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ARTICLE

Characteristics of carcass and meat of heifers finished on pasture amended with different supplement frequency and soybean oil source

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Abstract: Our objective was to investigate the effects of supplements with soybean oil source, administered either daily or three times per week, on meat quality and carcass characteristics of heifers finished on pasture. Forty-two crossbred heifers aged 17 ± 3 mo with an initial mean body weight (BW) of 297.1 ± 13.5 kg were selected for pasture finishing. The animals were randomly assigned to six treatments. Each treatment consisted of two feeding frequencies, daily (DAI; seven times per week, supplemented at 0.75% BW) or alternating (MWF; three times per week, on Monday, Wednesday, and Friday, supplemented at 1.75% BW on each day), and three different soybean oil sources: soybean oil (SO), rumen-protected fat (RPF), and whole soybean (WS). There was no interaction ($P > 0.05$) between the feeding frequencies and soybean oil source on meat quality and carcass characteristics. Supplements with WS resulted in a greater C18:0 concentration in the *longissimus* muscle compared with those with SO or RPF ($P = 0.02$). Supplements with SO increased the rumenic acid deposition, the total monounsaturated fatty acids, and the monounsaturated:saturated fatty acids ratio ($P < 0.05$). We conclude that reduction in the supplementation frequency does not alter the carcass and meat quality traits.

Key words: conjugated linoleic acid, *Brachiaria brizantha*, protected fat, soybean oil, supplementation.

Résumé : Notre objectif était d'étudier les effets des suppléments ayant comme source l'huile de soja, administrés quotidiennement ou trois fois par semaine, sur la qualité de viande et les caractéristiques de la carcasse chez les génisses en finition dans les pâturages. Quarante-deux génisses croisées âgées de 17 ± 3 mo avec une moyenne de poids corporel (BW — « body weight ») initiale de 297,1 ± 13,5 kg ont été sélectionnées pour la finition en pâturage. Les animaux ont été assignés aléatoirement à six traitements. Chaque traitement était constitué de deux fréquences d'alimentation, tous les jours (DAI — « daily », sept fois par semaine, suppléments à 0,75 % BW) ou en alternance (MWF — « Monday, Wednesday, Friday », trois fois par semaine, lundi, mercredi et vendredi, suppléments à 1,75 % BW chaque jour), ainsi que trois sources différentes d'huile de soja : l'huile de soja (SO — « soybean oil »), gras protégés dans le rumen (RPF — « rumen-protected fat »), et le soja entier (WS — « whole soybean »). Il n'y avait pas d'interaction ($P > 0,05$) entre les fréquences d'alimentation et les sources d'huile de soja sur la qualité de viande ni les caractéristiques de la carcasse. Les suppléments de WS se sont soldés par une plus grande concentration de C18:0 dans le muscle *longissimus* par rapport aux suppléments SO ou RPF ($P = 0,02$). Les suppléments SO ont augmenté le dépôt d'acide ruménique, la quantité totale d'acides gras mono-insaturés et le rapport d'acides gras mono-insaturés:saturés ($P < 0,05$). Nous concluons donc que la réduction de la fréquence de supplémentation ne change pas la qualité de viande ni les caractéristiques de la carcasse. [Traduit par la Rédaction]

Mots-clés : acide linoléique conjugué, *Brachiaria brizantha*, gras protégé, huile de soja, supplémentation.

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Introduction

The Brazilian beef industry is specialized in raising grass-fed cattle (Millen et al. 2009). Despite the variations in quantity and quality of forages, the growth of cattle can be compromised in the dry season. During these periods, supplementation of beef cattle raised on pastures is a common practice used to enhance the performance of the animals, reduce slaughter age, and improve meat quality by increasing concentration of polyunsaturated fatty acids (PUFA) in meat products, especially *n*-3 PUFA and conjugated linoleic acid (CLA) (San Vito et al. 2015). In addition, optimization of the feeding frequency of animals raised on pasture has been suggested to reduce production costs without reducing productivity (Canesin et al. 2007). Previously, it was considered that feeding significantly alters fermentation parameters (Canesin et al. 2014); however, literature data (Morais et al. 2014; Santana et al. 2015) demonstrate that cattle that are fed low-quality forage and supplemented infrequently are capable of maintaining their performance, nutrient intake, and characteristics of carcass and meat when compared with animals supplemented on a daily basis.

Previous research has shown that grass-fed beef contains a higher concentration of unsaturated fatty acids (UFA), especially *n*-3 fatty acids, than feedlot beef, which can increase rumenic acid concentration in milk and in beef intramuscular fat (Yang et al. 2002; Dannenberger et al. 2006; Kazama et al. 2008). Rumenic acid is the main form of CLA present in foods originating from ruminant animals (*cis*-9, *trans*-11; Kramer et al. 1998). Although increased content of UFA in meat is important, it should be emphasized that their physical and chemical properties directly affect the sensorial qualities and preservation of meat (Oliveira et al. 2011). The PUFA are susceptible to oxidation with the methylene bridge being the main target of free radicals; their oxidation can be harmful to meat, affecting characteristics associated with color and reducing its shelf life (Madruga 2004).

Another nutritional strategy for improving beef quality is manipulation of ruminal fermentation through the inclusion of lipid sources in diet. This strategy may increase the escape of UFA from the rumen (Duckett and Gillis 2010), potentially reducing the deposition of saturated fatty acids (SFA) and increasing the concentration of UFA in beef.

Among the different feeds available for ruminant nutrition in Brazil, soybean grain (high in lipids) is widely available and cost efficient given its high nutrient content (Barletta et al. 2012). Currently, Brazil is the world's largest producer of soybeans (United States Department of Agriculture 2014). Feeding with supplements high in lipid content has been used to increase the energetic value of diets in highly productive animals to enhance the yield and nutritional value of the meat (Rosa et al. 2013). However, inclusion of high levels of whole soybean, rich in unsaturated oils, can decrease animal performance when the fat content of the diet exceeds 6% of dry matter (DM) (Palmquist and Jenkins 1980; Hess et al. 2008). This is attributed to the coating of fiber particles by lipids and to the antimicrobial effects, which result in toxic effects on Gram-positive bacteria in the rumen, especially the cellulolytic and methanogen populations (Jenkins 1993).

Digestion of lipid forms and deposition of fatty acids in tissues can be altered by ruminal availability of lipids, the amount of supplement, and the composition of the basal diet (Jenkins and McGuire 2006). Diverse types of lipids with different fatty acid composition or availability can be used in ruminant diets. Among them oils are not protected, oil seeds are partially protected, and protected fats are the most protected from microbial action.

Protected fats from soybean oil may decrease ruminal biohydrogenation of UFA, which is a limiting factor for the deposition of UFA in the meat and dairy products of ruminants (Herdmann et al. 2010). Thus, supplementation of different soybean oil sources may be a nutritional strategy to increase the ruminal escape of UFA (Duckett and Gillis 2010), reduce the deposition of SFA, and enhance the levels of UFA in animal tissues.

Considering the pasture as the basal diet, a limited number of studies have evaluated the effects of soybean oil source on meat and carcass quality in *Bos indicus* breeds grazing on tropical pastures. We hypothesized that the reduction in feeding frequency of dietary supplements and higher protection of soybean oil will increase the deposition of UFA in beef from heifers fed on pastures. Thus, the objective of this study was to evaluate the meat and carcass quality of heifers finished on pasture of *Brachiaria brizantha* 'Marandu' and supplemented at different feeding frequencies with various soybean oil sources (soybean oil, rumen-protected fat, and whole soybean).

Materials and Methods

Animal, experimental design, and diets

The protocol used in this experiment was carried out following the guidelines laid down by the Canadian Council on Animal Care and was approved by the Ethics, Bioethics, and Animal Welfare Committee (Comissão de Ética e Bem Estar Animal, CEBEA, Brazil) of the Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal Campus (protocol no. 012799).

The experiment was performed for 135 d (July 2007 to November 2007) on the farm owned by the São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Jaboticabal. The mean monthly rainfall during the experimental period was 61.1 mm, with a minimum temperature of 16.5 °C, maximum temperature of 30.5 °C, and mean of 22.6 °C.

Table 1. Composition of the supplements in experimental diets.

	Diet supplement[a]		
	WS	SO	RPF
Ingredients (g kg^{-1} as fed)			
Whole soybean	522	0.00	0.00
Soybean oil	0.00	86.0	0.00
Megalac-E®	0.00	0.00	104
Ground corn	432	408	386
Soybean meal	0.00	461	464
Mineral supplement[b]	46.0	46.0	46.0
Chemical composition			
Dry matter (DM) (g kg^{-1} as fed)	878	891	889
Organic matter (g kg^{-1} of DM)	872	881	872
Crude protein (g kg^{-1} of DM)	264	290	299
Ether extract (g kg^{-1} of DM)	138	132	133
Neutral detergent fiber (g kg^{-1} of DM)	266	287	286
Lignin (g kg^{-1} of DM)	29.3	26.6	25.5
Metabolizable energy (MJ kg^{-1})	12.0	12.3	12.6

[a]Diets containing whole soybeans (WS), rumen-protected fat (RPF) (Megalac-E®), or soybean oil (SO) as the fat source.
[b]Composition of the product expressed in grams or milligrams per 100 g of supplement: calcium, 210 g; phosphorus, 20 g; sulfur, 37 g; sodium, 80 g; copper, 490 mg; manganese, 1.424 mg; zinc, 1.830 mg; iodine, 36 mg; cobalt, 29 mg; selenium, 9 mg; and fluorine (maximum), 333 mg.

Forty-two crossbred heifers aged 17 ± 3 mo (one-quarter Nellore × one-quarter Santa Gertrudis × one-half Braunvieh) with an initial mean body weight (BW) of 297.1 ± 13.5 kg were selected for pasture finishing. The animals were randomly assigned to six treatments, with seven animals per treatment as experimental units (Robinson et al. 2006). Each treatment consisted of two feeding frequencies, daily (DAI; seven times per week, supplemented at 0.75% of BW) or alternating (MWF; three times per week, Monday, Wednesday, and Friday, supplemented at 1.75% of BW on each day), and three different soybean oil sources: soybean oil (SO; rumen-non-protected), rumen-protected fat (RPF; calcium salts of soybean oil; Megalac-E®, Arm and Hammer Animal Nutrition, Church & Dwight Co., Inc., affiliate Química Geral do Nordeste S/A, Rio de Janeiro, Brazil), and whole soybean, ground raw (WS; rumen-partially protected). Rumen-protected fat (Megalac-E®) is manufactured by a saponification process where fatty acids (based on soybean oil) react with calcium hydroxide. These ingredients were incorporated into the supplement, which was composed of corn and soybean meal. The soybean meal was not used in the WS-supplemented diet (Table 1). At the beginning of the treatment, the animals were weighed, identified, and treated against ecto- and endo-parasites by administering ivermectin (Ivomec, Merial, Paulínea, Brazil).

The animals were randomly allocated to six 2.0 ha paddocks of *B. brizantha* 'Marandu', resulting in a total of seven animals in each paddock, in a continuous stocking rate grazing system with variable stocking. Along with the experimental animals, a number of cohort cattle were allowed to the paddock to control pasture vegetation at 3.0 kg herbage DM kg^{-1} BW. Stocking rate was estimated using the average BW of each animal, calculated as a difference between the initial and final BW during an experimental period, and the amount of above ground portion of herbage DM in that paddock. Despite the homogeneity within the experimental area, to minimize the effects of forage variation among the paddocks, the animals from the experimental treatments were rotated among the paddocks every 15 d. The composition of the pastures was reported by Santana et al. (2015).

The respective supplements were provided to the animals in each paddock at 0800 in linear feeders allowing 50 cm of space per head per feeder. The supplements were formulated to include 5% lipid content of the total DM. The DM intake from the supplement was calculated using the mean consumption per group within each paddock (treatment). The fatty acid profiles of the experimental diets (forage and supplements) are presented in Table 2.

Sampling and data collection

Forage and supplements were collected and analyzed for nitrogen [N; Association of Official Agricultural Chemists (AOAC) Official Method 984.13], ash (AOAC Official Method 942.05), and ether extract (AOAC Official Method 954.02) contents in accordance with the AOAC (1990). The neutral and acid detergent fiber analysis were conducted according to the procedures described by Mertens (2002), except that the samples were weighed in polyester filter bags (porosity of 25 μm) and treated with neutral detergent in an autoclave at 110 °C for 40 min (Senger et al. 2008). Acid detergent lignin was determined by solubilization of cellulose with sulfuric acid according to the protocol described by Van Soest and Robertson (1985).

Slaughter, carcass data, and sample collection

After 135 d of feeding, all the animals (the average shrunk BW 360 ± 19.2 kg) were slaughtered at a commercial beef plant. The preharvest handling was in accordance with good animal welfare practices, and the slaughtering procedure followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil 1997).

After the slaughter, all the carcasses were weighed and refrigerated at 0 °C for approximately 24 h. After the postmortem chilling period, final carcass pH, backfat

Table 2. Fatty acid profiles (%) from pasture and diet supplements.

		Diet supplement[a]		
Fatty acid (% of total FAs)	Pasture	WS	SO	RPF
Capric (C10:0)	0.07	0.01	0.01	0.00
Lauric (C12:0)	0.00	0.00	0.00	0.00
Miristic (C14:0)	2.98	0.26	0.15	0.41
Palmitic (C16:0)	33.4	22.8	22.5	28.3
Margaric (C17:0)	0.63	0.12	0.07	0.12
Stearic (C18:0)	4.48	5.29	5.49	5.88
Palmitoleic (C16:1)	3.30	0.79	1.29	0.41
Oleic (C18:1 c9)	9.96	29.4	28.3	25.8
Linoleic (C18:2)	7.38	35.1	33.4	31.9
Linolenic (C18:3)	35.3	2.07	0.92	1.49
Saturated fatty acids (SFA)[b]	41.3	30.0	30.5	37.5
Unsaturated fatty acids (UFA)[c]	58.7	70.0	69.5	62.5
Monounsaturated fatty acids (MUFA)[d]	4.40	31.9	34.5	28.0
Polyunsaturated fatty acids (PUFA)[e]	54.3	38.1	35.0	34.5

[a]Diets containing whole soybeans (WS), rumen-protected fat (RPF) (Megalac-E®), or soybean oil (SO) as the fat sources.
[b]SFA = C4:0, C6:0, C8:0, C10:0, C12:0, C13:0iso, C13:0ant, C14:0iso, C14:0, C15:0iso, C15:0ant, C15:0, C16:0iso, C16:0, C17:0iso, C17:0, C18:0, C20:0, C22:0, and C24:0.
[c]UFA = Sum of MUFA and PUFA.
[d]MUFA = C14:1c9, C16:1c9, C17:1, C18:1t6, C18:1t9, C18:1t10, C18:1t12, C18:1c9, C18:1c11, C18:1c12, C18:1c13, C18:1t16, C18:1c15, C20:1, C22:1, and C24:1.
[e]PUFA = C18:2t11c15, C18:2c9c12, C18:3, C18:2c9t11, C20:3, C20:4, C22:2, C20:5, C22:5, and C22:6.

thickness (BFT), and 12th rib *longissimus* muscle area (LMA) were measured on the left side of each carcass. The final pH was measured at a depth of approximately 4 cm in the *longissimus* muscle of the left side of each carcass (12th rib) using a pH meter (Testo 230, Testo GmbH & Co, Lenzkirch, Germany). The LMAs were traced on acetate paper and later measured with a planimeter (Steiner et al. 2003). The BFT measurements on the *longissimus* muscle followed procedures outlined by Fiorentini et al. (2015).

A transverse section [Hankins and Howe section (HH)], including the 9th, 10th, and 11th thoracic rib, was removed from the carcass to evaluate body composition and evaluate the physical separation of muscles, bones, and fat according to the method described by Hankins and Howe (1946). After dissection, the following equations were used to predict the proportion of muscle, adipose tissue, and bone in the carcass (Hankins and Howe 1946):

$$\text{Muscle proportion}: Y = 16.08 + 0.80X;$$

$$\text{Adipose tissue proportion}: Z = 3.54 + 0.80X; \text{ and}$$

$$\text{Bone proportion}: W = 5.52 + 0.57X,$$

in which X is the component percentage in the HH section obtained in the cold room.

A boneless *longissimus* section, 10 cm thick, was removed from the posterior end of the wholesale rib. *Longissimus* muscle samples were vacuum packaged individually and stored at −20 °C for 2 d (48 h after slaughter). Each *longissimus* sample was standardized from the posterior end into one 2.54 cm thick steak sample [American Meat Science Association (AMSA 1995)] for measurement of Warner–Bratzler shear force (WBSF) and other analyses as described below.

Meat color

Meat color was determined according to the method described by Houben et al. (2000) using a Minolta colorimeter (Model CR 300, Minolta Camera Co. Ltd., Osaka, Japan) to evaluate the lightness (L^*), redness (a^*), and yellowness (b^*). The color aspects were assessed by the CIE $L^*a^*b^*$ color system on a 25 mm diameter measurement area using a D65 illuminant and an angle of 0° as the standard observing point. Thirty minutes prior to the assessment, cross sections were made at the samples' surface to expose the myoglobin to oxygen. The same steps were repeated for the fat color measurement. After these steps, the color was measured at three different points and average values were calculated. The colorimeter was calibrated against white and black standards before analyzing the samples.

Shear force measurement, water-holding capacity, and cooking loss

The steaks were thawed at 4 °C for 24 h and oven-broiled in an electric oven (Luxo Inox, Layr, Sáo Paulo,

Brazil) preheated to 150 °C. Internal steak temperature was monitored by 20-gauge copper–constantan thermocouples (Omega Engineering, Stamford, CT, USA) placed in the approximate geometric center of each steak and attached to a digital monitor. When internal steak temperature reached 35 °C, the steak was turned over and allowed to reach an internal temperature of 70 °C before its removal from the oven. Cooked steaks were cooled for 24 h at 4 °C (AMSA 1995). Six cylindrical samples were removed from each steak using a 2.5 cm diameter punch. A texturometer (Texture Analyzer TA-XT2i, Godalming, Surrey, UK) attached to a 3.38 mm thick Warner–Bratzler blade was used to measure the force necessary to transversally cut each cylinder. The average cutting force was calculated, representing the shear force of each sample.

Cooking loss was evaluated on the steaks that were also used for WBSF measurements. Water-holding capacity was calculated as the percentage of sample weight remained after pressure treatment. Total cooking loss was calculated as the difference between the weight of the steaks before and after oven broiling, thawing loss was calculated as the difference between the weight of the steaks before and after thawing, and total loss was calculated by adding up the cooking loss and thawing loss.

Fatty acid profile

To determine the fatty acid composition of the feed, approximately 1 g of frozen sample was homogenized in 20 mL of chloroform and methanol solution (2:1) using a Turrax homogenizer, disintegrator, and emulsifier (Folch et al. 1957). In the next step, the lipid extract aliquot was methylated using the protocol described by Kramer et al. (1997). Fatty acids were quantified using a GC 2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) with an SP-2560 capillary column (100 m × 0.20 mm i.d. with 0.02 μm film thickness; Supelco, Bellefonte, PA, USA). The initial temperature was set to 70 °C for 4 min (13 °C min^{-1}) until it reached 175 °C, and then held for 27 min. After this, the temperature was increased 4 °C min^{-1} until it reached 215 °C and held there for 31 min. Hydrogen was used as the carrier gas at a flow of 40 cm^3 s^{-1}.

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. Lipids were extracted using a mixture of chloroform and methanol following a protocol described by Bligh and Dyer (1959), and fatty acid methyl esters (FAME) were obtained according to the ISO 5509 method [International Organization for Standardization (ISO) 1978]. Qualitative and quantitative measurements of the fatty acid content were performed by gas chromatography (Finnigan Focus GC, Thermo Fisher Scientific, Waltham, MA, USA) with fused silica capillary column (100 m × 0.250 mm, 0.20 μm film thickness; CP-Sil 88 for FAME, Agilent Technologies, Santa Clara, CA, USA) and hydrogen as a carrier gas at a constant flow of 1.5 mL min^{-1}. The flame ionization detector was set at 300 °C. The time and temperature program employed started with an initial temperature of 70 °C for 4 min, followed by 175 °C (at 13 °C min^{-1}) with a waiting time of 27 min and 215 °C (4 °C min^{-1} increment) with a waiting time of 9 min, and the final step at 230 °C for 4 min (at 7 °C min^{-1}), totaling to 65 min. Fatty acids were identified by comparing their retention times with those observed in commercial standards (C4–C22) of Supelco 18919-1AMP, a methyl ester mixture of 37 fatty acids (Sigma–Aldrich, St. Louis, MO, USA). Major fatty acids were identified by pure commercial standards: 05632 SIGMA, a mixture of CLA *cis*-9, *trans*-11, *trans*-10, and *cis*-12-octadecadienoic acid methyl esters; and V1381 SIGMA, *trans*-vaccenic acid methyl ester (Sigma–Aldrich, St. Louis, MO, USA).

Statistical analysis

A completely randomized design with a 3 × 2 factorial arrangement of treatments consisting of soybean oil source (SO, RPF, and WS) and feeding frequency (three and seven times per week) was employed. The carcass and meat characteristics were analyzed in a split plot arrangement, with frequencies as subplots and diet as the main plot. Supplementation diets, soybean oil source (2 df), frequency of supplementation (1 df), and the interaction of these two factors as fixed effects and a residual error as random effect were used in MIXED procedure implemented in SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). The effects of significant interactions between soybean oil source and each supplementation frequency were compared using Fisher's least significant difference (i.e., DIFF option LSMEANS command in SAS) at significance level of $P < 0.05$.

Results and Discussion

There was no interaction ($P > 0.05$) between the feeding frequencies and forms of oil for any of the variables evaluated. Therefore, the effects were discussed independently.

The supplementation frequencies and the soybean oil source of dietary supplements did not change the carcass characteristics of heifers and had no deleterious effects on total DM intake, shown average value 1.91% BW, and weight gain performance with the average of 0.58 kg BW d^{-1} (Santana et al. 2015). These observations indicate that the type of supplements and supplementation frequency do not affect the size of the organs or the gastrointestinal tract, resulting in no differences in animal weight and dressing percentages at slaughter among the treatments (Lage et al. 2012). Other studies on the supplementation of animals with different lipid sources also reported no significant effects on carcass traits (Zinn et al. 2000; Aferri et al. 2005;

Table 3. Carcass traits of crossbred heifers fed diets supplemented with different soybean oil sources at different frequencies.

	Diet (*D*)[a]			Frequency (*F*)			*P* value		
	WS	SO	RPF	DAI	MWF	SEM	*D*	*F*	*D* × *F*
No. of heifers	14	14	14	21	21	—	—	—	—
SW (kg)	354	372	367	363	366	8.62	0.14	0.38	0.94
HCD (%)	50.32	51.11	51.71	51.32	50.7	0.39	0.08	0.33	0.09
LMA[b] per 100 kg	35.73	35.21	36.50	36.02	35.71	0.89	0.79	0.97	0.12
LMA (cm^2)	62.42	65.53	67.25	65.51	64.92	1.06	0.23	0.65	0.36
BFT (mm)	3.69	4.65	4.63	4.04	4.64	0.24	0.07	0.30	0.62
Carcass composition (from 9-10-11 rib-cut dissection)									
Bone (%)	18.76	17.63	17.52	18.03	17.95	0.85	0.06	0.93	0.42
Muscle (%)	65.32	64.10	65.99	64.96	65.35	1.78	0.30	0.99	0.80
Adipose (%)	15.93	18.27	16.47	17.00	16.68	0.76	0.08	0.98	0.48

Note: No means differ significantly at the $P < 0.05$ level. DAI, daily supplementation; MWF, supplementation on Monday, Wednesday, and Friday; SEM, standard error of mean; SW, slaughter weight; HCD, hot carcass dressing; LMA, *longissimus* muscle area; BFT, backfat thickness.

[a]Diets containing whole soybeans (WS), rumen-protected fat (RPF) (Megalac-E®), or soybean oil (SO) as the fat sources.

[b]*Longissimus* muscle area in relation to cold carcass weight (LMA cm^2/100 kg).

Fiorentini et al. 2012*b*). The mean dressing percentage of 51.05% reported herein was similar to those of other studies; for example, Fiorentini et al. (2012*b*) reported a dressing percentage of 50.78% for one-quarter Nellore × one-quarter Santa Gertrudis × one-half Braunvieh heifers, which is considered typical and meets the standards of Brazilian markets (Eiras et al. 2014).

The LMA was similar for all treatments, with mean values of 65.10 cm^2 total area and 35.81 cm^2 per 100 kg ($P > 0.05$; Table 3). According to Sainz (1996), the LMA can accurately estimate the muscular development of an animal because the final BW at slaughter is the main factor that affects the LMA. Thus, heifers supplemented with varying levels of soybean oil forms under different feeding frequencies had similar BWs at slaughter and consequently similar LMAs. Fiorentini et al. (2012*b*) reported similar values of LMA (35.45 cm^2 per 100 kg) for heifers finished with different lipid sources in a feedlot, which exceeded the minimum value of 29 cm^2 per 100 kg of carcass recommended by Luchiari Filho (2000) to indicate sufficient muscle development.

All the carcasses from animals evaluated in this study had similar BFT ($P > 0.05$; Table 3), with a mean value of 4.32 mm, which is classified as uniform and within the standards required by the Brazilian beef industry (Kuss et al. 2008). The fat layer is an important thermal insulator because it minimizes the shortening of muscle fibers caused by abrupt fall in temperature on the surface of the muscle (Fiorentini et al. 2012*a*). Zinn et al. (2000), in their study on Holstein steers, did not observe a correlation between the BFT and the diet containing protected fat or animal fat as a lipid source at up to 60.0 g kg^{-1} of feed. In addition, Santana et al. (2014) observed that the feed with different forms of soybean oil did not affect carcass BFT in feedlot heifers.

The dietary supplementation of grass-fed animals with different lipid forms did not affect the proportion of bone, adipose, and muscle tissues ($P > 0.05$; Table 3). The fat, muscle, and bone proportions in animals are of great interest to the producer, the industry, and, in particular, the consumer (Hankins and Howe 1946) and determine the commercial value of the carcasses of meat-producing animals (Berg et al. 1978; Aaslyng 2009). The protein:fat ratio of a carcass can be altered by increasing the size at maturity, administering hormones or hormonal modifiers, limiting energy intake during the growing period or finishing period, or by slaughtering the cattle at an earlier stage of maturity (Owens et al. 1995). Therefore, in the present study, we used animals of the same breed, sex, and age; the treatments (feeding frequency or oil availability in the supplement) did not affect the BW at slaughter, dressing percentage, LMA, or BFT, indicating that there were no differences in body composition before and after the treatment.

With regard to the meat quality, the water-holding capacity, cooking loss, and shear force of the beef did not differ among the treatments, indicating that these parameters are not modified by diet supplements or feeding frequency ($P > 0.05$; Table 4). However, the mean value of the observed shear force (8.25 kg cm^{-2}) is not considered an acceptable level of tenderness according to Belew et al. (2003), because its values exceeded 6.00 kg cm^{-2}, signifying hard meat texture. In our experiment, the shear force value was greater than the average value of 7.6 kg cm^{-2} obtained by Fiorentini et al. (2012*a*). In other studies, the shear force of heifer meat varied between 3.0 and 6.2 kg cm^{-2}

Table 4. Meat traits measured on *longissimus* muscle of crossbred heifers fed diets supplemented with different soybean oil sources at different frequencies.

	Diet (*D*)[a]			Frequency (*F*)			*P* value		
	WS	SO	RPF	DAI	MWF	SEM	*D*	*F*	*D* × *F*
No. of heifers	14	14	14	21	21	—	—	—	—
SF (kg cm^{-2})	8.31	8.82	7.63	8.04	8.32	0.56	0.34	0.54	0.25
WHC (%)	72.01	72.71	72.91	73.21	71.80	1.89	0.78	0.20	0.84
CL (%)	33.91	33.72	32.62	33.23	33.67	1.28	0.31	0.58	0.81
L^* (lightness)	36.42	36.02	36.92	36.91	36.06	0.82	0.46	0.10	0.70
a^* (redness)	17.90	17.61	18.33	18.24	17.75	0.41	0.26	0.18	0.71
b^* (yellowness)	3.42	3.33	4.12	3.83	3.53	0.34	0.09	0.26	0.58
pH	5.71	5.70	5.84	5.76	5.77	0.24	0.12	0.87	0.08

Note: No means differ significantly at the $P < 0.05$ level. DAI, daily supplementation; MWF, supplementation on Monday, Wednesday, and Friday; SEM, standard error of mean ; SF, shear force; WHC, water-holding capacity (percentage of sample weight remained after pressure treatment); CL, cooking loss.

[a]Diets containing whole soybeans (WS), rumen-protected fat (RPF) (Megalac-E®), or soybean oil (SO) as the fat sources.

(Restle et al. 2001; Aferri et al. 2005). The higher value of the shear force observed in the present study may be due to the thicker Warner–Bratzler cell blade used in this study (3 mm) compared with the standard Warner–Bratzler blade (1.016 mm or 0.04 inches), since WBSF method has increased sensitivity to blade thickness when assessing meat texture (Pinto et al. 2010). Meat tenderness varies with the rate of glycolysis, the post-slaughter rigor onset, and the extent of glycolysis, which are all related to muscle temperature as well as muscle fiber characteristics, processing, and storing of carcasses, while the flavor is influenced by fat content and can be manipulated by genetic methods, growth performance control, and dietary supplementation (Joo et al. 2013). Collagen in muscle composition and marbling in fat content are two principal components that are responsible for meat tenderness and flavor, respectively, both of which vary with age and breed (Listrat et al. 2016). The greater shear force observed in our study may also be attributed to heifers' age or the difference in enzymatic activity, since free-range animals on pasture have greater level of exercise and (or) physical activity (Varnam and Sutherland 1995; Campo et al. 2008), which alters muscle fibers and affects meat quality (Lefaucheur and Gerrard 2000).

The carcass pH at 5.75 was considered ideal, because the pH normally decreases postmortem from the initial 7.0–7.2 to final values of 5.4–5.8 in the first 48 h after slaughter in unstressed animals with large reserves of muscle glycogen (Young et al. 2004). The pH value is higher in carcasses with low, generally <3 mm, rib BFT (Immonen et al. 2000; Velasco et al. 2004). In the present study, the BFT was measured at 4.32 mm, which is sufficient to allow normal decline in pH. Thus, muscle acidification occurred as expected, and because of the lack of differences in BFT values, it was not affected by feeding frequency or lipid forms in the supplement.

Evidence suggests that ingredients in ruminant diets can play a significant role in color and lipid stability of meat (Realini et al. 2004; Warren et al. 2008), which are most likely mediated by the dietary supply of UFA (Woods and Fearon 2009). In the present study, supplementation with different soybean oil source or feeding frequencies did not affect beef color (mean values 36.45, 17.95, and 3.62 for L^*, a^*, and b^*, respectively) ($P > 0.05$; Table 4). Our values fall within the ranges reported by Muchenje et al. (2009) for L^*, a^*, and b^* (33–41, 11.1–23.6, and 6.1–11.3, respectively). These authors stated that the luminosity and coloration of meat are directly related to the pH after cooling. In the present study, the pH values remained within the ideal limits, and the L^*, a^*, and b^* characteristics presented values that are considered typical for beef. Similar lack of an effect of supplementation by different lipid sources on meat color has been reported previously (Granit et al. 2001; Oliveira et al. 2012).

Animals fed WS supplement had greater C18:0 concentration in the *longissimus* muscle than animals fed SO or RPF ($P = 0.02$). The rumenic acid (18:2 c9 t11) concentration ($P = 0.01$), monounsaturated fatty acid (MUFA) concentration ($P = 0.02$), and MUFA:SFA ratio ($P = 0.01$) were greater in the *longissimus* muscle of animals fed SO supplement than in animals fed the other two supplements. The other fatty acids and ratios were not affected by the soybean oil source in the supplements or by the feeding frequency ($P > 0.05$; Table 5).

We hypothesized that diets with rumen-protected soybean oil would increase the deposition of UFA in beef. Thus, the levels of PUFA and MUFA were expected to increase in beef from animals fed diets with WS and RPF. However, the higher content of protected fat in

Table 5. Fatty acid contents (%) of *longissimus* muscle of crossbred heifers fed diets supplemented with different soybean oil sources at different frequencies.

	Diet (*D*)[a]			Frequency (*F*)			*P* value		
	WS	SO	RPF	DAI	MWF	SEM	*D*	*F*	*D* × *F*
No. of heifers	14	14	14	21	21	—	—	—	—
Saturated fatty acids (SFA) (% of total FAs)									
Capric (C10:0)	0.049	0.047	0.045	0.045	0.049	0.004	0.82	0.39	0.41
Lauric (C12:0)	0.12	0.10	0.11	0.11	0.11	0.01	0.12	0.72	0.77
Miristic (C14:0)	2.32	2.35	2.34	2.34	2.29	0.19	0.94	0.72	0.55
Palmitic (C16:0)	22.4	22.0	22.4	22.3	22.2	0.02	0.75	0.77	0.33
Margaric (C17:0)	0.99	0.94	1.02	0.99	0.98	0.03	0.37	0.89	0.47
Stearic (C18:0)	18.32a	15.77b	16.89b	17.47	16.34	0.55	0.02	0.11	0.12
Monounsaturated fatty acids (MUFA) (% of total FAs)									
Miristoleic (C14:1 c9)	0.46	0.55	0.51	0.51	0.51	0.05	0.34	0.99	0.09
Palmitoleic (C16:1 c9)	2.23	2.39	2.21	2.21	2.36	0.01	0.72	0.40	0.16
Heptadecenoic (17:1)	0.67	0.71	0.67	0.69	0.68	0.03	0.66	0.96	0.95
Octadecenoic (C18:1 t10-t11-t12)	7.46	10.91	7.63	9.14	8.16	2.24	0.06	0.49	0.16
Oleic (C18:1 c9)	33.2	33.2	33.6	33.1	33.6	0.02	0.97	0.79	0.20
Polyunsaturated fatty acids (PUFA) (% of total FAs)									
Linoleic (C18:2 c9 c12)	9.75	8.39	10.5	9.06	10.1	0.56	0.45	0.51	0.56
α-Linolenic (C18:3 n3)	0.59	0.56	0.55	0.57	0.56	0.02	0.76	0.92	0.40
Rumenic acid (C18:2 c9 t11-CLA)	0.79b	1.41a	1.01b	1.08	1.07	0.19	0.01	0.94	0.18
EPA (C20:5)	0.42	0.37	0.27	0.33	0.37	0.07	0.17	0.56	0.85
DPA (C22:5 n3)	0.88	0.77	0.79	0.77	0.86	0.05	0.75	0.46	0.48
DHA (C22:6 n3)	0.12	0.10	0.07	0.09	0.11	0.01	0.33	0.15	0.26
Total SFA[b]	46.6	43.8	45.4	45.8	44.7	1.09	0.09	0.23	0.75
Total MUFA[c]	40.0b	44.2a	41.0b	41.7	41.8	0.99	0.02	0.92	0.12
Total PUFA[d]	13.4	12.0	13.6	12.5	13.5	0.48	0.63	0.60	0.58
Total unsaturated fatty acids (UFA)[e]	53.4	56.2	54.6	54.2	55.3	1.06	0.28	0.44	0.80
PUFA:SFA	0.29	0.28	0.31	0.28	0.31	0.05	0.81	0.51	0.66
MUFA:SFA	0.86b	1.01a	0.90b	0.91	0.94	0.04	0.01	0.41	0.15
UFA:SFA	1.15	1.28	1.20	1.18	1.24	0.07	0.18	0.32	0.82

Note: Means within a column not sharing a lowercase letter differ significantly at the $P < 0.05$ level. DAI, daily supplementation; MWF, supplementation on Monday, Wednesday, and Friday; SEM, standard error of mean; EPA, eicosapentaenoic fatty acid; DPA, docosapentaenoic fatty acid; DHA, docosahexaenoic fatty acid.

[a]Diets containing whole soybeans (WS), rumen-protected fat (RPF) (Megalac-E®), or soybean oil (SO) as the fat sources.

[b]SFA = C4:0, C6:0, C8:0, C10:0, C12:0, C13:0iso, C13:0ant, C14:0iso, C14:0, C15:0iso, C15:0ant, C15:0, C16:0iso, C16:0, C17:0iso, C17:0, C18:0, C20:0, C22:0, and C24:0.

[c]MUFA = C14:1c9, C16:1c9, C17:1, C18:1t6, C18:1t9, C18:1t10, C18:1t12, C18:1c9, C18:1c11, C18:1c12, C18:1c13, C18:1t16, C18:1c15, C20:1, C22:1, and C24:1.

[d]PUFA = C18:2t11c15, C18:2c9c12, C18:3, C18:2c9t11, C20:3, C20:4, C22:2, C20:5, C22:5, and C22:6.

[e]UFA = Sum of MUFA and PUFA.

the rumen was not sufficient to increase UFA in beef. The slow release rate of fatty acids in oilseed is probably within the biohydrogenation capabilities of ruminal microbes (Coppock and Wilks 1991), resulting in lower MUFA and higher stearic acid (C18:0) content compared with those originating from soybean oil; similar results were reported by Fiorentini et al. (2012*a*). The protected soybean oil did not increase the concentration of MUFA compared with the soybean oil supplements. This is directly related to the protection of oil that does not allow fatty acids to pass intact through the rumen and be absorbed in the intestine and subsequently deposited in tissues (Oliveira et al. 2012). Lundy et al. (2004) assessed the effect of addition of 2.75% soybean oil calcium salts in the diet of dairy cows and found biohydrogenation values of 77.9% and 92.2% for 18:1 (*n*-9) and 18:2 (*n*-6) fatty acids, respectively. The level of dissociation of rumen-protected fat varies with the source of protected oil. Protected fats provide inconsistent results and limited ruminal protection (Jenkins and Bridges 2007). The protected fat used in this study originated from soybean oil rich in UFA. Calcium salts with high content of SFA are more efficiently protected than those with low SFA content (Jenkins and Bridges 2007). Therefore, diets supplemented with WS result in beef with higher C18:0 content, which represents approximately 30% of total SFA (Scollan et al. 2006). Although the saturated fat in beef may increase cholesterol

content in humans, fats rich in stearic acid do not exhibit this characteristic; C18:0 is thus considered to be neutral in terms of the control of plasma cholesterol levels (Scollan et al. 2006). The concentrations of C18:0 fatty acids found in this study are higher than those reported by Fiorentini et al. (2012*a*) for feedlot heifers with same oil availability in the rumen (average 14.56%). The greater C18:0 concentration found in the *longissimus* muscle of heifers fed on pastures is possibly related to the greater concentration of oleic acid in the diet and, consequently, results in greater biohydrogenation of this acid, which may have been converted to C18:0 by ruminal microorganisms.

Individual fatty acids within the same class (i.e., *cis* or *trans* MUFA, SFA, and PUFA) may have different effects on human health. Greater intake of MUFA benefits human health and is linked to reduction in cholesterol levels in the blood (United Kingdom Department of Health and Social Security 1994). In contrast, a recent review by Souza et al. (2015) suggests that SFA are not associated with all-cause mortality and cardiovascular disease, although the evidence is heterogeneous and with methodological limitations. However, trans fats are associated with all-cause mortality, total coronary heart disease (CHD), and CHD mortality probably due to higher intake of industrial trans fats than ruminant trans fats. Thus, the greater MUFA concentrations, or PUFA:SFA and MUFA:SFA ratios are not conclusive indicators of the nutritional value of feedstuff when healthier diets are considered. In the present study, higher MUFA:SFA ratios were detected in animals fed diet with soybean oil, which is likely due to the combination of soybean oil source (availability of soybean oil in rumen), the rate of ruminal biohydrogenation, and the ruminal passage rate of UFA. Similarly, Oliveira et al. (2012) observed that the meat of animals fed diets containing unprotected soybean oil had higher MUFA and rumenic acid levels.

Most likely the diets such as SO (characterized by fast oil release in the rumen) produce greater amount of linoleic or linolenic acids in the rumen, resulting in incomplete biohydrogenation. This, in turn, allows intermediate fatty acids to escape biohydrogenation, which has the potential to increase the rumenic acid concentration in meat (Fiorentini et al. 2015). Besides the rumenic acid that escapes ruminal biohydrogenation, the vaccenic acid (*trans* C18:1) may also be converted into rumenic acid in adipose and muscle tissues in the reaction catalyzed by the desaturase enzyme (C18-Δ9) in both animals and humans (Griinari et al. 2000).

Diets with soybean oil have a potential to elevate the concentration of rumenic acid, likely due to higher concentration of UFA in the rumen, which is negatively related to the rate of biohydrogenation and release of linoleic or linolenic acids for biohydrogenation. These findings are in agreement with Beam et al. (2000), who observed that the rate of biohydrogenation depends on the quantity and source of lipids. Working with pasture-finished Angus bulls and steers, Padre et al. (2006) observed rumenic acid values of 0.13% and 0.45%, respectively, in the resultant meat, whereas Oliveira et al. (2012) reported the average rumenic acid value of 0.72% for the meat of animals fed diets containing different types of oil in feedlot. These percentages are lower than those found in the present study. The total CLA content of specific foods may vary widely (Dhiman et al. 2005); for example, it varies from 0.17% to 1.35% in beef (Dhiman et al. 2005). Although the minimum effective intake of rumenic acid for disease prevention and overall health in humans is unknown, beef provides more than 30% of its current intake (Scollan et al. 2014).

The treatments did not influence the linolenic acid proportions in the present study. Scollan et al. (2014) reported increased levels of linolenic acid and long-chain PUFA in beef lipids in animals fed forage. Similar increase of linolenic acid (0.55–0.59) in the *longissimus* muscle was detected when the results were compared with those reported by Fiorentini et al. (2012*a*) (0.17–0.21) for heifers fed with the same supplements used herein.

The average PUFA concentration found in this study was greater than the values obtained by Fiorentini et al. (2012*a*) in their evaluations of oil availability in the rumen. However, their study was conducted with feedlot animals fed corn silage; hence, the greater values obtained in the present study can be attributed to the intake of fresh forage. The level of *n*-3 PUFA in muscles of cattle fed fresh grass is usually greater than that of cattle fed conserved grass, and it increases with the intake of pasture and the feeding time at pasture (Scollan et al. 2006).

In this present study, the mean PUFA/SFA ratio was 0.30. Although there are strategies to improve the quality of beef, such as the addition of fish oil to diet to increase the *n*-6:*n*-3 PUFA ratio, these supplements do not generally increase the PUFA/SFA ratio of meat beyond the typically observed range of 0.10–0.15 (Scollan et al. 2014). The PUFA/SFA ratio for beef is generally low (approximately 0.10), except in very lean animals (<1% of intramuscular fat) in which the PUFA/SFA ratio is much higher, ranging from approximately 0.5 to 0.7 (Scollan et al. 2006).

Conclusion

The reduction of supplementation frequency (from daily to three times a week) in our experimental conditions did not alter meat quality and carcass characteristics. The supplementation with soybean oil is a strategy to improve the fatty acid composition of meat, especially to increase the amount of MUFA and improve the MUFA:SFA ratio.

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