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Quality traits and lipid composition of meat from Nellore young bulls fed with different oils either protected or unprotected from rumen degradation

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ABSTRACT

The qualitative characteristics, lipids and chemical composition of the meat of 35 Nellore young bulls were analyzed. These bulls had an average slaughter weight and fat thickness of 532.17 ± 30.2 kg, and 7.00 mm, respectively. Significant differences were found only in the meat's water holding capacity (WHC), which was higher for animals fed with fresh linseed oil. More conjugated linoleic acid (CLA) was found in the meat of animals fed with unprotected soybean oil, while better omega-6/omega-3 ratios were noted for those fed unprotected linseed oil. The addition of different vegetable oils to the bulls' diet (soybean or linseed, either protected or not protected from rumen digestion) did not interfere with the qualitative characteristics of their meat while improving the lipid composition of the longissimus muscle. Of the oils examined, unprotected linseed oil most improved the omega-6/omega-3 ratio, thus producing the healthiest meat for human consumption.

1. Introduction

Studies in meat science are advancing the development of foods with characteristics that benefit human health. According to Mir et al. (2003), bovine meat can be considered as a "functional" element since it provides high-quality proteins and B-complex vitamins in addition to available minerals. However, the fat composition of ruminants' meat has been criticized, mainly due to the high saturated fat content, which has been deemed unhealthy (Bessa, 1999). Thus, the production of higher quality, healthier meat has become a priority (Abrahão, Prado, Perotto, & Moletta, 2005).

According to Felício (2004), meat produced in a tropical environment exhibits lean muscle, is devoid of marbling, and contains only 2–3% lipids, yet can have a good finish of subcutaneous and intermuscular fat. The taste depends on the consumer and may also be affected by the facility that physically separates the fat before or after grilling the meat.

A key part of the improvement of meat quality and composition is regulation of the animal's diet. According to Silva et al. (2007), grains have been substituted for fats and oils in the food of ruminants to increase energy density and nutritional efficiency. The importance of fats or oils in the diet of bovines tends to receive more emphasis than other nutrients, such as protein, fiber, minerals, and vitamins (Sarriés, Murray, Moloney, Troy, & Berlain, 2009). Many studies (Fernandes, Sampaio, Henrique, Oliveira, et al., 2009; Mir et al., 2003; Scollan et al., 2006) showed that animals fed with fresh roughage and concentrates containing different oils (linseed and fish), sources of omega-3 and omega-6 fatty acids, could produce meat with a desirable fatty acid composition.

However, the biohydrogenation in ruminant animals makes it difficult to alter the tissue fatty acid composition (Herdmann, Martin, Nuernberg, Dannenberger, & Nuernberg, 2010). A method to protect the polyunsaturated fatty acids (PUFA), mainly the omega-3 (n-3) fatty acids, from rumen biohydrogenation could be important to improve the omega-6:omega-3 (n6:n3) ratio and promote a better image for beef in world markets.

In light of previous observations, this study sought to evaluate the effects of either protected or unprotected oils from rumen digestion, rich in n-3 and n-6 fatty acids, on quality traits, chemical composition, cholesterol content, and fatty acid composition in *M. longissimus thoracis* from Nellore young bulls.

2. Methods and materials

2.1. Management of the animals and their diets

The experiment was conducted at the Beef Cattle Sector of the Department of Animal Sciences at the College of Agrarian and Veterinary Sciences of Unesp (Jaboticabal, SP, Brazil). A total of 35 Nellore-bred bulls were used, with an average body weight of 402.69 ± 14.90 kg and an average age of 18 ± 2 months. These animals were separated by weight, randomly assigned to different treatment groups, and subsequently adapted to specific management conditions and diets for

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28 days. During this period, their diet was composed of 50% concentrate (corn, soybean meal, citrus pulp, and a mineral mixture) and 50% forage (sugarcane only). The experimental period consisted of 96 days, over which the diet was composed of two meals: one at 8:00 am (40% of the total diet) and the other at 2:00 pm (60% of the total diet).

The experimental diets were formulated from different lipid sources or a control treatment using RLM® (Esalq/USP, Piracicaba, São Paulo, Brazil) software, with nutritional demands estimated by the CNCPS system (Fox, Sniffen, O'Connor, Russell, & Van Soest, 1992) with the aim of maximum weight gain (Table 1). All treatments exclusively used sugarcane forage variety IAC 86 2480, which was harvested and chopped daily. Meanwhile, plant oils were also used in the diets, with the objective of increasing energy concentration without augmenting the portion of concentrate. Megalac-E® was specifically used, which is a commercial product 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 to obtain this product from regular linseed oil at the Laboratory of Applied Enzymology of the College of Agrarian and Veterinary Sciences. The linseed oil was saponified with sodium hydroxide in 65% ethanol using an unheated plastic drum and agitated until glycerol and soap was produced. Once saponification 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 dried at room temperature. The resulting product contained large quantities of unsaturated fatty acids, with 85% protection at a pH level close to that of the rumen. According to Oser (1965), such saponification of fat results in a product with a high total fat composition, a low content of free fatty acids, and almost no oxidation.

At the end of the experimental period, the animals were transported to a slaughterhouse 200 km away and stunned and slaughtered. At the time of slaughter, the bulls exhibited an average weight of 532.17 ± 30.2 kg, an average carcass yield of 55.32%, and an average fat thickness of 7.00 mm. Immediately after slaughter, half-carcasses were stored at 4 °C for 24 h. From the left-hand half-carcasses, cuts between the ninth and thirteenth ribs were removed and taken to the Ruminant Laboratory of the College of Agrarian and Veterinary Sciences for subsequent analysis. All experimental procedures were approved by the Commission on Ethics and Animal Welfare (CEBEA) of the College of Agrarian and Veterinary Sciences (process no. 021167-07).

Table 1
Composition (% of dry matter (DM)), nutritional characteristics, and fatty acid content of the experimental diets.

Foods	Diets (% of DM)				
	Control	Soybean oil	Linseed oil	Megalac-E®	PLO[b]
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®[a]	–	–	–	4.5	–
PLO[b]	–	–	–	–	4.5
Mineral core[c]	2.5	2.5	2.5	2.5	2.5
Limestone	0.5	0.5	0.5	–	–
Nutrient fraction	Nutritional characteristics[d]				
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
EE (% 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	Fatty acid composition of experimental diets (mg/100 g)				
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
C18:1n9c	30.27	27.14	30.74	23.51	28.48
C18:2n6c	45.45	49.36	26.06	38.04	25.68
C18:3n3	3.78	4.97	22.98	3.86	10.96

[a] Protected soybean oil.
[b] Protected linseed oil.
[c] 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.
[d] Nutritional characteristics estimated by RLM® software. CP — Crude Protein; TDN — Total Digestible Nutrients; EE — Ether Extract; ME — Metabolizable Energy.

2.2. Chemical and qualitative analyses of the meat

Samples of 10 g between the 11th and 12th ribs from the left half carcasses were used for chemical analysis (moisture, crude protein, ether extract, and mineral content) which were determined according to AOAC (1995).

Two longissimus muscle steaks, 2.54 cm thick between the 12th and 13th ribs were obtained for the determination of pH, meat and fat color, water holding capacity (WHC), shear force (SF), and cooking loss (CL).

The determination of meat and fat color was performed as described by Houben, Van Dijk, Eikelenboom, and Hoving-Bolink (2000), using a Minolta colorimeter (Model CR 300, Minolta Camera Co. Ltd., Osaka, Japan) evaluating the lightness (L*), redness (a*), and yellowness (b*). The color aspects were assessed by the CIE L*a*b* color system using 0°/45°. Thirty minutes prior to the assessment, cross sections were made at the samples' surface to expose the myoglobin to oxygen, as described by Abularach, Rocha, and Felício (1998), the same steps were made for the fat color measurement. After this step the color was measured at three different points and average values calculated. The colorimeter was calibrated before analyzing the samples against white and black standards.

After evaluating each steak's color, approximately 2 g was collected to determine the WHC. This value was the difference between the weights of the sample before and after it was subjected to a pressure of 10 kg for 5 min (Hamm, 1986).

The meat samples were cooked in a gas oven at 175 °C until they reached 70 °C at their geometric centers measured by digital thermometer. The weights of the steaks before and after cooking were measured to calculate the cooking losses (CL).

After 24 h of cooling, six cylindrical samples were removed from the steaks using a 2.5 cm-diameter drawn punch. A texturometer (Texture Analyzer TA — XT2i, Godalming, Surrey, UK) attached to a Warner–Bratzler blade 3.38 mm thick was used to measure the force necessary to transversally cut each cylinder. The average cutting force was calculated, representing the SF of each sample as described by Abularach, Rocha, and Felício (1998).

For the sensory analysis, two steaks of longissimus muscle (13th rib) 2.54 cm thick were taken and cooked in an oven at 175 °C until their geometric centers reached 70 °C, as measured by a digital thermometer. After this procedure, the meat was cut into cubes, packed in foil and served to a panel of 13 trained panelists in individual cabins. The appearance, meat taste, fat taste, tenderness, and juiciness were evaluated. Scores varied from 1 to 9, with 1 being the minimum score and 9 being the maximum score (Meilgaard, Civille, & Can, 1991).

2.3. Cholesterol content of the meat

Determination of the cholesterol content of the loin was performed spectrophotometrically Genesys (Spectronic® 20 — Genesys, Jakarta, Indonesia), as described by Bragagnolo and Rodrigues-Amaya (1995). The total lipids were measured by extraction from approximately 10-g samples of loin 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 80 °C and agitated for 15 min. 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 separation of the phases, 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 absorbance was determined at 538 nm.

2.4. Fatty acid composition of the meat

To determine the fatty acid composition of the fresh meat, samples of the transversal section were collected from the longissimus muscle, freeze–dried, and frozen for lipid extraction and methylation. The fatty material was extracted using a mixture of chloroform–methanol, as reported by Bligh and Dyer (1959) and the fatty acids methyl esters (FAME) were obtained by ISO 5509 method (1978).

Qualitative and quantitative measurements of fatty acid content were performed by gas chromatography using a chromatograph (Shimadzu, Kyoto, Japan — Model GC-14B with a Communication Bus Module — CBM 102) with a flame ionization detector (FID) and fused silica capillary column (Omegawax 250), which was 30 m in length and 0.25 mm in diameter and had a film thickness of 0.25 μm (Supelco SP-24136). Helium was used as a carrier gas at a flow of 1 mL/min. A 1-μL aliquot of the sample was injected into a "split" at a division ratio of 1/100 and a temperature of 250 °C. The temperature of the oven was programmed to remain at 100 °C for 2 min and then increase to 220 °C at 4 °C/min for 25 min, while the detector was at 280 °C. Identification and quantification of the methyl esters of the fatty acids was achieved by comparison with the retention times and concentrations of methyl esters of standard fatty acids.

2.5. Statistical analysis

Results were subjected to analysis of variance using the general linear model (GLM) (SAS Institute Inc., 2001). A randomized block design was adopted for five treatments with seven repetitions. The means were compared using orthogonal contrasts. To evaluate the sensory characteristics of the meat, a Kruskal–Wallis non-parametric test was used, as described by Sampaio (2002). Differences for which $P<0.05$ were considered statistically significant.

3. Results and discussion

For the results pertaining to the meat's chemical composition (Table 2), no significant interactions were found. The meat of the longissimus muscle did not exhibit differences in moisture, mineral, or protein content among treatments. Yet, the content of the ether extract from the meat of the animals fed with fresh linseed oil (2.12%) was significantly lower than that of the meat of those fed with protected linseed oil (3.41%), and even lower among the unprotected oils.

The average value of 3.02% for the concentration of ether extract was greater than the 2.2% reported by Sampaio et al. (2008) for Nellore bovines and the 1.61% found by Moreira, Souza, Matsushita, Prado, and Nascimento (2003) for crossbred animals finished on pasture. The greater value found in this work was due the greater energy concentration of the bulls' diet compared from the other studies. In addition, the animals slaughtered in the present study were heavier than in the other studies, resulting in more body fat.

Table 2
Chemical composition of longissimus muscle of Nellore young bulls fed with different oils either protected or not protected from rumen degradation.

Treatment	Moisture	Protein	Ether extract	Minerals
	(%)			
Control	65.24	32.32	3.24	1.61
Soybean oil	64.99	32.94	3.18	1.59
Megalac-E®	64.67	32.44	3.15	1.55
Linseed oil	64.51	34.01	2.12	1.59
Protected linseed oil	65.10	31.92	3.41	1.60
Contrasts	Probability			
Control vs. diets with oil	0.7643	0.0940	0.5131	0.6032
Soybean oil vs. linseed oil	0.9820	0.8225	0.2937	0.6340
Protected oils vs. unprotected oils	0.9162	0.2963	0.1033	0.7585
Interaction (oil protection)	0.7167	0.5223	0.0924	0.6876
Protected or not for soybean oil	0.8584	0.2715	0.9621	0.6407
Protected or not for linseed oil	0.7462	0.7853	0.0123	0.9500
Type of protected oil	0.8138	0.7833	0.5900	0.5639
Type of unprotected oil	0.7898	0.5699	0.0349	0.9609
CV[a] (%)	5.03	9.80	32.64	7.82

[a] CV — coefficient of variation.

According to Lawrie (2004), 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.

The interactions and contrasts between meat traits were not significant ($P>0.05$; Table 3), except for WHC. The average SF found in this work was 5.9 kgf/cm^2, which classifies the meat as less tender, as the limit for the tenderness in beef is 4.5 kgf/cm^2. The SF between the treatments did not show differences ($P>0.05$) due to the similarity of the diets, the use of only one breed and the uniform fat cover (average of 7 mm) of the carcass avoiding cold shortening. Therefore, the higher values for SF were related to the Nellore breed which has more crosslinks of collagen and more calpastatin, that decreases the activity of the calpains.

The SF value of 5.97 kgf/cm^2 was greater than that obtained by Fernandes et al. (2009) (3.87 kgf/cm^2), who also evaluated the qualitative characteristics of the meat of Nellore bulls fed two concentrate levels (40% and 60%). According to Pereira (2002), meat tenderness may be explained by the presence of connective tissue proteins and myofibrils. Connective tissues increase hardness due to the cross-linking of collagen, increasing the meat's stability and resistance to chemical and thermal attacks. According to Coró, Youssef, and Shimokomaki (1999), *Bos taurus indicus* animals, in addition to possessing larger muscle fibers, possess more evident cross-links than the meat of animals of European origin, compromising tenderness. All these factors may contribute to the high SF value reported in this study.

Significant differences were not noted between pH values resulting from different treatments. All values were in the normal range for beef cattle (5.4–5.8) (Gregory, 1998). Abnormal pHs (over 6.0) are normally associated with pre-slaughter problems, sexual condition and breed. The pH average in the present work was 5.66 (Table 2). In the present study, during the loading, transportation and handling at the slaughterhouse, there was no event that would influence negatively the animals, thus, the pH values were the normal range for beef. There were no differences between the pH values for the diets offered.

Significant differences in the lightness (L*), redness (a*), or yellowness (b*) of the loin muscle and fat of the different groups (Table 3) were not observed, probably because the animals were from the same breed, had similar ages and diets, and were of normal pH. Costa et al. (2002) commented that the high variation of meat color in beef cattle could be explained by differences in age and sexual condition.

Table 3
Shear force (SF), pH, lightness (L*), redness (a*), and yellowness (b*) of the meat and fat, water holding capacity (WHC), cooking losses (CL) of the longissimus muscle of Nellore bulls fed with different oils, either protected or unprotected from rumen degradation.

Treatment	SF kgf/cm^2	pH	Meat			Fat			WHC	CL
			L*	a*	b*	L*	a*	b*	%	
Control	6.329	5.727	38.550	17.293	6.934	77.170	3.935	8.676	71.63	33.86
Soybean oil	6.086	5.613	38.330	16.959	6.287	79.104	3.342	7.413	70.64	31.06
Megalac-E®	5.493	5.546	38.407	17.566	6.943	78.146	3.487	7.196	71.08	31.38
Linseed oil	6.195	5.667	36.807	15.595	5.275	77.343	3.342	6.740	73.67	32.10
Protected linseed oil	5.760	5.772	39.505	16.819	6.892	78.390	3.651	7.961	70.19	33.79
Contrasts	Probability									
Control vs. diets with oil	0.3377	0.3968	0.8303	0.6384	0.5937	0.3271	0.4177	0.1321	0.8357	0.2456
Soybean oil vs. linseed oil	0.6481	0.0942	0.8596	0.3243	0.5876	0.4378	0.8222	0.9529	0.2806	0.2071
Protected oils vs. unprotected oils	0.2183	0.8162	0.2543	0.3912	0.2516	0.9634	0.6250	0.5231	0.1295	0.4560
Interaction (oil protection)	0.8471	0.2958	0.2808	0.7714	0.6240	0.3078	0.9401	0.3620	0.0543	0.6110
Protected or unprotected for soybean oil	0.3075	0.5754	0.9637	0.7018	0.6320	0.4883	0.7588	0.8132	0.7121	0.8477
Protected or unprotected for linseed oil	0.4514	0.3841	0.1213	0.4435	0.2454	0.4493	0.6762	0.1953	0.0082	0.3199
Type or protected oil	0.6419	0.0703	0.3707	0.6382	0.9700	0.8593	0.9117	0.4102	0.4572	0.1626
Type of unprotected oil	0.8496	0.6501	0.5163	0.3943	0.4620	0.2096	0.8242	0.4680	0.0188	0.5380
CV[a] (%)	18.02	3.76	8.20	16.466	39.59	3.26	40.55	26.94	3.58	10.86

[a] CV — coefficient of variation.

According to Ramos and Gomide (2007), color and appearance are two of the main attributes indicating food quality. Meat color is observed by the consumer at the time of purchase, with dark red meat normally being rejected since dark color is intuitively associated with deterioration.

In the current study, it was observed that the meat of bulls fed with linseed oil had the highest WHC value ($P<0.05$) in comparison to the other oils and types of protection (Table 3). There was no obvious explanation for this and it requires further investigation. The WHC values for all of the treatments were satisfactory. Fernandes, Sampaio, Henrique, Oliveira, et al. (2009), who examined Nellore bulls of the same age and genetic pattern, found smaller WHC values and fluid losses due to cooking, possibly due to the high pH of their samples.

There were no significant differences ($P<0.05$) in cooking losses (CL) (Table 3) among treatments. However, the average value found for this attribute was high (32.48%) which can lead to problems of consumer acceptance after cooking. Fernandes, Sampaio, Henrique, Oliveira, et al. (2009) found CL of 22.6%, lower than in this experiment. However these authors had samples of high pH which can lead to lower values for CL. A trained sensory panel assigned the highest scores for the characteristics of appearance, tenderness, and juiciness ($P<0.05$) to the meat of bulls fed with soybean oil, while the lowest scores were attributed to steaks from animals fed with protected linseed oil (Table 4).

The results for CL (Table 3) can help explain the higher grades obtained for unprotected soybean oil for the variables: appearance, tenderness and juiciness since these samples had the lowest LC compared to the other treatments (31.06% vs. 32.78%, respectively) giving a higher moisture content in the meat, providing greater juiciness, therefore better appearance and softness.

Fernandes et al. (2008) fed Canchim animals food containing sugarcane and sunflower seeds and scored the taste, appearance, and tenderness of the resultant meat (7.17, 7.14, and 7.00, respectively) similarly to the meat examined here (Table 4). The same scoring was reported by Fernandes, Sampaio, Henrique, Oliveira, et al. (2009) for beef from Nellore bulls fed with different proportions of concentrate in their diets.

Scheeder et al. (2001) evaluated the meat of bulls fed different sources of fat and found that the meat of animals fed with linseed oil tended to be juicier and to possess a more agreeable aroma. These results may be due to the higher proportions of omega-3 fatty acids in these animals, triggering odor precursors that are activated by oxidation during heating.

Significant interactions were observed for cholesterol content between the oil source and the type of oil protection (Table 5). In particular, the meat of animals fed with soybean or linseed oil possessed lower cholesterol contents than the same oil protected from rumen degradation ($P<0.05$). However, the cholesterol levels in this study were generally lower than those considered normal for different bovine cuts (58.3–83.4 mg/100 g) according to Werdi Pratiwi, Murray, and Taylor (2006). From the current study, it can be inferred that young, non-castrated zebus fed on a diet with different oils rich in polyunsaturated fatty acids reduced the resultant meat's cholesterol content. Such animals are likely using cholesterol during this stage of their development to produce hormones, for which cholesterol is a precursor.

Moreira et al. (2003) reported a cholesterol content of 37.46 mg/100 g, similar to that noted here, when examining crossbred *Bos taurus indicus* or *B. taurus taurus* animals finished on a pasture with or without protein supplementation. Rule, MacNeil, and Short (1997) evaluated the cholesterol concentration of the longissimus muscle of 18-month-old male bovines, which were feedlot-finished on a high-energy-content diet based on corn and found an average of 52.7 mg/100 g of cholesterol, approximately 50% greater than in the current study.

Cholesterol has important functions in the membrane, increasing its solubility when large proportions of saturated fatty acids are present. As an example, the membranes of erythrocytes contain high levels of cholesterol and low quantities of unsaturated fatty acids, while membranes with greater metabolic activity possess higher quantities of unsaturated fatty acids and lower cholesterol contents.

Table 4
Sensory evaluation of the meat of the longissimus muscle of Nellore bulls fed with different sources of oil, either protected or not protected from rumen degradation.

Variable[a]	Treatment[b]					Probability
	C	SO	M	LO	PLO	
Appearance	5.44	8.18	5.02	5.77	3.66	<0.0001
Taste of meat	6.87	7.58	7.73	7.90	7.79	0.3439
Taste of fat	6.44	5.22	5.81	5.97	5.87	0.5062
Tenderness	6.73	7.68	7.30	6.71	4.36	0.0035
Juiciness	6.53	8.08	5.95	6.03	5.06	0.0028

[a] Scores attributed by a tasting panel: 9 — maximum score; 1 — minimum score.
[b] Treatments: C — control; SO — soybean oil; M — Megalac-E®; LO — linseed oil; PLO — protected linseed oil.

Table 5
Cholesterol concentration and saturated fatty acid composition of the longissimus muscle of Nellore bulls fed with diets containing different sources of oils, either protected or unprotected from rumen degradation.

Treatment	Cholesterol mg/100 g	Fatty acids (mg/100 g)							
		C10:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0
Control	31.33	0.04	0.06	3.47	0.30	26.50	0.76	13.81	0.09
Soybean oil	29.40	0.04	0.05	3.23	0.26	24.62	0.72	15.26	0.10
Megalac-E®	31.16	0.04	0.06	3.29	0.27	25.89	0.69	15.03	0.09
Linseed oil	31.99	0.03	0.05	2.93	0.24	24.20	0.60	12.74	0.12
Protected linseed oil	30.76	0.04	0.05	3.47	0.28	25.71	0.69	14.72	0.10
Contrasts		Probability							
Control vs. diets with oil	0.2690	0.2727	0.0942	0.3931	0.0414	0.0376	0.0285	0.3453	0.2508
Soybean oil vs. linseed oil	0.0100	0.5001	0.4434	0.8097	0.5910	0.6103	0.1197	0.0231	0.3891
Protected vs. unprotected oils	0.5060	0.5001	0.3091	0.2332	0.2361	0.0219	0.3718	0.1448	0.2706
Interaction (oil protection)	0.0009	0.8214	1.0000	0.3217	0.4338	0.8342	0.0879	0.0695	0.7103
Protected or not for soybean oil	0.0001	0.5530	0.5072	0.8825	0.7199	0.1462	0.4255	0.7902	0.6073
Protected or not for linseed oil	0.0030	0.7659	0.5072	0.1300	0.0974	0.0877	0.0212	0.0317	0.3093
Type of protected oil	0.2830	0.5530	0.6180	0.5920	0.8295	0.8386	0.8783	0.7209	0.7313
Type of unprotected oil	<0.001	0.7659	0.6180	0.3845	0.2590	0.6275	0.0051	0.0081	0.3948
CV[a](%)	3.38	20.12	24.83	19.63	16.35	5.91	13.11	10.75	28.76

[a] CV — coefficient of variation.

As shown in the present study, different oils in the diets of Nellore bulls influence this intracellular content.

Costa et al. (2002) evaluated cholesterol in the longissimus muscle of Angus bulls, which were slaughtered at four different stages of maturity but at the same weights, and determined an average cholesterol content of 43.07 mg/100 g, a higher concentration than in the present study. Here, it was observed that the animals' diet caused significant changes in the lipid composition of the longissimus muscle (Tables 5 and 6), with significant interactions for some fatty acids by contrasting protected and unprotected soybean oils, protected and unprotected linseed oils, and the types of protected or unprotected oils.

The addition of oils to the Nellore bulls' diet, independent of the type or whether the oil was protected, decreased their muscular content of pentadecanoic (C15:0), palmitic (C16:0), palmitoleic (16:1), heptadecanoic (17:0), and heptadecenoic (C17:1) fatty acids ($P<0.05$; Tables 5 and 6). In contrast, statistically significant increases were observed in the content of elaidic (C18:1 n7), conjugated linoleic (CLA; C18:2 c9 t11), alpha-linolenic (C18:3 n3), and gamma-linolenic (C18:3 n6) fatty acids, which are beneficial to humans since they decrease the levels of low-density lipoproteins (LDLs) circulating in the blood (Bessa, 1999).

The decrease of saturated fatty acids and increase of unsaturated fatty acids with the addition of oil in the diets was due to the fatty acid composition of oils added, which had higher amounts of unsaturated fatty acids, mainly n-3 and n-6 fatty acids (Table 1). Therefore, the addition of oils in this experiment provided meat with higher levels of unsaturated fatty acids and reduction of some saturated fatty acids.

Among the fatty acids which increased its content, CLA should be highlighted. The increase of CLA was also due to the addition of oils presenting large quantities of its precursor C18:2 n6 (linoleic fatty acid) in diets with unprotected soybean and linseed oils (Table 1).

Additionally, the *B. taurus indicus* breed has high activity of Delta 9-desaturase and elongase which transforms saturated fatty acids containing 16 or 18 carbons into their respective unsaturated versions (Fernandes, Sampaio, Henrique, Oliveira, et al., 2009).

Working with pasture-finished Angus bulls and steers, Padre et al. (2006) observed CLA values of 0.13% and 0.45%, respectively, in the resultant meat. These percentages are lower than found in the present study, with an average CLA value of 0.72% for the meat of animals fed with diets containing different types of oil. Fernandes et al. (2009), in comparing Canchim-breed animals fed diets containing silage and concentrate or sugarcane and sunflower seeds, found an improvement

Table 6
Unsaturated and polyunsaturated fatty acid compositions of the loin meat of Nellore bulls fed with diets containing different sources of oils, either protected or unprotected from rumen degradation.

Treatment	Fatty acids (mg/100 g)													
	C14:1	C16:1	C17:1	C18:1	C18:1	C18:2	C18:2	C18:3	C18:3	C20:1	C20:2	C20:3	C20:3	C20:5
				ω7	ω9	ω6	c9, t11	ω3	ω6	ω9		ω3	ω6	ω3
Control	0.89	3.38	0.69	2.23	39.2	5.66	0.35	0.32	0.09	0.20	0.07	1.28	0.30	0.21
Soybean oil	0.76	2.90	0.58	3.96	38.75	5.43	0.94	0.33	0.07	0.19	0.07	1.15	0.27	0.22
Megalac-E®	0.73	2.80	0.51	2.85	37.05	7.82	0.60	0.38	0.08	0.19	0.08	1.07	0.25	0.15
Linseed oil	0.89	3.42	0.63	3.15	40.08	6.48	0.80	1.21	0.10	0.20	0.06	1.40	0.30	0.28
Protected linseed oil	0.84	3.09	0.57	2.66	39.21	5.42	0.55	0.72	0.18	0.19	0.06	0.92	0.20	0.23
Contrasts	Probability													
Control vs. diets with oil	0.315	0.013	0.0006	0.0002	0.606	0.450	<0.0001	0.001	0.006	0.415	0.786	0.436	0.273	0.864
Soybean oil vs. linseed oil	0.123	0.001	0.033	0.001	0.026	0.364	0.176	<0.0001	<0.0001	0.881	0.166	0.753	0.715	0.139
Protected oils vs. unprotected oils	0.573	0.060	0.012	0.0003	0.095	0.374	0.0003	0.020	<0.0001	0.460	0.423	0.112	0.138	0.223
Interaction (oil protection)	0.904	0.316	0.737	0.112	0.585	0.027	0.545	0.005	<0.0001	0.881	0.423	0.24	0.313	0.890
Protected or not for soybean oil	0.740	0.485	0.031	0.001	0.134	0.038	0.007	0.706	0.706	0.701	0.256	0.725	0.690	0.346
Protected or not for linseed oil	0.610	0.036	0.086	0.124	0.429	0.335	0.035	0.002	0.002	0.566	1.000	0.040	0.055	0.449
Type of protected oil	0.283	0.057	0.062	0.575	0.061	0.037	0.631	0.020	0.020	1.000	0.124	0.504	0.277	0.261
Type of unprotected oil	0.213	0.002	0.159	0.015	0.231	0.342	0.224	<0.0001	<0.0001	0.847	0.665	0.254	0.603	0.346
CV[a] (%)	24.44	9.23	11.13	17.06	5.03	31.59	28.34	38.33	16.64	19.07	26.07	38.10	36.20	55.31

[a] CV — coefficient of variation.

in the fatty acid composition of the longissimus muscle of the second group. Specifically, this meat had higher levels of CLA (0.73 g/100 g of total fatty acids, vs. 0.34 g/100 g) and polyunsaturated fatty acids (8.12 g/100 g of total fatty acids, vs. 6.31 g/100 g). Similarly, when Barton, Marounek, Kudrna, Bures, and Zahradkova (2007) included extruded linseed in the diets of Limousin and Charolais cattle, they found an increase in the concentrations of alpha-linolenic acid (C18:3 n3) and CLA, a decrease in the omega-6/omega-3 ratio, and a decline in saturated fatty acids accompanied by an increase in polyunsaturated fatty acids in the subcutaneous fat.

In this work, the meat of animals fed with diets based on soybean oil, independent of protection, presented lower levels of C16:1, C17:1, C18:1 n9, C18:3 n3, and C18:3 n6 ($P<0.05$).

The lowest levels ($P<0.05$) of C18:3 n3 and C18:3 n6 fatty acids were found in the soybean oil treatments due to its low concentration in those diets, while the diets containing linseed oils were richer in these fatty acids.

Herdmann et al. (2010) found significant increases in the concentrations of n-3 fatty acids (alpha linolenic acid, C20: 5 n3 and C22:5 n3 and C22:6 n3) in meat from German Holstein Bulls fed 3% linseed oil and 12% rapeseed cake.

For C14:0 and C14:1, no change was observed when the oil type and processing were varied (Tables 5 and 6). However, the concentrations of myristic and meristoleic fatty acids were lower than those published (4.39% and 1.11%, respectively) for meat from Nellore bulls fed with sugarcane and concentrate containing sunflower seeds, which are rich in polyunsaturated fatty acids (Fernandes, Sampaio, Henrique, Oliveira, et al., 2009). The greater reduction in these fatty acids noted here is valuable due to the fats' hypercholesterolemic function (Bessa, 1999).

Unprotected oils, regardless of the type, provide meat with significantly higher levels of C17:1, C18:1 n7, CLA, C18:3 n3, and C18:3 n6 and significantly decreased the levels of C16:0. The meat of animals fed diets containing unprotected soybean oil had higher values for all of these fats except C18:2 n6. This may be related to the greater degradation of C18:2 n6 in the rumen, leading to CLA formation (Bauman, Baumgard, Corl, & Griinari, 2000).

When consumed by ruminants, dietetic lipids undergo two important transformations in the rumen (Church, 1988). The initial transformation is the hydrolysis of the ester bond by microbial lipases. This initial step is a prerequisite for the second transformation, the bio-hydrogenation of unsaturated fatty acids.

Interactions ($P<0.05$) were found for the fatty acids C18: 2 n6, C18: 3 n3 and C18:3 n3. Assessment showed that the meat from animals fed protected soybean oil (Megalac-E®) had higher levels of C18:2 n6. This is directly related to the protection of the oil that allowed the fatty acid to pass intact through the rumen allowing its absorption in the intestine and subsequent deposition in the tissues. The assessment of the interaction for the C18:3 n3 fatty acid showed that the meat from animals fed with unprotected linseed oil had higher levels of this fatty acid compared to the other treatments. Therefore, the expected result for the treatment with protected linseed oil was not reached. The protection of this oil was not effective in providing meat with higher concentrations of omega-3 fatty acids compared with the unprotected oil, which would be the expected result.

The fatty acid composition of the diets (Table 1) shows that the protection process of linseed oil provided important losses of fatty acids, especially linolenic acid (C18:3 n3). Therefore, the diet with protected linseed oil had lower levels of C18:3 n3 compared to the same unprotected oil, which influenced the levels of C18:3 n3 in the meat.

Differences were not detected in the quantities of saturated, unsaturated, and polyunsaturated fatty acids in the longissimus muscle of animals fed with diets containing oil, as compared to the control diet (Table 7). However, larger quantities of unsaturated fats were observed in the meat of animals specifically fed with unprotected oils or fresh linseed oil ($P<0.05$). The result for this variable was directly related to the large amount of omega-3 fatty acids that diets with linseed oil had compared with the other diets (Table 1).

In contrast, when Barton et al. (2007) fed Charolais- or Simmental-breed steer diets supplemented with extruded linseed (70% extruded linseed and 30% wheat bran), they found quantities of saturated fats in the meat greater than reported here (48.54% vs. 42.60%, respectively).

There were higher ($P<0.05$) amounts of unsaturated (UFA) and monounsaturated (MUFA) fatty acids in meat from animals fed unprotected oils. This provided better ($P<0.05$) ratios of unsaturated: saturated fatty acids (UFA:SFA), monounsaturated:saturated (MUFA: SFA) fatty acids and n-6:n-3, than meat from animals fed protected oils.

Table 7
Fatty acid sum and ratios of the loin meat of Nellore bulls fed with diets containing different sources of oils, either protected or not protected from rumen degradation.

Treatment	Sum and ratio of the fatty acids[b]									
	Sat	Unsat	Mono	Poly	Unsat :sat	Mono:sat	Poly:sat	ω3	ω6	ω6:ω3
Control	45.07	54.92	46.61	8.31	1.22	1.03	0.18	1.82	6.05	3.43
Soybean oil	44.31	55.68	47.17	8.51	1.26	1.06	0.19	1.70	5.79	3.49
Megalac-E®	45.38	54.61	44.14	10.47	1.21	0.97	0.23	1.61	8.17	5.05
Linseed oil	40.94	59.05	48.40	10.65	1.45	1.18	0.26	2.90	6.88	2.36
Protected linseed oil	45.09	54.90	46.58	8.31	1.22	1.03	0.18	1.88	5.80	3.10
Contrasts	Probability									
Control vs. diets with oils	0.391	0.391	0.964	0.329	0.381	0.534	0.326	0.504	0.487	0.662
Soybean oil vs. linseed oil	0.130	0.130	0.025	0.993	0.110	0.036	0.657	0.012	0.419	<0.0001
Protected oils vs. unprotected oils	0.034	0.034	0.004	0.858	0.034	0.006	0.535	0.051	0.406	<0.0001
Interaction (oil protection)	0.198	0.198	0.436	0.052	0.165	0.448	0.064	0.999	0.034	0.007
Protected or not for soybean oil	0.533	0.533	0.016	0.214	0.580	0.149	0.372	0.812	0.046	<0.0001
Protected or not for linseed oil	0.024	0.024	0.132	0.142	0.020	0.019	0.089	0.014	0.347	0.0003
Type of protected oil	0.868	0.868	0.047	0.173	0.878	0.339	0.311	0.478	0.048	<0.0001
Type of unprotected oil	0.061	0.061	0.301	0.176	0.046	0.057	0.112	0.005	0.339	<0.0001
CV[a] (%)	6.979	5.520	4.381	30.230	12.721	9.952	36.429	35.786	31.181	12.22

Unsat — Unsaturated — C14:1 + C16:1 + C17:1 + C18:1ω7 + C18:1ω9 + C18:2 ω6 + C18:2 c9, t11 + C18:3ω3 + C18:3ω6 + C20:1ω9 + C20:2 + C20:3 ω3 + C20:3ω6 + C20:5 ω3.
Mono — monounsaturated — C14:1 + C16:1 + C17:1 + C18:1ω7 + C18:1ω9 + C20:1ω9.
Poly — polyunsaturated — C18:2 ω6 + C18:2 c9, t11 + C18:3ω3 + C18:3ω6 + C20:2 + C20:3ω3 + C20:3ω6 + C20:5 ω3.
ω3 — omega-3 fatty acids — C18:3ω3 + C20:3ω3 + C20:5ω3.
ω6 — omega-6 fatty acids — C18:2 ω6 + C18:3ω6 + C20:3ω6.
[a] CV — coefficient of variation.
[b] Sat — saturated — C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0.

Fernandes, Sampaio, Henrique, Oliveira, et al. (2009) reported lower ratios for Nellore bulls fed with sugarcane and sunflower seeds (1.02 and 1.27, respectively), indicating a less healthy fatty acid composition of the meat. Barton et al. (2007) reported a lower polyunsaturated/saturated fat ratio (0.13) than the present study (0.21). However, both of these ratios are lower than the 0.4 value recommended by the Department of Health of the United Kingdom for a healthy diet (Wood et al., 2003).

According to Simopoulos (2002), the excessive quantities of omega-6 fatty acids and the elevated omega-6/omega-3 ratio found in current Western diets may promote cardiovascular diseases, cancer, inflammatory diseases, and autoimmune disorders. Therefore, an increase in dietary omega-3 polyunsaturated fatty acids, resulting in a lower omega-6/omega-3 ratio, is beneficial for human health. Here, significantly greater quantities of omega-3 fatty acids were noted in the longissimus muscle of bulls fed with unprotected linseed oil (2.9% vs. an average of 1.75% for the other treatments). For the sum of the omega-6 fatty acids, there was a significant interaction, with the Megalac-E® diet achieving the greatest quantities of omega-6 (8.17% vs. an average of 6.13% for the other treatments). Additionally, the omega-6/omega-3 fatty acid ratio exhibited a significant interaction with the unprotected linseed oil-based diet, yielding a ratio of 2.36, and the protected linseed oil diet, resulting in a 3.13 ratio. Meanwhile, the meat of bulls fed fresh soybean oil possessed a better omega-6/omega-3 ratio than Megalac-E® (3.49 vs. 5.01, respectively). The average ratio of 3.4 reported here contrasts with the 6.08 average omega-6/omega-3 ratio published by Rodrigues, Bressan, Cardoso, and Fritas (2004) for both intact and castrated Nellore bulls, again due to differences in diet; in the present study, the animals were fed with oils that specifically narrowed the omega-6/omega-3 ratio, rendering their meat healthier.

4. Conclusions

In this study, the use of different sources of oil, soybean or linseed, protected or unprotected from rumen degradation in diets for Nellore young bulls affects only the WHC, while the other characteristics have not changed.

Feeding Nellore young bulls with unprotected soybean and linseed oils provides lower levels of cholesterol in the loin.

The use of linseed oil, regardless of the protection, is a strategy to improve the fatty acid composition of meat, especially to increase the amounts of n-3 and improve the ratios between n-6: n-3. The unprotected soybean oil is the best option for reducing cholesterol, to produce meat with better appearance, tenderness and juiciness in sensory analysis, besides the increase in conjugated linoleic acid levels.

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