef cuts were identified and quantified by comparison to retention times and concentrations of methyl esters from standard fatty acids. According to Bessa *et al*. (2008), the introduction and spread of a grouping criterion of fatty acids by the functionality would be preferred because classification only by the structure of the molecule (saturated and unsaturated) can often lead to errors in the nutritional assessment of foods. The functionality classification could put beef in evidence as a functional food. The equations are as follows: Hypercholesterolemic = C12:0 + C14:0 + C16:0 + C16:1; Hypocolesterolemic = C18:1n7 + C18:1n9 + C18:2 n6 + C18:3 n3 + C18:3 n6+ C20:3 n3 + C20:3 n6 + C20:5 n3; Neutral= C10:0 + C18:0.

All results were tested for normality using the Cramér-von Mises test (SAS Inst. Inc., Cary, NC), at 0.05 of probability. The experimental design was a randomized block with five treatments (control, soybean oil, linseed oil, protected soybean oil and protected linseed oil) and seven repetitions. Results were subjected to analysis of variance using the general linear model (GLM) (SAS Inst., Inc., Cary, NC) and means were compared by the following contrast: control diet vs. diets with oil, soybean oil vs. linseed oil, soybean oil vs. protected soybean oil, and linseed oil vs. protected linseed oil.

Table 1. Composition, nutritional characteristics and fatty acid content of the experimental diets

	Diets				
	Control	Soybean oil	Protected Soybean oil	Linseed oil	Protected Linseed oil
Ingredients (% of DM)					
Sugarcane	40.0	40.0	40.0	40.0	40.0
Corn grain	34.0	29.2	29.2	29.0	29.0
Soybean meal	12.0	13.0	13.0	13.0	13.0
Citrus pulp	10.0	10.0	10.0	10.0	10.0
Urea	1.0	1.0	1.0	1.0	1.0
Soybean oil	-	3.8	-	-	-
Linseed oil	-	-	3.8	-	-
Megalac-E®[1]	-	-	-	4.5	-
PL[2]	-	-	-	-	4.5
Mineral[3]	2.5	2.5	2.5	2.5	2.5
Limestone	0.5	0.5	0.5	-	-
Nutritional composition of the diet[4]					
DM (%)	47.6	47.6	47.7	46.5	47.7
CP (% of DM)	13.5	13.5	13.5	13.5	13.5
TDN (% of DM)	71.5	76.7	76.7	76.5	76.5
Fat (% of DM)	2.4	6.0	6.0	6.0	6.0
ME (MJ/kg DM)	11.5	12.2	12.2	12.2	12.2
Estimated gain (kg/day)	1.4	1.6	1.6	1.6	1.6
Fatty acid composition[5] (% of total fatty acids)					
C12:0	0.14	0.05	0.06	0.31	0.09
C14:0	0.19	0.12	0.12	0.93	0.26
C16:0	16.44	14.25	13.45	24.88	23.32
C16:1	0.18	0.13	0.15	0.49	0.16
C17:0	0.21	0.14	0.18	0.27	0.29
C17:1	0.30	0.11	0.13	0.10	0.28
C18:0	3.05	3.74	6.13	7.61	10.48
C18:1 n-9c	30.27	27.14	30.74	23.51	28.48
C18:2 n-6	45.45	49.36	26.06	38.04	25.68
C18:3 n-3	3.78	4.97	22.98	3.86	10.96
SFA	20.03	18.03	19.94	26.39	34.44
UFA	79.97	81.97	80.06	73.61	65.26
PUFA	53.23	54.33	49.04	41.90	36.64
Omega-3	3.78	4.97	22.98	3.86	10.96
Omega-6	45.45	49.36	26.06	38.04	25.68
Omega-6:Omega-3	12.02	9.93	1.13	9.93	2.34

[1]Protected soybean oil. [2]Protected linseed oil. [3]Composition per kg of product, phosphorus: 40 g; calcium: 146 g; sodium: 56 g; sulfur: 40 g; magnesium: 20 g; copper:350 g; zinc: 1.300 mg; manganese: 900 mg; iron: 1.050 mg; cobalt: 10 mg; iodine: 24 mg; selenium: 10 mg; fluorine: 400 mg. [4]Nutritional characteristics estimated by RLM® software. DM: dry matter, CP: crude protein, TDN: total digestible nutrients, ME: metabolizable energy. [5]SFA: saturated fatty acids; UFA: unsaturated fatty acids; PUFA: polyunsaturated fatty acids.

RESULTS

For the cholesterol content, the lowest (P<0.05) values were obtained for meat from animals fed diet containing linseed oil, when assessing only oil sources (protected or unprotected). The addition of protected linseed and protected soybean oils in the diet increased (P<0.05) the cholesterol levels of the *Biceps femoris* muscle, compared to the respective diets with no protected oil. Moisture and fat levels were not different (P>0.05) among diets. However, protein showed higher levels (P<0.05) for oil diets than control diet, and protected linseed oil compared with unprotected linseed oil (Table 2).

The addition of oil from different sources, whether protected or not, changed the concentration of some fatty acids of the rump cap. Oil supplementation increased the levels of alpha-linolenic (C18:3 n-3) (P<0.05) and gamma-linolenic (C18:3 n-6) (P<0.05). The treatments did not change (P>0.05) the levels of the myristic acid (C14:0) and other fatty acids, regardless of oil type and whether it was protected or not (Table 3).

The comparison between added oils (linseed or soybean, regardless the protection) showed that diets with soybean oil resulted in higher levels (P<0.05) of palmitic (C16:0), cis-11-octadecenoic acid (C18:1 n-7), CLA (C18:2 c9, t11) and eicosadienoic (C20:2) fatty acids, while linseed oil yielded higher levels of oleic (C18:1 n-9), alfa-linolenic (C18:3 n-3) and arachidic (C20:0) fatty acids (Table 3).

For unprotected soybean oil vs. por protected soybean oil, we observed increased levels (P<0.05) of the fatty acids C14:1, C16:1, C17:1, C18:1 n-7, CLA and C20:0 for unprotected soybean oil diet. The assessment of unprotected and protected linseed oil inclusion showed higher (P<0.05) levels of C18:0 fatty acids to protected oil. For monounsaturated C14:1, C16:1 and polyunsaturated C18:3 n-3 fatty acids, the highest levels (P<0.05) were observed for unprotected oil, regardless of oil source these fatty acids always had higher concentrations with unprotected oils (Table 3).

Both diets, control and oil added, did not differ (P>0.05) in relation to the sums of saturated (SFA), unsaturated (UFA), monosaturated (MUFA) and polyunsaturated (PUFA) fatty acids and to the ratios between unsaturated:saturated (UFA:SFA), monosaturated:saturated (MUFA:SFA) and polyunsaturated:saturated (PUFA:SFA) (Table 4). Differences (P<0.05) were found only for the omega-6:omega-3 ratio which presented the best ratio for meat from animals fed control diet. When the oil sources were compared between them, some differences (P<0.05) were observed for MUFA sum and MUFA:SFA and omega-6:omega-3 ratios (Table 4).

The results of the functionality showed that the addition of soybean oil whether protected or not decreased (P<0.05) hyper levels. Hypo fatty acid levels were not different (P>0.05) for all diets and the neutral fatty acids were higher (P<0.05) in the meat of young bulls fed linseed oil compared to protected linseed oil (Table 4). The comparison between added oils (linseed or soybean) showed that diets with soybean oil resulted in higher levels (P<0.05) of palmitic (C16:0), cis-11-octadecenoic acid (C18:1 n-7), CLA (C18:2 c9, t11) and eicosadienoic (C20:2) fatty acids, while linseed oil yielded higher levels of oleic (C18:1 n-9), alfa-linolenic (C18:3 n-3) and arachidic (C20:0) fatty acids (Table 3).

Table 2. Cholesterol level, moisture, protein, ether extract and ash of rump cap (*Biceps femoris*) from Nellore young bulls fed diets with oils from different sources, protected or unprotected from ruminal degradation

						Probability of contrasts				
	Control	Soybean oil	Protected Soybean oil	Linseed oil	Protected Linseed oil	C vs. O[1]	S vs. L[2]	S vs. PS[3]	L vs. PL[4]	SE[5]
Cholesterol mg/100g	41.86	39.96	47.64	33.99	41.42	0.5484	<0.0001	<0.0001	<0.0001	0.75
Moisture (%)	76.82	77.86	77.25	77.23	76.63	0.2597	0.3306	0.5633	0.3896	0.56
Protein (%)	18.06	17.92	18.40	18.74	19.77	<0.0001	0.6686	0.3096	0.0191	0.23
Ether extract (%)	4.16	3.25	3.41	3.14	2.73	0.1501	0.2090	0.2531	0.8712	0.51
Ash (%)	0.94	0.95	0.93	0.87	0.85	0.0011	0.1904	0.6391	0.0028	0.01

[1]Control diet vs. diets with oil. [2]Soybean oil vs. linseed oil. [3]Soybean oil vs. protected soybean oil. [4]Linseed oil vs. protected linseed oil. [5]Standard error.

Table 3. Fatty acid composition of rump cap (*Biceps femoris*) muscle of Nellore young bulls fed diets containing oils from different sources, protected or unprotected from rumen degradation

Fatty acids (% of total fatty acids)	Control	Soybean oil	Protected Soybean oil	Linseed oil	Protected Linseed oil	Probability of contrasts				
						C vs. O[1]	S vs. L[2]	S vs. PS[3]	L vs. PL[4]	SE[5]
C10:0	0.04	0.04	0.03	0.03	0.04	0.9303	0.5588	0.1065	0.4100	0.01
C12:0	0.07	0.06	0.06	0.06	0.06	0.7968	0.2124	0.2603	1.0000	0.01
C14:0	3.51	3.28	3.31	3.24	3.54	0.4051	0.4871	0.5180	0.8909	0.21
C15:0	0.37	0.32	0.33	0.32	0.33	0.7433	0.1815	0.2068	0.9606	0.02
C16:0	25.34	23.65	24.96	23.74	24.83	0.5192	0.0153	0.6341	0.9111	0.60
C17:0	0.84	0.82	0.80	0.74	0.77	0.5455	0.3999	0.4316	0.1798	0.04
C18:0	13.19	15.07	15.00	12.96	14.05	0.9988	0.9049	0.0635	0.0329	0.69
C20:0	0.08	0.10	0.11	0.12	0.11	0.3516	0.0442	0.0229	0.0989	0.01
C14:1	1.09	0.87	0.85	1.08	1.03	0.4905	0.9835	0.0192	0.0389	0.07
C16:1	3.95	3.22	3.17	3.77	3.65	0.4686	0.6919	0.0012	0.0168	0.15
C17:1	0.83	0.72	0.64	0.76	0.70	0.2563	0.8567	0.0002	0.3272	0.03
C18:1 n-7	2.48	4.01	3.08	3.49	2.82	0.0546	<0.0001	0.0406	0.0715	0.19
C18:1 n-9	39.41	39.72	38.13	41.11	40.11	0.5067	0.0257	0.2012	0.1673	0.72
C18:2 n-6	5.65	4.97	6.73	4.90	4.92	0.3521	0.0502	0.2223	0.9346	0.68
C18:2 c9 t11	0.46	1.01	0.76	0.98	0.66	0.1426	0.0001	0.0205	0.7569	0.08
C18:3 n-3	0.30	0.28	0.33	0.84	0.62	0.0114	0.0004	0.6595	<0.0001	0.06
C18:3 n-6	0.08	0.08	0.09	0.09	0.16	<0.0001	1.0000	0.3475	0.3475	0.01
C20:1 n-9	0.21	0.21	0.19	0.20	0.19	0.6293	0.8030	0.3509	0.7244	0.01
C20:2	0.07	0.06	0.07	0.05	0.05	0.3961	0.0252	0.8736	0.2107	0.01
C20:3 n-3	1.41	1.06	0.94	1.06	0.94	0.3721	0.4510	0.0666	0.9215	0.17
C20:3 n-6	0.30	0.22	0.21	0.19	0.17	0.1686	0.1811	0.1146	0.5223	0.03
C20:5 n-3	0.22	0.15	0.14	0.21	0.15	0.4015	0.9455	0.0627	0.1561	0.03

[1]Control diet vs. diets with oil. [2]Soybean oil vs. linseed oil. [3]Soybean oil vs. protected soybean oil. [4]Linseed oil vs. protected linseed oil. [5]Standard error.

Table 4. Fatty acid sums and ratios of the *Biceps femoris* muscle of Nellore young bulls fed diets containing oils from different sources, protected or unprotected from rumen degradation

Fatty acids (%)	Control	Soybean oil	Protected Soybean oil	Linseed oil	Protected Linseed oil	Probability of contrasts				
						C vs. O[1]	S vs. L[2]	S vs. PS[3]	L vs. PL[4]	SE[5]
SFA[6]	43.47	43.36	44.62	41.24	43.76	0.6486	0.1373	0.4812	0.1977	1.13
UFA[7]	56.52	56.63	55.37	58.75	56.23	0.6486	0.13773	0.4812	0.1977	1.13
MUFA[8]	48.00	48.76	46.07	50.43	48.52	0.8269	0.0051	0.1137	0.1691	0.88
PUFA[9]	8.52	7.86	9.29	8.32	7.71	0.4277	0.3605	0.5352	0.7125	0.96
UFA:SFA	1.31	1.31	1.24	1.43	1.30	0.7093	0.1638	0.4655	0.1883	0.06
MUFA:SFA	1.11	1.12	1.04	1.22	1.12	0.8931	0.0335	0.2601	0.1372	0.04
PUFA:SFA	0.20	0.18	0.21	0.20	0.18	0.51006	0.6500	0.7480	0.5219	0.02
Omega-3[10]	1.94	1.49	1.42	2.10	1.71	0.9280	0.6380	0.1428	0.0893	0.25
Omega-6[11]	6.03	5.28	7.04	5.18	5.27	0.3984	0.0532	0.2795	0.9181	0.72
n-6:n-3	3.25	3.59	5.03	2.52	3.08	0.0080	<0.0001	<0.0001	<0.0001	0.16
Hyper[12]	33.98	31.09	32.36	31.90	33.13	0.3792	0.0450	0.1610	0.4781	0.85
Hypo[13]	49.88	50.52	49.68	51.89	49.93	0.6358	0.1894	0.8966	0.3658	1.06
Neutral[14]	13.24	13.00	14.09	15.11	15.04	0.9985	0.9026	0.0654	0.0330	0.69

[1]Control diet vs. diets with oil. [2]Soybean oil vs. linseed oil. [3]Soybean oil vs. protected soybean oil. [4]Linseed oil vs. protected linseed oil. [5]Standard error. [6]Saturated fatty acid: C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0. [7]Unsaturated fatty acid: C14:1+ C16:1+ C17:1+ C18:1 n-7+ C18:1 n-9 + C18:2 n-6+ C18:2 c9 t11+ C18:3 n-3+ C18:3 n-6+ C20:1 n-9+ C20:2+ C20:3 n-3+ C20:3 n-6+ C20:5 n-3. [8]Monounsaturated fatty acid: C14:1+ C16:1+ C17:1+ C18:1 n-7+ C18:1 n-9+ C20:1 n-9. [9]Polyunsaturated fatty acid: C18:2 n-6+ C18:2 c9 t11+ C18:3 n-3+ C18:3 n-6+ C20:2+ C20:3 n-3+ C20:3 n-6+ C20:5 n-3. [10]C18:3 n-3+ C20:3 n-3+ C20:5 n-3. [11]C18:2 n-6+ C18:3 n-6+ C20:3 n-6. [12]C12:0+C14:0+C14:1+C16:0+C16:1. [13]C18:1 n-7+C18:1 n-9+C18:2 n-6+C18:3 n-3+C18:3 n-6+C20:3 n-3+C20:3 n-6+C20:5 n-3. [14]C10:0+C18:0.

DISCUSSION

Supposedly, the lower cholesterol levels observed when non-protected oil was added (Table 2) may be resulted from the large amount of fatty acids acting on cholesterol metabolism, decreasing its production in the body; or else, the protected oils may have lost, during the protection process, important fatty acids with hypocholesterolemic effect or even prevented these fatty acids from being released and used in the metabolism to reduce cholesterol, but no references were found.

The cholesterol levels found for all treatments were lower (Table 2) than standard cholesterol levels (58.3 to 83.4 mg/100 g) for beef cuts, according to Werdi Pratiwi e*t al*. (2006). Spritz and Mishkel (1969) explained that this lower cholesterol level resulted from a diet rich in polyunsaturated fatty acids. According to these authors, serum lipids enriched with polyunsaturated fatty acids occupy more space within the lipoprotein particles, and since lower amounts of ester-cholesterol molecules are found inside the low density lipoprotein (LDL), it is not the LDL level that is reduced but the cholesterol levels inside each LDL particle.

The differences found for protein and ash contents (Table 2) may be related to levels of ether extract presented by the meat, despite statistical analysis showed no difference. According to Lawrie and Ledward (2006), the ether extract content varies most in meat, and once its concentration increases, there is a decrease in the levels of moisture, protein, and minerals. Keeton and Eddy (2004) stated that meat chemical composition varies according to animal breed, slaughtering age, diet and genetic predisposition to the appearance of dry, firm and dark meat (DFD), and anatomical cuts location on the carcass as well.

The long chain polyunsaturated fatty acids C18:3 n-3 and C18:3 n-6, which increased when oil was added to the diet, are important due to the beneficial effect they have on the human body, especially decreasing the LDL levels circulating in the organism.

The addition of linseed oil, rich in omega-3 polyunsaturated fatty acids, in the diet of bulls proved to be benefic, showing a meat quality improvement (Table 3), since those fatty acids are very important to human health. Simopoulos (2002) stated that the omega-3 fatty acids are essential for normal growth, to prevent and treat coronary heart disease, diabetes and arthritis. Maddock *et al.* (2006) evaluated 80 g/kg of linseed grains in the diets of feedlot cattle and reported an increase in the levels of C18:3 n-3 and C18:3 n-6 fatty acids. Raes *et al.* (2004) assessed the fatty acid composition of sirloin and shoulder from feedlot cattle fed diets containing linseed and soybean grains and reported improved lipid composition of meat when the animals were fed linseed.

The levels of myristic fatty acid (Table 3) were below those reported by González *et al.* (2014) (4.3%), who studied Rubia Gallega calves fed different oil sources, rich in polyunsaturated fatty acids, especially C18:2 n-6 and C18:3 n-3. Reducing beef fatty acids such as C14:0 has become an important goal, mainly due to its hypercholesterolemic effect on the human body (Bessa *et al*., 2008).

It´s possible to observe that differences found in fatty acids for soybean vs. linseed (Table 3) are directly related to the dietary lipid composition, except for long chain fatty acids C20:0 and C20:2, which are under elongase enzymatic action.

The highest CLA content in the meat of animals fed diet containing unprotected soybean oil is related to the amount of C18:2 n-6 present in this oil (Table 3). The formation of CLA occurs with the incomplete biohydrogenation of C18:2 n-6 by the action of microorganisms in the rumen (Harfoot and Hazlewood, 1997). Among the fatty acids, CLA should be highlighted, since the observed increase can be credited to the addition of linseed and soybean oils, which contain large amounts of its precursor, the linoleic fatty acid (C18:2 n-6) (Table 1). Khanal and Dhiman (2004) stated that the ingestion of CLA has positive effects on human health as reducing body fat deposits and atherosclerosis, changing nutrient partitioning, improving bone mineralization and immunological system, and has anti-diabetic properties. The results of this study (Table 3) were similar to those of Gillis *et al*. (2004), who reported that corn oil (rich in C18:2 n-6) added diets fed to heifers increased the levels of CLA and some unsaturated fatty acids in the meat.

The fatty acid composition of diets (Table 1) shows that protected linseed oil resulted in loss of important fatty acids, especially C18:3 n-3. This loss may be related to the heating process that occurs during saponification. This higher level of unsaturated fatty acids obtained with non-protected oils, probably resulted from the fact that the lipids were readily available for uptake and metabolization by the body (Table 3).

Besides, when compared to the use of unprotected oil, protected linseed oil did not result in meat cuts with higher levels of omega-3 fatty acids (Table 4). Increasing levels of omega-3

PUFA is desirable to improve the composition of beef since a large amount of SFA is present in beef. According to SCOLLAN *et al.* (2001), although beef has lower levels of omega-3 when compared to fish, it is still an important proven source of this fatty acid.

The unprotected oil benefited the deposition of the largest concentrations of MUFA more than protected form. Probably in any of the steps of calcium salts formation of fatty acids (protected oils), due to actions as excessive heating, favored the loss of the double bonds of the carbon chains (Table 3). Comparing linseed and soybean oils the highest value for MUFA and consequently the highest relation MUFA:SFA found in the meat of animals fed linseed are due to the higher levels of C18:1 n-9c fatty acid presented in the provided diet (Table 4).

The best omega-6:omega-3 ratio obtained in the rump cap of animals fed linseed oil (Table 4), compared to soybean oil, is directly related to the large amount of fatty acids from the omega-3 chain present in linseed oil, which favored its deposition on the meat also directly related to the high levels of omega-6 fatty acids found in soybean oil. RAES *et al.* (2004) also reported improved omega-6:omega-3 ratio in sirloin and shoulder beef cuts when linseed was added. The data from this study corroborates the results reported by those authors, confirming the beneficial effect of linseed and its byproducts on meat lipid composition. Animals fed unprotected linseed oil compared to protected form provided higher levels of omega-3 fatty acids and consequently lower omega-6:omega-3 ratio. The higher level of omega-3 is due to higher concentration of this fatty acid family in the diet of animals that were fed unprotected oil.

The oil protection process supposedly modifies its digestion site as large amounts of protected oil should pass through rumen without suffering any modification. Consequently, differences in the intestinal absorption and tissue deposition of omega-3 PUFA are expected. However, our results did not show benefic modifications of meat compared to unprotected oil. The poor omega-6:omega-3 ratio observed in the meat of animals fed protected oil (Table 4) do not justify its use, especially linseed oil, included that the protection process cost overburdens the final price of the product.

The evaluation of fatty acids and their functionality (hypercholesterolemic, hypocholesterolemic and neutrals) was also performed (Table 4). This classification has been recommended by BESSA *et al.* (2008) and although not commonly used, it may be important to show how fatty acids act on the human body and might help to promote beef as a functional food. Studies related to fatty acids functionality are scarce at literature; however, we believe that such assessment is important and needs further attention. BESSA *et al.* (2008) stated that emphasizing positive aspects and nutritional value of fats from ruminants in order to rehabilitate its image are welcome.

CONCLUSION

The use of protected oils against ruminal degradation is not efficient, particularly for the linseed oil, when compared to the same unprotected oil. The unprotected linseed oil is the best option to improve the omega-6:omega-3 ratio, however, the unprotected soybean oil is the best option to produce higher amounts of conjugated linoleic acid.

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