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# Impact of ground soybean and starch levels on the quality of meat from feedlot young Nellore bulls



MEAT SCIENCE

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# 1. Introduction

Change of 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 (Aldai et al., 2006; Ladeira et al., 2014). Research has mainly considered a simultaneous reduction of SFA and increase in polyunsaturated fatty acids (PUFA) in order to prevent health problems (Wood et al., 2003; Sierra et al., 2008). This alteration can be achieved by manipulating lipid sources in the diet (Daley, Abbott, Doyle, Nader, & Larson, 2010). However, lipids may undergo rumen biohydrogenation and be transformed from unsaturated (UFA) to SFA. One method to partially prevent rumen biohydrogenation and to increase the passage of UFA to the duodenum is the use of high-starch diets. Increased starch decreases the rumen pH, allowing more UFA to reach the intestine and be absorbed and incorporated into milk fat carcass (Kmicikewycz & Heinrichs, 2015).

Of the various lipid sources available for ruminant nutrition in Brazil, the soybean is valued for its wide availability, low cost, and high nutritive content (Barletta et al., 2012). Brazil is currently the world's largest soybean producer (USDA, 2014). According to Lee, Wu, Shannon, Sleper, and Nguyen (2007), the oil present in soybeans contains approximately 85% UFA, especially oleic, linoleic, and linolenic acids. The inclusion of lipid sources in cattle feeding may thus increase the energy density of the diet without reducing its fiber content, resulting in increased energy

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# ABSTRACT

Twenty-eight young Nellore bulls ( $395 \pm 32$  kg initial body weight) were assigned to a completely randomized design ( $2 \times 2$  factorial design with 7 animals per treatment) to evaluate effects on the quality of meat. Diet treatments consisted of high (about 25%) or low (about 16%) starch levels, with or without ground soybean addition. Shear force was decreased in the meat from animals fed a low-starch diet (P = 0.0016). Meat from animals fed a high-starch diet had increased total concentrations of unsaturated (P = 0.0029) and monounsaturated fatty acids (P = 0.0253). Polyunsaturated fatty acid content increased in the meat from animals fed a diet containing soybean (P = 0.0121). High starch diets (>25%) decreased the concentration of saturated fatty acids and increased the amount of unsaturated fatty acids in the meat from young Nellore bulls.

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intake, enhanced productive and reproductive performance, and modification of the fatty acid profile of the meat from these animals (Oliveira et al., 2011; Fiorentini et al., 2012; Fiorentini et al., 2015).

The present study therefore examined the hypothesis that maintenance of low rumen pH through the use of high-starch diets can protect supplemental sources of unsaturated lipids derived from ground soybean against biohydrogenation, allowing an increase in UFA deposition in the *longissimus* muscle. We thus proposed to evaluate the quality of meat from feedlot-finished young Nellore bulls fed diets containing ground soybean and different levels of starch.

# 2. Materials and methods

The procedure used in this trial was in accordance with the Brazilian College of Animal Experimentation (COBEA – Colégio Brasileiro de Experimentação Animal) guidelines and was approved by the Ethics, Bioethics, and Animal Welfare Committee (CEBEA – Comissão de Ética e Bem Estar Animal) of the FCAV–UNESP–Jaboticabal campus (protocol number 021119/11).

# 2.1. Animals and feeding

A total of 28 Nellore young bulls (initial body weight =  $395 \pm 32$  kg;  $20 \pm 3$  months) were housed in individual  $21 \text{ m}^2$  (7 × 3 m) pens provided with a concrete trough and drinker. The experiment was completely randomized in a 2 × 2 factorial design (high or low starch; with or without ground soybean), totaling 4 treatment conditions (n = 7 per treatment). Although animals would have been fed and penned separately, we felt penning animals within diet and using individual animal as the



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experimental unit would be appropriate because dietary effects were anticipated and only the degree of difference was of interest (Robinson, Wiseman, Udén, & Mateos, 2006). The animals spent 21 days acclimating to diets, facilities, and management. After this period, animals were confined for 119 days (experimental period).

Feeds were formulated to be isonitrogenous and to provide an average gain of 1.30 kg/day (Valadares-Filho, Marcondes, Chizzotti, & Paulino, 2010). Diet treatments consisted of high (HS; around 25%) or low (LS; around 11%) starch levels, with (WSB; around 6.2% ether extract) or without (NSB; around 2.8% ether extract) ground soybean addition. The main ingredients of HS and LS diets were corn and soybean hulls, respectively. Ground soybean was added to diets as a lipid source. Maize silage was used with diet containing 40% silage and 60% concentrate on a DM basis. All diets contained 10% crude glycerin in dry matter, replacing corn or soybean hulls. Glycerin is a byproduct from the biodiesel industry and can be used in ruminant diets without compromising performance (Parsons, Shelor, & Drouillard, 2009; Drouillard, 2012). The proportion of dietary ingredients and composition of dietary fatty acids are displayed in Table 1. Animals were fed with maize silage and experimental concentrates once daily at 08:00 h and quantities were adjusted to allow a surplus of approximately 100 g/kg relative to the total amount consumed on the previous day.

## 2.2. Chemical analyses of feedstuff

Samples of maize silage and concentrates were analyzed to determine dry matter (DM; method 934.01) and mineral matter (MM; method 942.05), and to obtain ether extract (EE; method 954.02) according to AOAC (1990). Nitrogen was determined using an LECO FP-528

#### Table 1

Composition of experimental diets (g/kg on a dry matter basis).

	HS		LS	
	WSB	NSB	WSB	NSB
Ingredient, %				
Maize silage	40.00	40.00	40.00	40.00
Ground corn	24.09	30.34	0.00	0.00
Soybean meal	0.00	17.61	0.00	15.68
Soybean hulls	0.00	0.00	20.68	23.86
Ground soybean	23.86	0.00	27.27	0.00
Crude glycerine	10.00	10.00	10.00	10.00
Mineral supplement <sup>a</sup>	2.05	2.05	2.05	2.05
Chemical composition, %				
Dry matter	60.1	60.3	58.9	58.6
Organic matter	95.7	95.9	94.7	94.7
Starch	24.0	25.9	12.0	9.78
Crude protein	14.0	14.7	15.6	15.2
Ether extract	5.87	2.48	6.46	3.14
Non fiber carbohydrates <sup>b</sup>	48.5	51.2	38.6	40.3
Total carbohydrates <sup>c</sup>	75.8	78.0	72.6	76.3
NDF	17.7	17.2	24.4	26.5
Metabolizable energy <sup>d</sup> (Mcal/kg)	2.62	2.58	2.68	2.57
Fatty acids, g/100 g of total fatty acids				
C12:0 (lauric)	0.15	0.15	0.15	0.15
C14:0 (myristic)	0.13	0.13	0.13	0.13
C16:0 (palmitic)	15.7	16.8	17.2	17.9
C16:1 (palmitoleic)	0.11	0.11	0.11	0.11
C18:0 (stearic)	3.19	2.71	3.67	4.20
C18:1 <i>n</i> -9, <i>c</i> (oleic)	27.5	30.6	28.4	28.6
C18:2n-6,c (linoleic)	44.4	42.3	41.8	40.0
C18:3n-6 (linolenic)	5.09	3.99	4.76	4.91
Saturated fatty acids	21.1	21.4	22.8	24.4
Unsaturated fatty acids	79.9	79.6	78.2	75.6

HS = high starch; LS = low starch; WSB = with ground soybean; NSB = without ground soybean.

<sup>a</sup> Composition of product: Calcium, 120 g; Phosphorus, 30; Sulfur, 25 g; Sodium, 80; Copper, 330 mg; Manganese, 950 mg; Zinc, 1220 mg; Iodine, 24 mg; Cobalt, 20 mg; Selenium, 6 mg; Fluorine (Maximum), 300 mg.

<sup>b</sup> By Hall (1998).

<sup>c</sup> Total carbohydrates = 100 - (CP + EE + Ash).

<sup>d</sup> Metabolizable energy by National Research Council, 2001.

nitrogen analyzer (LECO Corp., St. Joseph, MI, USA). Neutral detergent fiber was determined using  $\alpha$ -amylase, without the addition of sodium sulfite, following Van Soest, Robertson, and Lewis (1991) and adapted for the Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NY).

Determination of the fatty acid composition of feed offerings was performed on approximately 1 g of sample (Folch, Lees, & Sloane-Stanley, 1957). The lipid extract aliquot was methylated following Kramer, Fellner, and Dugan (1997). Fatty acids were quantified using a GC 2010 gas chromatograph (Shimadzu Corp., Kyoto, Japan) with an SP-2560 capillary column (100 m  $\times$  0.20 mm i.d. with a 0.02-µm film thickness; Supelco, Bellefonte, PA).

# 2.3. Slaughter, carcass data collection and meat sampling

At the end of the trial period, young bulls (average body weight  $590 \pm 34$  kg) were fasted for 18 h on the day prior to transportation to the slaughterhouse. They were transported to the slaughterhouse (Minerva Foods, Barretos, Sao Paulo, Brazil) 90 km from the feedlot and 01:30 h transport. On arrival at the slaughterhouse, they were kept in resting pens and were humanely harvested under Brazilian federal inspection. After slaughter, carcass were transferred to a cooler at 4 °C and chilled for 24 h. Following which meat samples were collected through a perpendicular cut in the *longissimus* muscle between the 12th and 13th ribs on the right half of the carcass. Each chilled longissimus sample was standardized from the cranial end of the loin break into one 2.54 cm thick steak sample (AMSA, 1995) for Warner-Bratzler shear force (WBSF) measurement, and two 1 cm thick steaks for further analysis. All steaks were vacuum-packed (99% vacuum, with a 200 Selovac Sealer machine (Selovac, São Paulo, SP, Brazil)), in Polyamide/ Polyethylene pouches of 120  $\mu$ m and 1 cm<sup>3</sup>/m<sup>2</sup>/24 h 0<sub>2</sub> permeability,  $3 \text{ cm}^3/\text{m}^2/24 \text{ h } \text{CO}_2$  permeability measured at 5° and 75% relative humidity; water vapor transmission rate (WVTR) was 3 g/m<sup>2</sup>/24 h at 38 °C and 100% RH. The vacuum value 20 (50 Pa) was used to pack the steaks and held at -20 °C for 10 days until analysis.

#### 2.4. Analysis of longissimus muscle

The pH was measured (in triplicate) in the muscle portion of the longissimus muscle for 24 h after slaughter (taken before vacuum packaging) using a Testo 205 coupled penetration electrode. The WBSF steaks were thawed at 4 °C for 24 h and oven-broiled in an electric oven (Layr, Luxo Inox) preheated to 150 °C. Internal steak temperatures were monitored by 20-gauge copper-constantan thermocouples (Omega Engineering, Stamford, CT) 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 removal from the oven. Cooked WBSF steaks were cooled for 24 h at 4 °C (AMSA, 1995). Eight round cores (1.27 cm diameter) were removed from each steak parallel to the long axis of the muscle fibers (AMSA, 1995). Each core was sheared once through the center, perpendicular to the fiber direction, using a Warner-Bratzler shear machine (G-R Manufacturing Company, Manhattan, KS – USA). Cooking losses (CLs) were evaluated on the steaks that were also used for WBSF measurement. Total CL was calculated as the difference between the weight of the steaks before and after oven broiling.

Water holding capacity (WHC) was obtained by the difference between the weights of a meat sample (approximately 2 g) before and after being subjected to a 10 kg pressure for 5 min (Hamm, 1986). Steaks were removed from the vacuum packaging and were allowed to bloom for 30 min. CIE L\* (lightness), a\* (redness), and b\* (yellowness) values were measured on the surface at three random locations using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Osaka, Japan) with illuminant C, 8 mm aperture, and 2° observer angle. In this space, L\* indicates luminosity, and a\* and b\* are the chromaticity coordinates as follows: the axis that runs from  $-a^*$  to  $+a^*$  varies from green to red, and the axis that runs from  $-b^*$  to  $+b^*$  varies between blue and yellow.

To determine the fatty acid composition of the fresh meat samples, transversal sections 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 after Bligh and Dyer (1959), and the fatty acids methyl esters were obtained by ISO (1978) 5509 method. Qualitative and quantitative measurements of fatty acid content were performed by gas chromatography (CG-2010 Plus - Shimadzu, auto injector AOC 20i) with a flame ionization detector and fused silica capillary column (Omegawax 250); 30 m length and 0.25 mm diameter, with a film thickness of 0.25 µm (Supelco SP-24,136, USA). Helium was used as a carrier gas at a flow rate of 1 mL/min. A 1 µL aliquot of 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 a rate of 4 °C/min for 25 min, while the detector was at 280 °C. Identification and guantification 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.

The activity index was calculated for elongase and  $\Delta^9$ -desaturase enzymes on fatty acids with 16 and 18 carbons, which are responsible for the conversion of saturated fatty acids (SFA) with 16 and 18 carbons into their respective monounsaturated correspondents with double bond in carbon 9 as described by Malau-aduli, Siebert, Bottema, and Pitchford (1997). The atherogenecity index was calculated as described by Ulbricht and Southgate (1991) as an indicator of risk of cardiovascular disease. Calculations were performed as follows:

$$\begin{split} &\Delta^9 \text{-desaturase C16 activity} = 100[(16:1\ cis-9)/16:1\ cis-9+16:0)]; \\ &\Delta^9 \text{-desaturase C18 activity} = 100[(18:1\ cis-9)/18:1\ cis-9+18:0)]; \\ &\text{Elongase activity} = 100[(C18:0+C18:1\ cis-9)/(C16:0+C16:10)]; \\ &\text{Tris-9} + C18:0+C18:1\ cis-9)]; \\ &\text{Atherogenicity index} \end{split}$$

 $= [C12:0+4(C14:0)+C16:0]/(\Sigma SFA+\Sigma polyunsaturated \ fatty \ acids).$ 

## 2.5. Statistical analysis

The experimental design was completely randomized, with a  $2 \times 2$  factorial arrangement (two levels of each factor consisting of 4 treatments and 7 animals per treatment), in which data were analyzed using the MIXED procedure of SAS software (version 9.4; SAS Institute Inc., Cary, North Carolina, USA) with the animal identity as a random effect. The model included the fixed effects of starch level, inclusion of lipid source, and interaction between starch level and lipid source. Results were subject to analysis of variance with means compared by Tukey's test at the 5% probability level.

#### 3. Results

No influence of dietary treatment was detected on pH or meat color characteristics (P = 0.0691). However, the yellow intensity of the fat (b\*) was lower in meat from animals that received WSB (P = 0.0002; Table 2). No impact of dietary treatment was apparent on WHC (P = 0.6535), CL (P = 0.3234), or the percentage of fat in the meat (P = 0.6321). WBSF was reduced in the meat from animals fed LS in comparison to HS (P = 0.0016; Table 3).

Palmitic acid concentration decreased in the meat from animals that received HS (P = 0.0053), while the stearic acid content in the meat from animals fed WSB increased (P = 0.0064). Treatments had no influence on other meat SFAs (P > 0.05). Among the monounsaturated fatty acids (MUFA), *cis* pentadecanoic acid level was reduced in the meat from animals fed WSB (P = 0.0009) and increased in those fed HS (P = 0.0138). The WSB diet resulted in an increase in the concentration of palmitoleic acid in meat (P = 0.0023). Oleic acid concentration

#### Table 2

Color evaluation of muscle *longissimus* and subcutaneous fat, and pH of feedlot young Nellore bulls across treatment diets.

	Ground Soybean		Starch	Starch		<i>P</i> -value				
	WSB	NSB	HS	LS		GS	S	$GS\timesS$		
pН	5.6	5.6	5.5	5.6	0.07	0.6465	0.2018	0.0658		
Color, muscle										
L*	35.1	34.8	34.4	35.5	1.13	0.7983	0.3887	0.3094		
a*	16.6	16.8	16.7	16.7	0.27	0.4093	0.9792	0.8923		
b*	4.00	4.10	3.90	4.10	0.17	0.8670	0.3661	0.9428		
Color,	fat									
L*	78.5	77.5	77.9	78.1	0.75	0.2320	0.8086	0.5154		
a*	3.9	4.4	3.8	4.5	0.52	0.4486	0.2493	0.5971		
b*	10.1 <sup>b</sup>	11.8 <sup>a</sup>	10.9	11.0	0.39	0.0002	0.8834	0.3101		

HS = high starch; LS = low starch; WSB = with ground soybean; NSB = without ground soybean.

 $L^*$  – Luminosity;  $a^*$  – intensity of the color red;  $b^*$  – intensity of the color yellow SEM = Standard mean error.

GS = effect of ground soybean, S = effect of starch,  $GS \times S$  = interaction effect ground soybean and starch.

 $^{a,b}\,$  Means within a row lacking the common superscript letter differ by Tukey's test at  $\alpha=0.05.$ 

increased in the meat from animals fed NSB (P = 0.0282) and decreased in those fed LS (P = 0.0238; Table 4).

With regard to the concentration of PUFA, linoleic acid concentration increased in the meat from animals fed WSB (P = 0.0009), whereas the linolenic acid content increased in those fed LS (P = 0.0111; Table 5).

The meat from animals fed LS displayed an increase in SFA content (P = 0.0301). Diets containing HS resulted in an increase in the concentrations of UFA (P = 0.0029) and MUFA in the meat (P = 0.0253). The MUFA concentration in the meat from animals fed WSB increased (P = 0.0071). The UFA:SFA ratio was higher in the meat from animals fed HS (P = 0.0167). The concentration of fatty acids of the  $\omega 6$  family was higher in the meat from animals fed WSB (P = 0.0088), and the  $\omega 6:\omega 3$  ratio was lower in those fed NSB (P < 0.0001; Table 5).

The C16 and C18  $\Delta^9$ -desaturase enzyme activities were greater in the meat from animals that received NSB (P = 0.0036; P = 0.0030; Table 6) and lower in the meat from the animals fed LS (P = 0.0090; P = 0.0189). Elongase activity was greater in the meat from animals fed HS (P = 0.0138). A lower atherogenicity index was observed in the meat from WSB-fed animals in comparison to NSB (P = 0.0292).

# 4. Discussion

The pH recorded during this experiment (5.6) was within the ideal range recommended for beef. The final pH of the carcass is an important characteristic to be evaluated, as it is responsible for changes in meat quality aspects, such as color (Jeremiah, Tong, & Gibson, 1991; Wulf, O'Connor, Tatum, & Smith, 1997). Duration of the feeding period affects

# Table 3

Water holding capacity (WHC), cooking losses (CL), Warner–Bratzler shear force (WBSF), and fat content of muscle *longissimus* of feedlot young Nellore bulls across treatment diets.

	Ground soybean		Starch		SEM	<i>P</i> -value			
	WSB	NSB	HS	LS		GS	S	$GS\timesS$	
WHC, % CL, % WBSF, kgf Fat, %	69.1 36.9 4.11 5.06	68.9 38.3 4.69 5.42	69.7 37.9 4.93 <sup>a</sup> 5.25	68.2 37.3 3.87 <sup>b</sup> 5.24	1.57 2.08 0.32 0.82	0.9130 0.4683 0.0588 0.5955	0.3571 0.7477 0.0016 0.9798	0.6535 0.3234 0.7294 0.6321	

HS = high starch; LS = low starch; WSB = with ground soybean; NSB = without ground soybean.

SEM = Standard mean error.

GS = effect of ground soybean, S = effect of starch,  $GS \times S = interaction$  effect ground soybean and starch.

 $^{a,b}\,$  Means within a row lacking the common superscript letter differ by Tukey's test at  $\alpha=0.05.$ 

# Table 4

Main saturated and monounsaturated fatty acids of muscle longissimus of feedlot young Nellore bulls across treatment diets.

Fatty acid		Ground so	ybean	Starch		SEM	<i>P</i> -value		
		WSB	NSB	HS	LS		GS	S	$GS\timesS$
Saturated									
Myristic	C14:0	2.56	2.58	2.44	2.70	0.15	0.8563	0.1043	0.6147
Pentadecanoic	C15:0	1.76	1.99	1.98	1.77	0.22	0.3174	0.3600	0.2531
Palmitic	C16:0	22.9	23.3	22.5 <sup>b</sup>	23.7 <sup>a</sup>	0.45	0.3898	0.0053	0.0889
Margaric	C17:0	2.04	1.63	1.93	1.74	0.19	0.0524	0.3492	0.2225
Stearic	C18:0	13.5 <sup>a</sup>	12.0 <sup>b</sup>	12.3	13.1	0.50	0.0064	0.0923	0.3629
Behenic	C22:0	0.07	0.13	0.14	0.07	0.03	0.0725	0.0525	0.4978
Lignoceric	C24:0	0.21	0.24	0.24	0.21	0.04	0.4137	0.4485	0.2665
Monounsaturated									
Myristoleic	C14:1	0.70	0.77	0.75	0.71	0.06	0.3011	0.5454	0.3876
Pentadecanoic cis	C15:1 cis10	0.06 <sup>b</sup>	0.15 <sup>a</sup>	0.13 <sup>a</sup>	0.08 <sup>b</sup>	0.02	0.0009	0.0138	0.8648
Palmitoleic	C16:1	2.62 <sup>b</sup>	3.04 <sup>a</sup>	2.92	2.74	0.13	0.0023	0.1592	0.2488
Heptadecanoic cis	C17:1 cis10	0.91 <sup>b</sup>	1.17 <sup>a</sup>	1.09	0.99	0.06	0.0006	0.1292	0.3833
Elaidic	C18:1 n9 trans	0.80	0.71	0.61 <sup>b</sup>	0.89 <sup>a</sup>	0.08	0.2867	0.0048	0.7759
Oleic	C18:1 n9 cis	36.2 <sup>b</sup>	38.5 <sup>ª</sup>	38.5 <sup>a</sup>	36.1 <sup>b</sup>	1.03	0.0282	0.0238	0.0501
Nervonic	C24:1	0.22	0.21	0.19	0.18	0.03	0.7815	0.7815	0.2230

HS = high starch; LS = low starch; WSB = with ground soybean; NSB = without ground soybean.

SEM = Standard mean error.

GS = effect of ground soybean, S = effect of starch,  $GS \times S = interaction$  effect ground soybean and starch.

<sup>a,b</sup> Means within a row lacking the common superscript letter differ by Tukey's test at  $\alpha = 0.05$ .

fat color (Moloney, Keane, Dunne, Mooney, & Troy, 2008), and fresh pastures or preserved forages contain chemical compounds called carotenoids that intensify the yellow color of beef fat when consumed regularly (Dunne, Monahan, O'Mara, & Moloney, 2009). In the present study, the yellow color of the fat in animals fed the NSB diet (11.85) resulted from the larger amount of corn in that diet, which provided more carotenoids to produce the color observed than the carotenoids from the corn silage that was supplied in the same amount to all animals (40%).

The lack of dietary effect on WHC is likely related to the similar pH values observed across treatments. Although CL is an important quality associated with meat yield at the time of consumption that can influence tenderness; CL represents water losses plus a smaller part of rendered fat, nitrogen compounds, and minerals (Lawrie, 1981). The fat, in turn, can be influenced by the WHC in the meat structures (Pardi, Santos, Souza, & Pardi, 1993). During the present study, however,

neither of these characteristics differed between treatments, and thus the CL remained unchanged.

In the present study, WBSF decreased in the meat from animals fed LS (3.87) than HS (4.93), however, no alterations were observed in WHC. The meat is considered very tender when WBSF is lower than 3.2 kgf, tender between 3.2 and 3.9 kgf, medium from 3.9 to 4.6 kgf, and tough above 4.6 kgf (Belew, Brooks, Mckenna, & Savell, 2003). Therefore, the meat from animals that received WSB or LS can be classified as having medium tenderness, whereas the meat from animals fed NSB or HS was tough.

The palmitic acid content decreased in the meat from animals that received HS diets, given that these diets had a lower percentage of this acid. However, the higher percentage of stearic acid in the meat from WSB-fed animals was due to the higher level of linoleic acid in the diet, a precursor of stearic acid produced *via* biohydrogenation (Lee & Jenkins, 2011). Increase in concentration of stearic acid with the supply

#### Table 5

Main polyunsaturated fatty acids of muscle longissimus of feedlot young Nellore bulls across treatment diets.

		Ground so	ybean	Starch		SEM	<i>P</i> -value		
		WSB	NSB	HS	LS		GS	S	GS  imes S
Polyunsaturated									
Linolelaidic	C18:2 n6 trans	0.09	0.12	0.14	0.08	0.05	0.5553	0.3000	0.9325
Linoleic	C18:2 n6 cis	7.39 <sup>a</sup>	5.43 <sup>b</sup>	6.07	6.74	0.52	0.0009	0.1961	0.1171
α-Linolenic	C18:3 n3	0.44	0.41	0.38 <sup>b</sup>	0.47 <sup>a</sup>	0.03	0.4257	0.0111	0.3744
CLA	C18:2 cis9 trans11	0.42	0.35	0.37	0.40	0.07	0.2976	0.6790	0.4838
Eicosatrienoic	C20:3 n6	0.44	0.39	0.42	0.40	0.06	0.4075	0.7977	0.1618
Arachidonic	C20:4 n6	1.85	1.63	1.82	1.66	0.25	0.3822	0.5432	0.0998
DHA	C22:6 n3	0.54	0.59	0.60	0.54	0.08	0.6306	0.5001	0.2266
SFA		44.6	43.6	43.1 <sup>b</sup>	44.9 <sup>a</sup>	0.84	0.1524	0.0301	0.7113
UFA		55.4	56.4	56.9 <sup>a</sup>	54.6 <sup>b</sup>	0.60	0.1775	0.0029	0.5580
MUFA		42.9 <sup>b</sup>	45.8 <sup>a</sup>	45.7 <sup>a</sup>	42.9 <sup>b</sup>	1.08	0.0071	0.0253	0.2344
PUFA		12.5 <sup>a</sup>	10.5 <sup>b</sup>	11.3	11.7	0.85	0.0121	0.5615	0.2908
UFA/SFA		1.24	1.30	1.32 <sup>a</sup>	1.21 <sup>b</sup>	0.03	0.1361	0.0167	0.7187
ω-6		9.7 <sup>a</sup>	7.5 <sup>b</sup>	8.4	8.8	0.78	0.0088	0.5728	0.2345
ω-3		0.98	1.00	0.98	1.00	0.11	0.8682	0.8283	0.2442
ω-6/ω-3		10.0 <sup>a</sup>	7.76 <sup>b</sup>	8.85	8.92	0.42	< 0.0001	0.8561	0.1672

HS = high starch; LS = low starch; WSB = with ground soybean; NSB = without ground soybean.

SEM = Standard mean error.

 $GS = effect of ground soybean, S = effect of starch, GS \times S = interaction effect ground soybean and starch.$ 

SFA – saturated fatty acids = C14:0; C15:0; C16:0; C17:0; C18:0; C22:0; C24:0.

MUFA – monosaturated fatty acids = C14:1; C15:1*cis*10; C16:1; C17:1*cis*10; C18:1*n*9*trans*; C18:1*n*9*cis*; C24:1.

PUFA – polysaturated fatty acids = C18:2n6trans; C18:2n6cis; C18:3n3; C18:2cis9trans11; C20:3n6cis8,11,14; C20:4n6; C22:6n3cis4,7,10,13,16,19.

ω-6: Omega-6 fatty acids = C18:2n6trans; C18:2n6cis; C20:3n6cis8,11,14; C20:4n6.

 $\omega$ -3: Omega-3 fatty acids = C18:3*n*3; C22:6*n*3*cis*4,7,10,13,16,19.

<sup>a,b</sup> Means within a row lacking the common superscript letter differ by Tukey's test at  $\alpha = 0.05$ .

#### Table 6

Enzymes involved in the metabolism of fatty acids, and the index of atherogenicity in longissimus muscle of feedlot young Nellore bulls across treatment diets.

	Ground soybean		Starch	Starch		P-value	<i>P</i> -value		
	WSB	NSB	HS	LS		GS	S	$GS\timesS$	
∆ <sup>9</sup> -desaturase C16	10.25 <sup>b</sup>	11.54 <sup>a</sup>	11.46 <sup>a</sup>	10.33 <sup>b</sup>	0.43	0.0036	0.0090	0.6353	
∆ <sup>9</sup> -desaturase C18	72.74 <sup>b</sup>	76.19 <sup>a</sup>	75.76 <sup>a</sup>	73.16 <sup>b</sup>	1.04	0.0030	0.0189	0.0762	
Elongase	66.00	65.69	66.65 <sup>a</sup>	65.05 <sup>b</sup>	0.65	0.6076	0.0138	0.9856	
Atherogenicity	0.61 <sup>b</sup>	0.67 <sup>a</sup>	0.63	0.65	0.02	0.0292	0.4903	0.0554	

HS = high starch; LS = low starch; WSB = with ground soybean; NSB = without ground soybean.

Index  $\Delta^9$ -desaturase C16 = 100 \* [C16:1*n*9/(C16:0 + C16:1*n*9)].

Index  $\Delta^9$ -desaturase C18 = 100 \* [C18:1*n*9/(C18:0 + C18:1*n*9)].

Elongase = 100[(C18:0 + C18:1 cis-9)/(C16:0 + C16:1 cis-9 + C18:0 + C18:1 cis-9)].

Index of atherogenicity =  $[C12:0 + 4(14:0) + C16:0/(\Sigma SFA + \Sigma PUFA)].$ 

SEM = Standard mean error.

GS = effect of ground soybean, S = effect of starch,  $GS \times S = interaction$  effect ground soybean and starch.

<sup>a,b</sup> Means within a row lacking the common superscript letter differ by Tukey's test at  $\alpha = 0.05$ .

of WSB can also be explained by greater exposure of the UFA during rumen biohydrogenation. In the ruminant diet without addition of lipids, linoleic acid is present at high quantities whereas stearic acid represents only approximately 2%. However, stearic acid forms a large fraction of the fatty acids that reach the small intestine, whereas linoleic acid is present only in a small amount, at a little >10% (Duckett & Andrade, 2000; Ladeira et al., 2014).

The predominating SFAs in beef are C14:0 (myristic), C16:0 (palmitic), and C18:0 (stearic), with C18:0 representing approximately 30% of the total saturated fatty acids (Scollan et al., 2006). Among them, myristic and palmitic acids are considered hypercholesterolemic, as they minimize the activity of the hepatic cholesterol receptors, reducing their removal and metabolization (Wood et al., 2003). An increase in the saturated fatty acid content is not desirable, since it can elevate both low- (LDL) and high-density (HDL) lipoproteins. Myristic acid (C14:0) is hypercholesterolemic, palmitic acid (C16:0) has a lower hypercholesterolemic action, and stearic acid (C18:0), whose presence in meat is considerable, has a neutral effect (Mensin & Kata, 1992). The effect is higher for C14:0, which has a potential to increase cholesterol concentrations 4 to 6 times greater than C16:0 (Mensin & Kata, 1992).

The lower percentage of *cis* pentadecanoic acid observed in the meat from animals that consumed WSB or LS was a result of the lower amount of corn in the diet, which possibly generated a smaller amount of propionate, the precursor of this acid. Mansbridge and Blake (1997) reported that pentadecanoic (C15:0) and heptadecanoic (C17:0) acids originate from *de novo* synthesis by rumen bacteria from propionate (C3:0), and are later incorporated into the microbial lipids. Lipids from microorganisms can account for 10 to 15% of the total reaching the small intestine.

Oleic acid acts positively to reduce the concentration of LDL cholesterol and increase HDL cholesterol in the blood (Mir et al., 2003). Thus, the production of meat rich in oleic acid may be beneficial to human health. *In vitro* studies have demonstrated that oleic acid can be isomerized to several C18:1 *trans* isomers, including vaccenic acid, a precursor of conjugated linoleic acid (CLA) (Dannenberger et al., 2004). Diets that lead to low rumen pH, like high-starch diets, have the potential to reduce lipolysis (Van Nevel & Demeyer, 1996) and biohydrogenation (Sackmann et al., 2003), and cause changes in rumen microbial populations (Klieve et al., 2003).

In the present study, HS diets provided a greater deposition of *cis* pentadecanoic and oleic acids in the meat, likely due to a decrease in pH in animals fed HS diets, which could have served to reduce lipolysis and biohydrogenation. Moreover, the diets containing HS had a higher concentration of oleic acid in their composition and provided a higher UFA/SFA ratio.

Alterations in the PUFA biohydrogenation pathways that lead to accumulation of *trans*10 C18:1 over vaccenic acid are usually caused when the diet contains linoleic acid (Aldai, Dugan, Kramer, Mir, & McAllister, 2008), as was the case for the WSB diet. It is known that CLA is produced in the rumen during biohydrogenation of linoleic acid (C18:2 *n*-6) and in tissues by the desaturation of vaccenic acid (C18:1 *trans*-11) through the action of the  $\Delta$ 9-desaturase enzyme (Griinari et al., 2000; Hayashi, Medeiros, Carvalho, & Lanna, 2007). Nevertheless, in the present study, the higher percentage of linoleic acid in the WSB diet was not associated with a change in meat CLA content, increasing only the percentage of linoleic acid in the lower concentration of linolenic acid in the meat, given the lower concentration of this acid in such diets.

The  $\omega$ -6: $\omega$ -3 ratio is used to classify the nutritional quality of fats, oils, and diets. In the present study, NSB diets promoted a positive  $\omega$ -6: $\omega$ -3 ratio in the animals' meat, reducing the percentage of linoleic acid. In a previous study, diets containing soybean provided higher deposition of linoleic acid in beef (Fiorentini et al., 2012).

An increase in the  $\omega$ -6: $\omega$ -3 ratio is undesirable from the humanhealth perspective, as despite it being an essential fatty acid, high levels of  $\omega$ -6 are responsible for triggering a number of physiological dysfunctions, such as formation of thrombi, atheromas, and immunological disorders (Haug, Høstmark, & Harstad, 2007). According to the nutritional recommendations published by the World Health Organization (2003), the  $\omega$ -6: $\omega$ -3 ratio should be between 4:1 and 5:1.

Correlations exist between  $\Delta^9$ -desaturase C16 and palmitoleic acid concentration and  $\Delta^9$ -desaturase C18 and oleic acid concentration (Oliveira et al., 2011). During the present study, there was therefore a higher concentration of both palmitoleic acid and oleic acid in the meat from animals fed NSB diets.

The atherogenicity index (AI) relates the pro- and anti-atherogenic acids and indicates the potential stimulation of platelet aggregation. As AI decreases, the amount of anti-atherogenic fatty acids present in the fat increases, as does the potential for prevention of the onset of coronary disease (Ulbricht & Southgate, 1991). In the present study, a reduced AI was observed for animals fed WSB diets, attributable to the higher percentage of PUFA in this treatment, with values in the acceptable range of around 0.7 (Ulbricht & Southgate, 1991).

# 5. Conclusion

High starch (25%) diets improved nutritional values of beef by decreasing amount of saturated and increasing levels of unsaturated fatty acids. Inclusion of approximately 25% soybean in the diets increased the amount of PUFA in the meat.

#### **Conflict of interest**

There is no conflict of interest in this article.

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