Effects of different forms of soybean lipids on enteric methane emission, performance and meat quality of feedlot Nellore

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Abstract

The aim of the current study was to evaluate the effect of different forms of soybean lipids on enteric methane emission, intake, performance, digestibility and meat quality of 40 young Nellore bulls (initial body weight (BW) 444 ± 10.2 kg and 24 ± 2.1 months). The dietary treatments were as follows: NF = no dietary additional fat (46.0 g ether extract (EE)/kg diet), SO = soybean oil (62.0 g EE/kg diet), SB = soy beans (without any processing; 62.0 g EE/kg diet) and RPF = rumen-protected fat based on soybean oil (62.0 g EE/kg diet). The intake of dry matter (DM), organic matter (OM), crude protein (CP) and neutral detergent fibre (NDF) was greater in the SO diet than those fed with NF. The SO diet decreased digestibility of NDF when compared with the NF and RPF diets. The diets did not affect digestibility of DM, OM, CP or emission of enteric methane. Animals fed with SO had greater average daily gain and feed efficiency in relation to the other diets tested. The SO diet increased hot and cold carcass weights and subcutaneous fat thickness of carcasses when compared with the NF diet. The proportions of saturated and unsaturated, mono and polyunsaturated, and n-3 and n-6 fatty acids were not affected significantly by treatments. The SO diets were demonstrated to be more beneficial for animal performance compared with diets without supplemental fat.

Introduction

Changing meat fatty acid profiles in order to reduce the proportion of saturated fatty acids (SFA) is of paramount importance in the production of healthier meat for consumers (Ladeira *et al.*, 2014). Thus, improving the nutritional quality of beef is a key challenge for the beef industry. The current challenge of global livestock production is to increase the productivity and quality of the end product, such as milk and meat, and reduce environmental impact. Thus, an alternative suggested for use in livestock to mitigate methane (CH_4) emissions and improve meat quality is the inclusion of lipids in diets of ruminants (Cottle *et al.*, 2011; Moloney *et al.*, 2012). This strategy could be an attractive target for manipulation because the reduction of CH_4 is usually linked to increased productivity (Leng, 1993) and improvement in meat fatty acid profiles.

Soybean is one of the most commonly used lipid sources in ruminant diets in Brazil due to its availability, low cost and high nutritive content (Barletta *et al.*, 2012). Brazil is currently one of the world's largest soybean producers (USDA, 2018). The lipids present in soy beans contain approximately 850 g/kg of unsaturated fatty acids (UFA), especially oleic, linoleic and linolenic acids. The inclusion of lipid sources in cattle feed may increase the energy density of the diet, resulting in increased energy intake, decreased methane emission and modification of the fatty acid profile of meat from these animals (Fiorentini *et al.*, 2012, 2014, 2015*a*). According to Grainger and Beauchemin (2011), the addition of 10.0 g fat/kg dry matter (DM) in the diet reduces the emission of methane by 1.0 g/kg DM intake by cows. In contrast, the high quantity or availability of fatty acids in the rumen may interfere with rumen fermentation, causing adverse effects on the digestibility and intake of nutrients (Fiorentini *et al.*, 2015*b*). However, the effects of fat in the rumen, the digestion of diet components (e.g. fibre) and the deposition of fatty acids in tissues may vary greatly because they are determined by the degree of saturation and availability of fat in the diet, the amount of supplement provided and composition of the basal diet (Jenkins and McGuire, 2006).

Thus, the hypothesis of the current study is that the addition of different forms of soybean lipids in the diet could reduce methane emissions, increase animal performance and improve the profile of UFA in the meat and the response will depend on the form in which the lipids are fed. So, the objective of the current study was to evaluate the effect of different forms of

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Table 1. Composition of the experimental diets

	Diet (g/kg on a DM basis)			
	NF	SO	SB	RPF
Ingredients				
Maize silage	300	300	300	300
Crude glycerine	100	100	100	100
Ground maize	479	457	465	454
Soybean meal	81.1	85.6	–	86.3
Soybean oil	–	17.3	–	–
Soybean	–	–	95.2	–
Megalac-E®	–	–	–	19.7
Urea	10.0	10.0	10.0	10.0
Mineral supplement[a]	30.0	30.0	30.0	30.0
Chemical composition				
Dry matter	619	620	620	620
Organic matter	954	955	956	945
Crude protein	160	160	161	164
NDF	293	286	296	286
Ether extract	46.0	62.0	62.0	62.0
Total digestible nutrients[b]	719	758	730	754
Fatty acids (g/100 g of total fatty acids)				
C10:0 (capric)	0.15	0.11	0.11	0.12
C12:0 (lauric)	0.24	0.18	0.18	0.21
C14:0 (myristic)	0.10	0.10	0.10	0.11
C16:0 (palmitic)	13.7	13.0	13.1	14.4
C16:1 (palmitoleic)	0.14	0.13	0.12	0.13
C17:0 (margaric)	0.15	0.13	0.14	0.15
C17:1 (heptadecenoic)	0.03	0.04	0.04	0.03
C18:0 (stearic)	2.76	3.08	3.16	3.17
C18:1n9c (oleic)	33.4	31.9	31.7	31.1
C18:2 n6,c (linoleic)	45.6	46.3	46.4	43.8
C18:3 n6 (linolenic)	1.48	2.56	2.58	1.79

NF, no additional fat; SO, soybean oil; SB, soy beans; RPF, rumen-protected fat; NDF, neutral detergent fibre. The guarantee levels of the RPF (Megalac-E®): 850 g/kg ether extract; linoleic acid (C18:2): 450 g/kg; Linolenic acid (C18:3): 60 g/kg.
[a]Composition of product expressed in g or mg per 100 g of supplement: Calcium, 210 g; Phosphorus, 20 g; Sulphur, 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; Fluorine (maximum), 333 mg.
[b]Estimated by NRC (2001).

soybean lipids in diets on enteric methane emission, intake, performance, digestibility and meat quality of feedlot young Nellore bulls.

Materials and methods

The study was carried out at the animal nutrition experimental sector of the Departamento de Zootecnia at FCAV/UNESP, Jaboticabal, São Paulo, located at 21°15′22″ S, 48°18′58″ W and 595 m asl.

Experimental design and animals

A total of 40 Nellore young bulls with an initial average body weight (BW) of 444 ± 10.2 kg (after the adaptation period and at 24 ± 2.1 months) were housed in individual 8 m^2 (4×2 m^2) pens and provided with a feed trough and a water trough. The animals spent 28 days acclimating to the diets, facilities and management. After this period, they were confined for 84 days (totalling 112 days – acclimatization plus experimental period), during which intake, weight gain and CH_4 emissions were evaluated. At the beginning of the trial (0 day – after 28 days of acclimatization period), all the animals were weighed after a 16-h fast and before the first feeding in the morning. Average daily weight gain (ADG) was obtained by weighing animals at the beginning and the end of the experiment (84 days), always after a 16-h fast.

Diets, sampling and data collection

Young bulls were fed with one of the following experimental diets: NF = no dietary additional fat (46.0 g ether extract (EE)/kg diet); SO = soybean oil (62.0 g EE/kg diet); SB = soy beans (without any processing; 62.0 g EE/kg diet); and RPF = rumen protected fat based on soybean oil (62.0 g EE/kg diet; Megalac-E® – Church and Dwight, affiliate Química Geral do Nordeste S/A, Camacari, Brazil). The RPF (Megalac-E®) is manufactured by a saponification process where fatty acids (based on soybean oil) react with calcium hydroxide. The diets were mixed weekly to avoid potential rancidity problems.

All four diets were composed of maize silage (300 g/kg DM), used as the only source of roughage and concentrate (700 g/kg DM). Concentrates were composed of maize, soybean meal, urea, crude glycerin (803 g/kg glycerol; 15.9 g/kg EE; 50.3 g/kg ash; 120 g/kg water and 0.20 g/kg methanol). The crude glycerin was acquired from a soybean oil-based biodiesel production company (ADM, Rondonópolis, Brazil; Table 1). All diets were formulated to provide 160 ± 4.4 g/kg of crude protein (CP) to meet the nutritional requirements of Nellore bulls with an average initial weight of 400 kg and ADG of 1.25 kg/day (Valadares Filho *et al.*, 2006).

Cattle were fed with maize silage and the experimental concentrates twice per day at 08:00 and 16:00 h as a total mixed ration. Throughout the entire experimental period, the quantities provided were adjusted to allow a surplus of approximately 100 g/kg in relation to the total amount consumed on the previous day. Daily weights and samplings were taken for the diet quantities provided. To estimate intake, samples of feed offered (maize silage and concentrates) and feed refusals from each animal were first composited weekly and then composited every period (every 28 days). Feed samples were frozen at −20 °C for later analysis. Samples (feed offerings and feed refusals) were dried at 55 °C for 72 h and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1 mm screen and analysed according to AOAC (1990) to determine DM (method 934.01) and mineral matter (MM; method 942.05) and to obtain EE (method 954.02). Nitrogen was determined using a LECO FP-528 nitrogen analyser (LECO Corp., St. Joseph, MI, USA). Neutral detergent fibre (NDF) was determined using α-amylase and without the addition of sodium sulphite following Van Soest *et al.* (1991) and adapted for the Ankom^{200} Fibre Analyser (Ankom Technology, Fairport, NY, USA).

The fatty acid composition of feed offerings was determined using approximately 1 g of dried sample. The frozen sample

was homogenized in 20 ml of a 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 of Kramer *et al.* (1997). Fatty acids were quantified using a gas chromatograph (GC 2010, Shimadzu Corp., Kyoto, Japan) with an SP-2560 capillary column (100 m × 0.20 mm internal diameter with a 0.02-µm film thickness; Supelco, Bellefonte, PA, USA). The initial temperature was set to 70 °C for 4 min (13 °C/min) until it reached 175 °C and then held for 27 min. After this, the temperature was increased by 4 °C/min until it reached 215 °C and held there for 31 min. Hydrogen was used as the carrier gas with a flow of 40 cm^3/s (Table 1).

Total tract diet digestibility coefficients were determined for all animals using the indigestible NDF (iNDF) marker technique, as described by Cochran *et al.* (1986). There were three *in vivo* digestibility measurement periods (every 28 days = 3 periods) with ten animals from each treatment sampled during each period. Faecal samples were collected directly from the rectum over 3 days (end each period – 26th day), at 07:00, 11:00 and 16:00 h, the first, second and third day of collection, respectively (Ferreira *et al.*, 2009). Faecal samples were dried at 55 °C for 72 h, ground and composited proportionately (on DM basis) for each of the 3 days of sampling, for each animal. The iNDF was obtained via an *in-situ* methodology after 240 h (Casali *et al.*, 2008) with incubated samples of feed offered, feed refusals and faeces (Wiley mill, 2 mm screen). The iNDF was analysed using an Ankom200 Fibre Analyser (Ankom Technology Fairport, NY, USA).

Methane measurements

Methane emissions were assessed using the sulphur hexafluoride (SF$_6$) tracer technique (Johnson *et al.*, 1994), where each animal was sampled daily for 5 consecutive 24-h days, beginning at 77 days of feeding. Twenty-four animals (six per treatment) were fitted with gas collection halters at 10 days before methane sampling to allow animals to adapt and facilitate sampling. The selection criterion utilized was the animal's tameness and ease of handling in the management centre (stockyard). The enteric methane was measured simultaneously on the 24 animals.

The release rate of gas from a permeation tube was known before its insertion into the rumen. The permeation tubes were maintained in a water bath at 39 °C and weighed for 6 weeks in the laboratory. The average release rate was 2.0 ± 0.52 mg SF$_6$/day, mean ± S.D. Brass permeation tubes filled with SF$_6$ and known release rates were administered orally to each of the 20 animals 72 h before methane sampling to allow the tracer gas to equilibrate in the rumen. The animals were fitted with gas collection halters connected to pre-evacuated polyvinyl chloride canisters designed to fill halfway over 24 h. Sampling started at 06:00 h daily (one by one), when the animals were removed from the pens and was conducted at the management centre (stockyard) to facilitate sampling.

The collection canisters were located above each animal to reduce the risk of equipment damage and were connected to the halter by tubing inside airline flexible-coil tubing. Canister pressure was measured after 24 h of collection and the halter replaced if the final pressure was beyond the expected range (from −12 000 PSI (initial pressure) to −6000 PSI (final pressure)); higher pressure indicated probable blockage of the halter, whereas lower pressure indicated a probable leak in the system. In both situations, a new halter was placed on the animal to achieve an average absorption rate within the stipulated range; filling halfway over 24 h. After sampling (approximately 30 min), each animal was taken to the original pen for feeding. The pressure readings were recorded and the canisters pressurized using pure nitrogen gas (N$_2$). The ambient air samples in the feedlot were collected twice daily to determine the background concentrations of methane and SF$_6$. These values were subtracted from the animal's values to calculate the net output in the expired breath. At the end of sample collection, concentrations of CH$_4$ and SF$_6$ were determined at the EMBRAPA Environment laboratory, located in Jaguariúna, Sao Paulo State, Brazil, by a GC HP6890 (Agilent, Delaware, USA), equipped with flame ionization detector at 280 °C, column megabore (0.53 mm × 30 m × 15 µm) Plot HP-Al/M (for CH$_4$), electron capture detector at 300 °C and for cleaning purposes. Soon after the collection period and prior to the determination of CH$_4$ and SF$_6$, the canisters were pressurized to between 1.3 and 1.5 PSI (g) using special nitrogen 5.0, and then readings of the initial and final dilution pressures were taken with a portable digital manometer (±0.01), certified for reading range from −1 to +2 bar (g) (model DPI705, Druck, Groby, UK), in order to obtain the dilution factor. Calibration curves were established using standard gases certified by the White Martins development laboratory (Jaboticabal, São Paulo, Brazil), with the concentration for CH$_4$ in ppm (5 ± 0.3, 10 ± 0.2 and 19 ± 0.7 ppm) and for SF$_6$ in ppt (34 ± 9.0, 91 ± 9.0 and 978 ± 98.0 ppt), according to Westberg *et al.* (1998). The emission of methane (kg/year) was calculated by multiplying the daily emission by 365 days. Methane emissions (g CH$_4$/kg DM intake (DMI, g/kg)) were calculated by dividing the daily CH$_4$ output of each animal by daily intake (during methane sampling).

Slaughter, carcass data and sample collection

At the end of the trial period (84 days), young bulls were fasted for 16 h on the day prior to transportation to the slaughterhouse. The slaughterhouse (Minerva Foods, Barretos, Sao Paulo, Brazil) was 90 km and 01:30 h transport time away from the feedlot. On arrival at the slaughterhouse, they were kept in resting pens and slaughtered after 6 h. Pre-harvest handling was in accordance with good animal welfare practices and slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997). After slaughter, carcasses were weighed and then refrigerated at 0 °C for approximately 24 h. After the post-mortem chill period, the cold carcass weight (CCW), final carcass pH (using a Testo 205 pH meter 205 coupled penetration electrode − Testo Inc., Sparta, NJ, USA), 12th rib subcutaneous fat thickness (SFT) and 12th rib *longissimus* loin-eye area (LEA) were measured on the left side of each carcass. The LEAs were traced on transparencies and measured later with a compensating polar planimeter; SFT measurements were taken at ¾ of the ventral length over the *longissimus* muscle (Steiner *et al.*, 2003). Cold carcass yield (CCY) was calculated using CCW divided by final slaughter weight and then multiplying the result by 100.

A boneless *longissimus* section was removed from the posterior end of the wholesale rib (between the 10th and 13th ribs on the right half of the carcass). *Longissimus* muscle samples were vacuum-packed individually and held at −20 °C for 2 days. After that, each frozen *longissimus* sample was standardized from the posterior end into one 2.54 cm thick steak sample

(AMSA, 1995) for Warner–Bratzler shear force (WBSF) measurement and other analyses as described later. All steaks were vacuum-packed and held at −20 °C for 10 days, then they were thawed at 4 °C for 24 h prior to analysis.

Meat and subcutaneous fat colour

The determination of meat and fat colour was performed as described by Houben *et al.* (2000), using a Minolta colorimeter (Model CR 300, Minolta Camera Co. Ltd., Osaka, Japan) evaluating lightness (*L**), redness (*a**) and yellowness (*b**). The colour aspects were assessed by the International Commission on Illumination *L**a*b* colour system with a 25-mm-diameter measurement area using a D65 illuminant (which corresponds roughly to the average midday light in Western Europe/Northern Europe) and 0° as the standard observing point. The samples were thawed before colour evaluation. Cross sections were made at the sample surface 30 min prior to the assessment to expose the myoglobin to oxygen; the same steps were performed for the fat colour measurement. After this step, the colour was measured at three different points and the average values calculated. The colorimeter was calibrated before analysing the samples against black and white standards.

Warner–Bratzler shear-force measurement, cook loss and sarcomere length

Warner–Bratzler shear-force steaks were thawed at 4 °C for 24 h and oven-broiled in an electric oven (Layr, Luxo Inox, Rio de Janeiro, Brazil) preheated to 150 °C. Internal steak temperatures were monitored by 20-gauge copper-constantan thermocouples (Omega Engineering, Stamford, CT, USA) placed in the approximate geometric centre of each steak and attached to a digital monitor. When the internal steak temperature reached 35 °C, the steak was turned over and allowed to reach an internal temperature of 70 °C before removal from the oven. Cooked WBSF steaks were cooled for 24 h at 4 °C (AMSA, 1995). Five round cores (1.27 cm diameter) were removed from each steak parallel to the long axis of the muscle fibres (AMSA, 1995). Each core was sheared once through the centre, perpendicular to the fibre direction, by a Warner–Bratzler shear machine (G-R Manufacturing Company, Manhattan, KS, USA). Cook loss was evaluated on the steaks used for the WBSF measurement. The 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 weights of the steaks before and after thawing, and total loss was calculated by adding the cooking loss and thawing loss. Sarcomere length (SL) was measured by laser diffraction using a 05-LHR-021 laser, Melles Griot, (Carlsbad, CA, USA) and calculated as described by Cross *et al.* (1981). From each cube, the SL of six fibre samples was determined and used for calculation of average SL.

Proximate analysis

After thawing at room temperature, the meat samples were lyophilized for 36 h to obtain homogeneous and moisture-free samples, ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1-mm screen and chemical composition determined according to AOAC (1990). Crude protein was quantified by the Kjeldahl method, EE was extracted by the Soxhlet

method and the ashes were obtained through a muffle furnace at 550 °C.

Lipid oxidation

For determination of thiobarbituric acid reactive substances (TBARS), meat samples (50 g) were collected from between the 10th and 13th ribs on the right half of the carcass, identified and vacuum-packed in polyethylene bags. A 10-g sample was ground in a multiprocessor and 0.2 ml of antioxidant butylated hydroxytoluene (0.30 g/kg) was added along with 50 ml of distilled water and 1 ml of an antifoaming solution (Sigma A5758, São Paulo, Brazil). The samples were then ground again and homogenized for 1 min. After homogenization, the samples were transferred to a 250 ml volumetric flask containing pieces of porcelain, to which 50 ml of a 4 M hydrochloric acid (HCl) solution was added. Subsequently, the samples were distilled in a blanket heater at 100 °C until 50 ml of the distillate was collected. From the distillate, 5 ml was transferred to a test tube and 5 ml of a 0.02 M thiobarbituric acid solution was added. The test tubes were kept in a boiling water bath for 35 min and the absorbance was measured at 530 nm in a spectrophotometer (Hitachi High Technologies America, Inc., model U-2,900, Pleasanton, USA). The TBARS value, expressed in mg malonaldehyde (MDA)/kg meat, was obtained using a conversion factor based on a standard curve using 1, 1, 3, 3-tetraethoxypropane.

Fatty acid profile

To determine the fatty acid composition of fresh meat, samples of the transverse 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 acid methyl esters were obtained by ISO method 5509 (ISO, 1978). The transmethylated samples were analysed with a GC (model Focus CG-Finnigan, Thermo Finnigan, San Jose, CA, USA) with a flame-ionization detector and a capillary column (CP-Sil 88; Varian, Palo Alto, CA, USA) measuring 100 m × 0.25 mm internal diameter with a thickness of 0.20 μm (Supelco, Bellefonte, PA, USA). Hydrogen was used as the carrier gas at a flow rate of 1.8 ml/min. The initial temperature of the oven was 70 °C and was increased by 13 °C/min to 175 °C, where it was maintained for 27 min. The temperature was then increased by 4 °C/min to 215 °C, where it was maintained for 9 min, followed by another increase of 7 °C/min to 230 °C, where it remained for 5 min. The temperature of the injector was 250 °C and of the detector was 300 °C. Identification of the fatty acids was performed by comparison of retention times with standards of fatty acids from butter, and the percentage of fatty acids was obtained using Chromquest 4.1 software (Thermo Electron, Milan, Italy).

Statistical analysis

The experimental design was completely randomized. Intake, digestibility, weight gain and quantitative carcass trait data were collected from 40 animals over four treatments with ten replications. Initial BW was used as a covariate for the statistical analysis of intake, digestibility, weight at slaughter, ADG, feed efficiency (FE), hot carcass weight (HCW), hot carcass yield (HCY), LEA, relative LEA and back fat thickness. The mathematical model

was represented by:

$$Y_{ij} = \mu + t_i + iw_j + e_{ij}$$

where Y_{ij} = observation of animal j subject to treatment i; μ = the overall mean; t_i = effect of treatment i; $i = 1$ to 4; iw_j = initial weight of the animal j; and e_{ij} = the residual experimental error. The CH_4 emission data were analysed without the use of the covariate. Statistical analysis was conducted using the GLM procedure of SAS (Statistical Analysis System, version 9.1). Treatment means were compared using the Tukey test and adopting $P < 0.05$. The standard errors of the mean derived from the model are reported in tables.

Results

The intake of DM, organic matter (OM), CP and NDF was greater ($P = 0.010$) in the SO diet than those fed with NF (Table 2). The SO diet decreased digestibility of NDF ($P = 0.010$) when compared with the NF and RPF diets. The diets did not affect the digestibility of DM, OM or CP. The animals fed with SO diet showed greater final weight, average daily gain and FE ($P < 0.05$) than those fed with the other diets (Table 3). Emission of enteric methane (g/d, kg/year and g/kg DM intake) was not affected by different forms of soybean lipids (Table 3). The animals with SO diet had greater slaughter weight ($P < 0.001$) when compared with the NF and SB diets. The HCW and CCW values were greater ($P < 0.001$) in SO diets compared with NF diet. The HCYs ($P = 0.040$) and CCYs ($P = 0.020$) were greater in RPF compared with the NF diets. No significant differences were observed among the treatments on carcass shrink loss, LEA and final pH of the meat. The animals fed with SO diet had thicker subcutaneous fat ($P = 0.040$) than those from the NF diet (Table 4).

The young bulls fed with SO and RPF diets had greater SL in the *longissimus* muscle ($P = 0.020$) than the SB supplemented animals (Table 5). No significant differences were observed for moisture, protein, ash or fat (Table 5). The lightness (L^*) of fat was greater ($P = 0.030$) in the RPF compared with SO diets (Table 5). The proportions of saturated and unsaturated, mono and polyunsaturated and n-3 and n-6 fatty acids were not affected significantly by the fat sources (Table 6).

Discussion

The inclusion of fat may cause adverse effects on rumen fermentation and alter ruminant intake (Fiorentini et al., 2015b). The fatty acids of the SO diet were probably more readily available in the rumen, and thus more prone to impair rumen functions. The reduction of fibre digestibility in SO diets compared with NF may be associated with unsaturated fat inclusion (Harvatine and Allen, 2006), which may have toxic effects on gram-positive bacteria, especially in the cellulolytic population (Nagaraja et al., 1997). Diets containing 70 g/kg or more of EE in the DM can cause negative effects on feed degradation, especially if there are large proportions of UFA contained within the EE. In addition to being toxic to ruminal microorganisms, fatty acids will adhere to food particles and create a physical barrier that prevents the action of microorganisms and microbial enzymes, which consequently impairs the performance of growing and finishing animals (Sullivan et al., 2004). Nevertheless, Zinn et al. (2000) and

Hess et al. (2008) reported that supplementing 50–60 g/kg of EE in the DM affected DMI. Thus, contrary to expectations, there was no negative effect with the addition of different forms of soybean lipids in diets (DMI and digestibility), with the exception of reduced NDF digestibility for the SO and SB diets. On the other hand, the SO diet increased DMI and consequently the performance of animals. Therefore, it can be concluded that there is no absolute amount of added dietary lipid that would be appropriate across all feed sources. The amount is variable and depends on the particular source used, the type of animal studied, the supply methods and other characteristics.

It has been postulated that high levels of fat can be used as supplements for beef cattle without deleterious effects in the form of saturated or protected fat (Fiorentini et al., 2014; Carvalho et al., 2016). This statement supports the results found in the present study, in which the SB diet, with grain as a physical barrier that reduces the release of triglycerides present within the cells, did not affect intake or digestibility (Doreau and Ferlay, 1995). The nutrient digestibility of RPF diet, whose triglycerides are partially protected from ruminal effects due to the saponification process, did not differ from the NF diet. Changes in fibre digestibility can have a great impact on intake in high fibre diets. The increase in fibre retention could result in lower total passage rates and affect intake. Jordan et al. (2006) verified similar DM intake between the control diet and a diet supplemented with soybean oil, using fat supplementation at levels >60 g/kg of EE in high grain diets with the addition of soy beans or refined soybean oil in diets of young bulls. These authors attributed the absence of harmful effects on intake to the low portion of roughage in the diets (<120 g/kg). However, the lower NDF digestibility coefficients in the current experiment apparently contributed to increasing the intake of animals fed the SO diet.

Supplementation of lipid sources is linked with a reduction of methane emissions (Martin et al., 2010; Patra, 2013; Fiorentini et al., 2014; Rossi et al., 2017). However, the current results differed from these statements and this may be explained by the similar intakes of DM and NDF. Some studies show that intensifying production system can reduce the enteric emission of CH_4, although the emission of nitrous oxide (N_2O) can be increased by the use of nitrogen fertilizers, either for grain cultivation or for pasture fertilization. This reduction of greenhouse gases per unit of product is mainly related to better utilization of feed and reduction of age at slaughter. The overall estimation of gas balance is complex and requires much research, including more efficient production systems such as silvopastoral and agrosilvopastoral that improve the recycling of gases emitted by the activities of beef cattle production.

Furthermore, glycerol may inhibit ruminal lipolysis (Edwards et al., 2012) and the amount of fatty acid released in the rumen. The lower concentration of free UFA may reduce deleterious effects in the *Archaea* group. It is reported that this corresponds to no change in methane production (g/kg DMI) in diets with soybean oil combined with crude glycerin (Jose Neto et al., 2015). However, decreases in CH_4 emissions have been reported in *in vitro* studies when increasing levels of glycerol were added to the diet (Lee et al., 2011) or combined with soybean oil (Castagnino et al., 2015). In the current study, CH_4 emissions remained constant regardless of soybean lipid source. A possible explanation for this lack of effect is that the current data refer to *in vivo* studies, in which the microbial population had the possibility of adapting to the new environment, and also because some of the added glycerol could be absorbed through the rumen wall.

Table 2. Effects of feeding diets containing different forms of soybean lipids on component intake and apparent total tract digestibility in young Nellore bulls (*n* = 10)

| | Diet | | | | | |
	NF	SO	SB	RPF	S.E.M.	*P* value
Intake (kg/d)						
Dry matter	6.6	8.8	7.6	7.7	0.43	0.010
Organic matter	6.1	8.2	7.1	7.2	0.40	0.010
Crude protein	1.1	1.4	1.3	1.2	0.07	0.010
NDF	2.1	2.7	2.4	2.4	0.13	0.030
Ether extract	0.34	0.60	0.51	0.56	0.030	<0.001
Intake (g/kg of BW)						
Dry matter	13	18	16	16	0.90	0.009
Digestible component (g/kg)						
Dry matter	731	742	724	753	8.8	0.061
Organic matter	751	762	744	773	8.9	0.162
Crude protein	700	721	713	704	1.2	0.571
NDF	690	653	662	695	2.0	0.010
Ether extract	920	940	923	925	1.7	0.463

NF, no additional fat; SO, soybean oil; SB, soy beans; RPF, rumen-protected fat; NDF, neutral detergent fibre.

Table 3. Effects of diets containing different forms of soybean lipids on the initial weight (IW), slaughter weight (SW), average daily weight gain (ADG), feed efficiency (FE) and methane (CH_4) emission in young Nellore bulls (*n* = 10 for SW, ADG and FE; *n* = 6 for methane emission)

| | Diet | | | | | |
	NF	SO	SB	RPF	S.E.M.	*P* value
IW (kg)	447	440	443	448	10	0.854
SW (kg)	511	545	512	516	8.5	<0.001
ADG (kg/d)	0.8	1.3	0.8	0.8	0.10	0.010
FE (kg ADG/kg DM)	0.11	0.14	0.11	0.11	0.010	0.031
Methane emission						
CH_4 (g/d)	133	137	135	110	18	0.690
CH_4 (kg/year)	49	50	49	40	6.5	0.690
CH_4 (g/kg DM intake)	21	16	18	18	1.9	0.400

NF, no additional fat; SO, soybean oil; SB, soy beans; RPF, rumen-protected fat; DM, dry matter. The feed efficiency (FE) was calculated as follows: Average daily weight gain (kg)/Dry matter intake (kg).

The changes in carcass characteristics are associated with the greater slaughter weight of animals supplemented with fat sources. Slaughter weight is an indicator of carcass yield in animals; therefore, heavier animals usually have greater HCW and CCW. Carcass yield depends directly on carcass weight, but indirectly on the BW of the animal. Feed type may cause changes in the size of organs and the gastrointestinal tract, which affects the animal's BW and carcass yield (Lage *et al.*, 2012).

Loin eye area is indicative of the muscle development of animals, especially when expressed as cm²/100 kg of the carcass. As this index increases, the edible portion of the carcass also increases. In this way, it can be inferred that the fat sources utilized did not affect the animals' muscle development. The similarities found in the current study for LEA, expressed as cm² (76.8 cm²) and as 100 kg of cold carcass (27.1 cm²/100 kg cold

carcass) are in agreement with Aferri *et al.* (2005), who also found no differences in LEA and LEA percent (average 68.4 and 28.3, respectively) of feedlot finished animals fed cottonseed and protected fatty acids.

The subcutaneous fat tissue acts as a thermal insulator and is an important quality factor, directly affecting the chilling time and conversion of muscle to meat (Savell *et al.*, 2005; Zuin *et al.*, 2012). For all the treatments, SFT met the guidelines of the Brazilian market, which requires that the meat has between 3 and 6 mm of fat (Eiras *et al.*, 2014) to prevent muscle fibres from shortening due to the cold.

The differences found among the treatments for SFT might have influenced shortening of the sarcomeres in the *longissimus* muscle. The SO and RPF diets presented greater SLs than the SB treatment, and thus lower WBSF values were expected for

Table 4. Hot carcass weight (HCW), hot carcass yield (HCY), cold carcass weight (CCW), cold carcass yield (CCY), shrink loss (SL) and loin-eye area (LEA) expressed as cm² and cm²/100 kg of cold carcass, subcutaneous fat thickness (SFT) and final pH of feedlot-finished young Nellore bulls fed with diets containing different forms of soybean lipids (n = 10)

	Diets					
	NF	SO	SB	RPF	S.E.M.	*P* value
HCW (kg)	281	304	286	294	5.5	<0.001
HCY (kg/100 kg)	54.8	55.8	56.0	56.8	0.42	0.040
CCW (kg)	274	298	280	287	5.4	<0.001
CCY (kg/100 kg)	53.5	54.7	54.8	55.6	0.42	0.020
SL (kg/100 kg)	2.4	2.1	2.1	2.2	0.10	0.170
LEA (cm²)	77	77	77	77	2.6	0.970
LEA (cm²/100 kg CCW)	28.2	25.8	27.6	26.7	0.93	0.320
SFT (mm)	3.6	5.7	4.0	4.3	0.56	0.040
Final pH	6.1	5.7	6.0	5.7	0.12	0.080

NF, no additional fat; SO, soybean oil; SB, soy beans; RPF, rumen-protected fat.

Table 5. Warner–Bratzler Shear Force (WBSF), cooking loss (CL), sarcomere length (SAL), thiobarbituric acid reactive substances (TBARS) and chemical composition of the longissimus muscle, and colour of meat and fat from the tenderloin of feedlot-finished young Nellore bulls fed with diets containing different forms of soybean lipids (n = 10)

	Diets					
	NF	SO	SB	RPF	S.E.M.	*P* value
WBSF (kg/cm²)	2.9	3.4	3.1	3.1	0.22	0.431
SAL (µm)	1.4	1.5	1.3	1.5	0.04	0.020
CL (g/100 g)	14	18	13	26	4.4	0.193
TBARS (mg malonaldehyde/kg meat)	0.6	0.9	0.8	1.0	0.17	0.334
Chemical composition of longissimus muscle (g/100 g)						
Moisture	72	72	71	72	0.4	0.882
Protein	22	21	22	21	0.2	0.052
Ash	1.1	1.1	1.1	1.1	0.02	0.891
Fat	5.0	5.6	5.1	5.5	0.23	0.220
Longissimus muscle						
L*	32	35	34	31	1.9	0.420
a*	15	17	16	16	0.6	0.390
b*	7.5	8.0	7.9	8.0	0.73	0.951
Fat						
L*	74	71	75	76	1.0	0.032
a*	5.9	6.3	4.6	6.0	0.96	0.620
b*	7.2	8.5	8.1	8.1	0.50	0.351

NF, no additional fat; SO, soybean oil; SB, soy beans; RPF, rumen-protected fat; *L** = lightness; *a** = red colour intensity; *b** = yellow colour intensity.

SO and RPF, given that shear force decreases exponentially as SL increases for lengths shorter than 2.00 µm (Wheeler *et al.*, 2000), such as the average of 1.44 µm in the present study. However, the difference found in the current study for SL did not influence meat tenderness.

The association of glycerin and fat in diets did not affect the WBSF. According to Belew *et al.* (2003), meat can be considered very tender when the shear force is below 3.20 kg/cm²; in the current study, all treatments showed meat with highly satisfactory average results for WBSF (3.13 kg/cm²). The higher frequency of problems related to meat toughness usually coincides with the final pH of the muscle being near 5.8–6.0 (Wulf *et al.*, 2002), but this was not observed in the present study, where the final meat pH (not affected by the fat inclusion in the diets) averaged 5.9.

Colour is the parameter most affected by the slaughter technique, feeding type and management (Dunne *et al.*, 2011), but

Table 6. Fatty acid profile of the *longissimus* muscle of feedlot-finished young Nellore bulls fed with diets containing different forms of soybean lipids (*n* = 10)

	Diets					
	NF	SO	SB	RPF	S.E.M.	*P* value
Fatty acid profile (g/100 g of fat)						
Lauric (C12:0)	0.07	0.08	0.07	0.07	0.006	0.940
Myristic (C14:0)	3.5	3.5	3.4	3.4	0.18	0.980
Pentadecanoic (C15:0)	0.43	0.42	0.41	0.41	0.026	0.920
Palmitic (C16:0)	28	28	28	28	0.6	0.962
Margaric (C17:0)	1.0	1.0	0.98	0.93	0.049	0.524
Stearic (C18:0)	15	14	14	14	0.6	0.264
Myristoleic (C14:1)	0.80	0.80	0.81	0.87	0.069	0.873
Palmitoleic (C16:1)	3.3	3.5	3.1	3.4	0.20	0.657
Heptadecenoic (C17:1)	0.88	0.96	0.88	0.84	0.043	0.214
Cis-vaccenic (C18:1 n-7)	1.9	1.9	1.8	2.1	0.10	0.120
Oleic (C18:1 n-9)	39	41	40	41	0.9	0.272
Linoleic (C18:2 n-6)	4.3	3.3	4.7	3.9	0.56	0.372
CLA (C18:2 c9, t11)	0.30	0.39	0.41	0.40	0.033	0.113
α-linolenic (C18:3 n-3)	0.19	0.15	0.20	0.16	0.016	0.092
Eicosenoic (C20:1 n-9)	0.15	0.16	0.18	0.17	0.011	0.263
Eicosatrienoic (C20:3 n-6)	0.21	0.14	0.19	0.15	0.039	0.561
Arachidonic (C20:4 n-6)	0.60	0.38	0.69	0.42	0.153	0.450
Docosatetraenoic (C22:4 n-6)	0.08	0.05	1.00	0.05	0.022	0.482
SFA	48	47	47	47	0.9	0.672
UFA	52	53	53	53	0.9	0.671
MUFA	46	48	47	48	0.9	0.190
PUFA	5.8	4.5	6.4	5.2	0.80	0.402
UFA:SFA	1.1	1.1	1.1	1.1	0.04	0.692
PUFA:SFA	0.12	0.11	0.14	0.11	0.020	0.551
n-6	5.3	3.9	5.7	4.6	0.77	0.392
n-3	0.22	0.17	0.23	0.17	0.025	0.150
n-6:n-3	23	23	25	27	2.0	0.461

NF, no additional fat; SO, soybean oil; SB, soy beans; RPF, rumen-protected fat; SFA, total saturated fatty acids; UFA, total unsaturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids; UFA:SFA, ratio between UFA and SFA; PUFA:SFA, ratio between PUFA and SFA; n-6, fatty acids of the omega-6 series; n-3, fatty acids of the omega-3 series; n-6:n-3, ratio between n-6 and n-3.

it is well reported that fat supplementation does not have an influence on the meat colour (Fiorentini *et al.*, 2012; Moloney *et al.*, 2012; Oliveira *et al.*, 2012), as also observed in the present study.

Although the diets with soybean had different forms of protection, there was no modification in the fatty acid composition of meat, which can be explained by the similar profile of all saturated and UFA fed to animals. Of the commonly present SFA in ruminant meat, myristic and palmitic acids have a high hypercholesterolemic potential (Daley *et al.*, 2010); myristic acid has the greatest potential (Scollan *et al.*, 2006) but is present at a lower concentration than palmitic acid.

Despite constant criticism about the level of SFA in beef, they account for <0.50 of the total fatty acids; 0.30 consists of stearic acid (C18:0), which although saturated, because of its long chain and absence of a double bond, does not behave as

such; it is therefore not related to heart disease and does not affect the level of circulating cholesterol (Scollan *et al.*, 2006). The addition of fat did not change the proportion of stearic acid (14.3 g/100 g fatty acid) in the *longissimus* muscle: this result is in line with Fiorentini *et al.* (2012) and Oliveira *et al.* (2012), who found no differences between the concentrations of stearic acid in the meat from zebu (Nellore) cattle and crossbred heifers with the inclusion of fat in their feedlot-finishing diets, obtaining mean values of 14.3 and 14.5 g/100 g fatty acid, respectively.

In conclusion, supplemental soybean oil at <0.02 of DM diet does not modify the emission of enteric methane and meat quality of young Nellore bulls. However, soybean oil diets have been demonstrated to be more beneficial for animal performance than diets without supplemental fat.

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