



## Fatty acid profile of beef from immunocastrated (BOPRIVA®) Nellore bulls



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### ABSTRACT

Twenty Nellore bulls (ABW = 357.7 ± 9.65 kg) were divided into 2 groups: intact and immunocastrated – Bopriva®. After the trial period, the cattle were slaughtered and carcass fat thickness was evaluated, ether extract and fatty acid composition of the *longissimus thoracis* analyses were performed, and the activity indices of relevant enzymes were calculated. The means were calculated and compared by Student's t-test and Pearson's correlation coefficients ( $p < 0.05$ ). The immunocastrated group showed higher back fat thickness, ether extract, monounsaturated fatty acids (MUFAs), and activity index of  $\Delta^9$  desaturase C18 and lower polyunsaturated fatty acids (PUFAs) and n – 6 fatty acids when compared to the intact group. The correlations between ether extract and the saturated and monounsaturated fatty acids were positive, and negative with polyunsaturated, n – 3, n – 6 and PUFA/SFA. Therefore, immunocastration may improve the fatty acid profile in the *longissimus thoracis* by increasing MUFAs, mainly oleic acid that is the most representative fatty acid in the meat and is considered beneficial to health.

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### 1. Introduction

Beef quality is affected by many factors, such as age, genetic and breed variation (Du Plessis & Hoffman, 2007), feeding regime (Belk, Scanga, Smith, & Grandin, 2002) and sex (Choat et al., 2006). In addition to being important determinants of muscle growth, these factors also affect different aspects of the quality of the meat (Lawrie, 2006).

Intact males have some advantages over castrated males, as they grow more rapidly and utilize feed more efficiently (Seideman, Cross, Oltjen, & Schanbacher, 1982). Surgical castration is also often considered a questionable practice from the perspective of animal welfare (Bonneau & Enright, 1995). However, intact males exhibit less fat deposition on the carcass, and expend energy on undesirable and sexual behaviors (Huxsoll, Price, & Adams, 1998; Price, Adams, Huxsoll, & Borgwardt, 2003).

An alternative method to inhibit sexual development and aggressive behavior and to enhance the fat deposition in the carcass of intact animals is immunocastration (Jago, Bass, & Matthews, 1997; Janett et al., 2012). According to Robertson, Wilson, and Fraser (1979) immunocastration involves actively immunizing the animal against gonadotrophin-releasing

hormone (GnRH). The antibodies raised against GnRH prevent the release of gonadotrophins from the pituitary gland and as a consequence testosterone production in the testes ceases temporarily.

The immunocastration vaccine (Bopriva®) is administered in two injected doses; the first dose prepares the bovine immune system and the second dose activates the immune response. The animal is considered immunocastrated only after the second dose (booster) has been administered (Hennessy, 2008).

According to Amatayakul-Chantler et al. (2012, 2013), the immunocastration vaccine Bopriva® may provide a welfare-friendly alternative to surgical castration and, compared to raising intact bulls, improves carcass and meat quality and controls unwanted behavior while maintaining performance.

Furthermore, the vaccine may increase subcutaneous fat thickness when compared with intact males (De Freitas et al., 2015) and maintain growth rates at levels similar to intact bulls and superior to surgically castrated bulls (Adams & Adams, 1992) due to the lower serum testosterone levels before being immunocastrated (Amatayakul-Chantler et al., 2013; De Freitas et al., 2015).

According to De Smet, Raes, and Demeyer (2004), the level of fatness of an animal affects the fatty acid composition of the meat. The contents of saturated (SFA) and monounsaturated fatty acids (MUFA) increase faster with increasing fatness than does the content of polyunsaturated fatty acids (PUFA), resulting in a decrease in the relative proportion of

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PUFA and consequently in the polyunsaturated/saturated fatty acids (PUFA/SFA) ratio.

Thus, the aim of the present study was to compare the fatty acid profile in beef from intact and immunocastrated Nelore bulls slaughtered at the same age. We hypothesized that at the same age of slaughter there would be differences in the fatty acid profile of the meat due to differences in fatness between the sexual conditions.

## 2. Materials and methods

The experiment was conducted in a feedlot of the Araucaria Farm, located in Luiziana – PR, Brazil. Twenty animals with an average body weight of  $357.7 \pm 9.6$  kg and with up to two permanent teeth (less than two years old) were used. These animals were divided randomly into two groups consisting of intact and immunocastrated bulls, each containing 10 animals. The average body weight of the groups was  $356.8 \pm 10.4$  kg for intact and  $358.7 \pm 8.7$  kg for immunocastrated animals.

The Bopriva<sup>®</sup> vaccine from Zoetis was used for the immunocastration. The first vaccination was conducted 30 days before the group entered the feedlot, and the second immunization was conducted when the group entered the feedlot (30 days after the first vaccination), where both groups remained for 67 days.

In the feedlot, the animals were maintained in semi-covered stalls containing a trough for feeding and automatic drinkers equipped with buoys, and they were fed on an ad libitum basis, a diet with forage: concentrate ratio of 10:90. Both groups fed the same diet five times a day (Table 1).

The contents of dry matter, crude protein, ash, ether extract, neutral detergent fiber and acid detergent fiber of the experimental diet were determined following the analytical procedures described by Silva and Queiroz (2002).

After 97 days, the period from the first application of the vaccine to last day of the experiment, the animals were sent to slaughter after a 24 h withdrawal of solid feed, with water still available (Brasil. Ministério da Agricultura, Pecuária e Abastecimento, 1968). The slaughter was performed in a commercial abattoir in Campo Mourão – PR, Brazil, overseen by the State Inspection Service.

The animals were stunned using a pneumatic penetration pistol and were then immediately exsanguinated (Chambers & Grandin, 2001).

At the end of the slaughter line, the left half of the carcass of each animal was marked to identify the treatment given. After 24 h the left halves of the carcasses were cut between the 12th and 13th ribs to measure back fat thickness using a digital caliper.

**Table 1**

Composition of the feed given to the cattle (intact and immunocastrated groups) during feedlot period.

Ingredients	g/kg DM
Bagasse of cane	105.0
Corn (grain)	113.5
Oats (grain)	771.5
Limestone	5.5
Core mineral <sup>a</sup>	4.5
<i>Nutrients</i>	
Dry matter	845.7
Ether extract	32.1
Mineral matter	26.7
Crude protein	142.3
Neutral detergent fiber	335.2
Acid detergent fiber	156.2
Total digestible nutrients	767.0

<sup>a</sup> Minimum composition of core: Ca 135 g/kg; Na 80 g/kg; S 150 g/kg; Co 30 mg/kg; Cu 2.000 mg/kg; I 120 mg/kg; Mn 2.500 mg/kg; Se 25 mg/kg; Zn 6.000 mg/kg; ionophore 5.000 mg/kg; yeast 63 g/kg; adsorbent 100 g/kg.

After that, the *longissimus thoracis* muscle was removed and two 1.0-cm thick steaks (samples) were obtained along the caudal–cranial axis of each animal and individually vacuum packed (0.18 mm nylon polyethylene vacuum bags of 24.5 × 20.3 cm) with Selovac 200s (99.8% of final vacuum). The first sample obtained was for ether extract analysis and the second one was for lipid extraction. All vacuum-packed samples were immediately frozen at  $-18$  °C pending further analyses.

The ether extract of the samples was quantified according to the methodology described by AOAC (1990).

The lipids were extracted from each animal according to the method described by Bligh and Dyer (1959), in which 40 g of crushed sample (meat) was homogenized in 80 ml of chloroform and 40 ml of methanol. After being stirred for 1 h, 40 ml of chloroform and 40 ml of distilled water were added and the homogenate was stirred for an additional 20 min.

The homogenate was then filtered through a Whatman filter paper No. 1 held in a Buchner funnel using vacuum pressure. The filtrate was transferred to a separator funnel and 40 ml of an aqueous solution of 0.9% NaCl was added. After the phase separation was complete, the chloroform phase and all of the lipid was collected in a round-bottom volumetric flask and the solvent was evaporated using a rotary evaporator with the temperature held at 33–34 °C.

The hydrolytic and transesterification processes were performed according to ISO method 5509 (1978). Two milliliters of n-heptane was added to 200 mg of lipid and the mixture was vigorously stirred until the lipids were completely solubilized. Then, 2 ml of 2 M NaOH in methanol were added and the mixture was stirred. After the phase separation was complete, the upper phase that contained n-heptane and the fatty acid methyl esters was removed using an automated pipette, transferred to an amber vial, and stored at  $-18$  °C until gas-chromatographic analysis was performed.

The fatty acid methyl esters were analyzed using a Shimadzu 17A gas chromatograph equipped with a flame-ionization detector and a capillary column (100 m × 0.25 mm) containing 0.25 µm particles in IBS CP 88 cyanopropyl polysiloxane. The column temperature program was as follows: 65 °C for 15 min; 10 °C min<sup>-1</sup> to 165 °C and held for 2 min; 4 °C min<sup>-1</sup> to 185 °C and maintained for 8 min; 4 °C min<sup>-1</sup> to 235 °C and maintained for 5 min. The detector and the injector were maintained at 260 °C, and the split ratio was 1/100. The gas flow rates were 1.2 ml min<sup>-1</sup> for the carrier gas (H<sub>2</sub>), 30 ml min<sup>-1</sup> for the auxiliary gas (N<sub>2</sub>), 30 and 300 ml min<sup>-1</sup> for the flame gases, H<sub>2</sub> and synthetic air, respectively. To identify the fatty acids, the relative retention times of the peaks of the samples were compared with those of fatty acid methyl ester standards (Sigma). The results were expressed as the percentage of the normalized area of the fatty acid peak.

The activity index of the  $\Delta^9$  desaturases C16 and C18, which are responsible for the conversion of C16 and C18 fatty acids via their respective monounsaturated double bond at C9, were calculated using the following formulas:  $\Delta^9$  desaturase C16 =  $100 [C16:1 / (C16:0 + C16:1)]$  and  $\Delta^9$  desaturase C18 =  $100 [C18:1 / (C18:0 + C18:1)]$  (Malau-Aduli, Siebert, Bottema, & Pitchford, 1997; Pitchford, Deland, Siebert, Malau-Aduli, & Bottema, 2002).

The experimental design was completely randomized. The means were compared by Student's t-test ( $p < 0.05$ ) and the Pearson's correlation coefficients ( $p < 0.05$ ) of the fatty acids with the back fat thickness and ether extract, and were calculated using the SAEG statistical program (UFV. Universidade Federal de Viçosa, 2000).

## 3. Results and discussion

The immunocastrated animals showed higher back fat thickness and ether extract percentage compared with intact animals (Table 2). These results are in accordance with De Freitas et al. (2015) who found that immunocastrated bulls had greater subcutaneous fat thickness (4.90 mm) than intact bulls (3.61 mm). De Freitas et al. (2015) attributed the greater subcutaneous fat thickness to the lower serum testosterone

**Table 2**  
Fatty acid composition and activity indices of desaturases enzymes from *longissimus thoracis* muscle of intact and immunocastrated (Bopriva®) groups.

Variables	Treatment groups		p Value
	Intact	Immunocastrated	
Back fat thickness (mm)	3.34 ± 0.86	4.22 ± 0.71	*
Ether extract (%)	1.02 ± 0.34	1.58 ± 0.42	*
<b>Saturated (%)</b>			
C14:0	2.03 ± 0.90	2.63 ± 0.43	NS
C15:0	0.28 ± 0.12	0.31 ± 0.06	NS
C16:0	22.56 ± 1.85	23.97 ± 1.38	NS
C17:0	0.73 ± 0.28	0.83 ± 0.19	NS
C18:0	15.36 ± 2.45	14.54 ± 2.18	NS
C21:0	0.37 ± 0.20	0.40 ± 0.10	NS
<b>Monounsaturated (%)</b>			
C14:1	0.42 ± 0.25	0.60 ± 0.18	NS
C15:1	0.11 ± 0.06	0.09 ± 0.06	NS
C16:1 n-7	0.15 ± 0.06	0.15 ± 0.01	NS
C16:1 n-9	2.62 ± 0.82	3.37 ± 0.54	*
C17:1	0.55 ± 0.22	0.63 ± 0.16	NS
C18:1 n-9	37.84 ± 3.57	42.86 ± 3.40	*
<b>Polyunsaturated (%)</b>			
C18:2 n-6	9.60 ± 5.41	5.17 ± 2.85	*
C18:3 n-3	2.68 ± 0.94	1.21 ± 0.50	NS
C20:3 n-6	0.38 ± 0.23	0.31 ± 0.16	NS
C20:4 n-6	2.29 ± 1.00	1.34 ± 0.74	*
C20:5 n-3	0.77 ± 0.47	0.61 ± 0.39	NS
C22:6 n-3	1.27 ± 0.77	0.98 ± 0.55	NS
Δ <sup>9</sup> Desaturase C16	10.33 ± 2.70	12.40 ± 1.84	NS
Δ <sup>9</sup> Desaturase C18	71.22 ± 2.51	74.70 ± 3.01	*

NS – not significant differences between treatments ( $p > 0.05$ ).

\* Significant difference at 5% probability ( $p < 0.05$ ).

levels of immunocastrated bulls at feedlot entry compared with intact bulls.

The results found in this study were probably due to the same factors mentioned by De Freitas et al. (2015). These differences are possibly due to the effect of testosterone on intact males that promotes muscular development throughout life due to increased nitrogen retention (Galbraith, Demspter, & Miller, 1978). Prior et al. (1983) suggested that testosterone has an inhibitory effect on lipogenic enzyme activities in adipose tissue inducing higher basal lipolytic rates.

Fat thickness can serve as a barrier against moisture loss and plays a significant role in the reduction of cold shortening during the chilling processes of beef (Smith & Carpenter, 1973; Dolezal, Smith, Savell, & Carpenter, 1982). According to Smith and Carpenter (1973), postmortem shrinkage may be accompanied by surface dehydration and color deterioration, which significantly influence product shelf-life and ultimate consumer acceptability.

This way, it is possible that the immunocastrated animals have an improvement in meat quality due to the factors cited above as a result of the higher fat thickness observed when compared to the intact group.

There was no significant difference between groups for the percentages of myristic (C14:0), pentadecylic (C15:0), palmitic (C16:0), margaric (C17:0), stearic (C18:0) and n-heneicosoico (C21:0) acids (Table 2). Saturated fatty acids may raise total and low-density lipoprotein (LDL) cholesterol, increasing the risk of cardiovascular disease (Lichtenstein et al., 2006). In this way, these results are important because immunocastration did not change the profile of saturated fatty acids ( $p > 0.05$ ) in meat when compared to the intact group.

Palmitic (C16:0), stearic (C18:0) and oleic (C18:1 n-9) acids together represent approximately 76 and 81% of the total fatty acids in the beef of the intact and immunocastrated groups, respectively (Table 2), i.e., the majority of the fatty acids of the *longissimus thoracis* muscles of the cattle was represented by these three fatty acids. Monteiro, Bessa, Navas, and Lemos (2013) also found that together

they are the most representative fatty acids, accounting for approximately 61 and 62% of the total fatty acids in beef of bulls and steers, respectively.

Palmitic acid is the predominant saturated fatty acid in the diet (Jonnalagadda, Egan, Heimbach, Harris, & Kris-Etherton, 1995). However, the hypercholesterolemic effect of this acid has recently been questioned by a number of researchers, so its effect is debatable (Clandinin, Cook, Konard, & French, 2000). Although stearic acid has little effect on plasma lipid levels, it exerts a neutral effect similar to that of some monounsaturated fatty acids (Grundy & Denke, 1990).

There was no significant difference ( $p > 0.05$ ) between the groups for the percentages of monounsaturated fatty acids C14:1, C15:1, C16:1 n-7 and C17:1. However, the C16:1 n-9 and C18:1 n-9 fatty acids percentages differed between the intact and immunocastrated groups (Table 2).

The beef from the immunocastrated group had greater levels of 7-hexadecenoic (C16:1 n-9) and oleic (C18:1 n-9) acids compared with the intact group (Table 2). These results were most likely due to the immunocastrated animals having larger deposits of carcass fat than the intact males (Amatayakul-Chantler et al., 2012; De Freitas et al., 2015) and according to De Smet, Raes and Demeyer (2004) the concentration of monounsaturated fatty acids increases faster with increasing fatness than does the concentration of polyunsaturated.

Contrary to the results obtained in this study, Ruiz et al. (2005) found no significant differences ( $p > 0.05$ ) in the percentage of these fatty acids in immunocastrated and intact males. The divergence of the results is possibly a consequence of how the experiments were conducted. Ruiz et al. (2005) kept the animals on pasture during the whole experimental period and used three doses of a luteinizing hormone-releasing hormone fusion protein vaccine, while in this study the animals were kept at a feedlot on a feedlet diet, and it were given only two doses of the Bopriva® immunocastration vaccine.

According to Jansen et al. (2000), a monounsaturated fatty acid diet produced a marked improvement in the lipid profile in humans, lowering total plasma cholesterol and LDL-cholesterol concentrations when compared to a saturated fatty acid diet.

Oleic acid is quantitatively the predominant monounsaturated fatty acid in the diet (Grundy & Denke, 1990; Lopez-Huertas, 2010). Oleic acid has a beneficial effect upon cholesterol metabolism and exerts a protective role against cardiovascular diseases (De Lacruz et al., 2000).

Gilmore et al. (2011) demonstrated that ground beef rich in oleic acid (27 g/d) can increase the HDL-cholesterol concentration when compared to a diet poor in oleic acid (22 g/d). According to Lopez-Huertas (2010) an increase in oleic acid intake may be beneficial as it limits the intake of saturated fat. This can be achieved by changing dietary patterns or by using food technology to modify the fatty acid profile of foods naturally rich in saturated fatty acids in favor of oleic acid.

Therefore, immunocastration could be used as an alternative to castration in order to improve the fatty acid content of beef, by increasing the percentage of monounsaturated fatty acids, mainly oleic found in the meat (42.86% immunocastrated × 37.84% intact).

Among the polyunsaturated fatty acids, linoleic (C18:2 n-6) and α-linolenic (C18:3 n-3) acids were predominant (Table 2), making up approximately 72% and 66% of the total polyunsaturated fatty acids in the beef of the intact and immunocastrated groups, respectively.

Linoleic and linolenic fatty acids are representatives of the omega 6 (n-6) and omega 3 (n-3) series. These are considered essential fatty acids because they are not synthesized by animals (Kahveci & Xu, 2011), so they must be obtained from their diet.

The levels of linoleic (C18:2 n-6) and arachidonic (C20:4 n-6) fatty acids were lower ( $p < 0.05$ ) in the meat from the immunocastrated group than in intact group (Table 2). These results were possibly due to immunocastrated animals having higher fat deposits on the carcass, and according to Chow (2007) one important consequence of increasing fat deposition is that the proportion of phospholipid is reduced, leading to a decrease in the proportion of the major PUFA, 18:2 n-6.

However, Ruiz et al. (2005) found no significant differences between the levels of these fatty acids in immunized and intact males. The contradictions among studies may be due to the animals from the study of Ruiz et al. (2005) being raised on pasture and receiving three doses of immunocastration vaccine while in this study the animals were finished in a feedlot and received two doses of Bopriva<sup>®</sup> vaccine.

Thus, it would be interesting to compare studies that have applied the same methodology; however, few studies have evaluated the fatty acid profile in beef from immunocastrated animals.

The essential fatty acids play a role in the formation of long-chain polyunsaturated fatty acids, e.g., linoleic acid gives rise to arachidonic (C20:4 n-6) acid, which is a precursor of the eicosanoids, which are responsible for triggering inflammatory processes and pro-aggregatory (Hata & Breyer, 2004). Linolenic acid gives rise to several fatty acids, including eicosapentaenoic (C20:5 n-3), docosapentaenoic (C22:5 n-3) and docosahexaenoic (C22:6 n-3) acids, which are involved in the formation of eicosanoids that act as anti-inflammatory and anti-aggregatory agents (Sinn et al., 2012).

The beef from the immunocastrated group exhibited higher activities of  $\Delta^9$  desaturases C18 than the intact group (Table 2). The C16 and C18 enzymes are important steps in the conversion of palmitic (C16:0) and stearic (C18:0) acids into their corresponding monounsaturated fatty acids (n-9), 7-hexadecenoic and oleic acids, respectively (Kazala et al., 1999; De Smet, Raes, & Demeyer, 2004). These results are consistent with those of Padre et al. (2006), who observed higher ( $p < 0.05$ ) concentrations of C18:1 n-9 fatty acid in steers than in bulls and attributed this phenomenon to the increase in adipose tissue which increases this fatty acid (Leat, 1975) and consequently the activity of  $\Delta^9$  desaturase C18, because the higher oleic content will result in a higher percentage of this enzyme.

Nevertheless, oleic acid is not an essential fatty acid because most animals possess  $\Delta^9$  desaturase and can synthesize the  $\Delta^9$  series by chain elongation and desaturation. Consequently, oleic acid can compete with linoleic and linolenic acid and their immediate products, for the reactions mediated by desaturase and elongase (Woutersen, Appel, Van Garderen-Hoetmer, & Wijnands, 1999). Due to bovine adipose tissue being a major site of fatty acid elongation and desaturation at the  $\Delta^9$  desaturase (John, Lunt, & Smith, 1991), the immunocastrated animals, that showed higher level of fatness, had a higher amount of oleic fatty acid which was the result of increased activities of  $\Delta^9$  desaturases C18, and consequently led to a lower percentage of linoleic fatty acid.

The total percentages of monounsaturated, polyunsaturated and n-6 fatty acids differ ( $p < 0.05$ ) between the treatment groups (Table 3), while the percentages of saturated, n-3 fatty acids and ratio PUFA/SFA and n-6/n-3 did not differ.

The intact group had a lower level ( $p < 0.01$ ) of monounsaturated fatty acids and a higher level ( $p < 0.05$ ) of polyunsaturated fatty acids than the immunocastrated group. These results were possibly obtained because according to Marmer, Maxwell, and Williams (1984) the lower

fat content (that was found in intact group in our study) is associated with fewer and smaller adipocytes, containing fewer triglycerides, which is accompanied by a relative increase in the proportion of phospholipids in total lipids and an increased PUFA content.

However, the percentages of unsaturated fatty acids (polyunsaturated + monounsaturated) of the intact (58%) and immunocastrated (57%) groups were numerically similar.

Unsaturated fatty acids produce degradation products, such as aliphatic aldehydes, ketones, and alcohols, which may have intrinsic flavors. These degradation products may react further with Maillard products to produce other compounds that may contribute to flavor (Mottram & Edwards, 1983; Elmore, Mottram, Enser, & Wood, 1997). Moreover, lipid oxidation is a major cause of meat spoilage and is considered one of the main limiting shelf-life factors of these products (Frankel, 1998).

The intact group had a higher average level of n-6 fatty acids than the immunocastrated group, but this value did not affect the n-6/n-3 ratio, which was not significantly different (Table 3). The recommended dietary n-6/n-3 ratio is 4.0 or less (Wood et al., 2003), because a high intake of n-6 fatty acids promotes the development of cardiovascular diseases, inflammatory disorders and cancers (Horrobin, Jenkins, Bennett, & Christie, 2002). The n-6/n-3 ratio in the both groups was within the recommended range.

The recommended dietary PUFA/SFA ratio should be greater than 0.4 (Wood et al., 2003) because polyunsaturated fatty acids produce nutritional benefits to human health (Lopez-Huertas, 2010). Therefore, only the beef of the intact group was in accordance with the recommended ratio; however, according to Wood et al. (2003), many meats naturally have a PUFA/SFA ratio of approximately 0.1.

There was a significant moderate negative correlation between the fatty acid C15:1 ( $p = 0.05$ ;  $r = -0.37$ ) and the back fat thickness (Table 4). As the fatness degree increased, there was a decrease in this fatty acid. There was a moderate positive correlation between the ether extract and C14:0 ( $p = 0.00$ ;  $r = 0.54$ ), C16:0 ( $p = 0.02$ ;  $r = 0.44$ ) and C14:1 ( $p = 0.05$ ;  $r = 0.36$ ) fatty acids and a strong positive correlation for C16:1 n-9 ( $p = 0.00$ ;  $r = 0.67$ ) and C18:1 n-9 ( $p = 0.00$ ;  $r = 0.74$ ) fatty acids (Table 4). With the increase in beef ether extract there were also increases in the saturated and monounsaturated fatty acids mentioned above. According to De Smet, Raes and Demeyer (2004) the contents of monounsaturated fatty acids increase faster with increasing fatness than does the content of polyunsaturated.

However, the polyunsaturated fatty acid contents were inversely correlated to the saturated and monounsaturated fatty acid contents in relation to the beef ether extract, presenting moderate negative correlation to: C20:3 n-6 ( $p = 0.01$ ;  $r = -0.50$ ), C20:5 n-3 ( $p = 0.01$ ;  $r = -0.49$ ) and C22:6 n-3 ( $p = 0.01$ ;  $r = -0.52$ ) and strong negative correlation to: C18:2 n-6 ( $p = 0.00$ ;  $r = -0.66$ ) and C20:4 n-6 ( $p = 0.00$ ;  $r = -0.72$ ) (Table 4). Thus, there was an increase in the percentage of ether extract and a decrease in polyunsaturated fatty acids.

Wood et al. (1996) found high negative correlations between lipid concentration in pork and the concentrations of C18:2 n-6 (-0.83) and C20:4 n-6 (-0.81), but not with C18:3 n-3 (0.14), which suggests different control factors for the concentrations of linoleic and  $\alpha$ -linolenic acids (Wood et al., 1996). These findings support the results of our study.

There was a moderate positive correlation ( $p = 0.00$ ;  $r = 0.61$ ) between the activity of C16 desaturase and the ether extract percentage of beef. As the ether extract increased, there was an increase in the activity index of this enzyme.

Significant positive correlations were observed between the ether extract and the total of saturated and monounsaturated fatty acids, respectively, moderate ( $p = 0.04$ ;  $r = 0.39$ ) and strong ( $p = 0.00$ ;  $r = 0.74$ ). However, a strong negative correlation was found for the total of polyunsaturated fatty acids ( $p = 0.00$ ;  $r = -0.66$ ) and n-6 ( $p = 0.00$ ;  $r = -0.71$ ) and a moderate negative for n-3 ( $p = 0.00$ ;  $r = -0.55$ ) and PUFA/SFA ( $p = 0.00$ ;  $r = -0.59$ ) (Table 4).

**Table 3**

Total of saturated fatty acids, monounsaturated, polyunsaturated, omega 3 (n-3) and omega 6 (n-6) from *longissimus thoracis* muscle of intact and immunocastrated (Bopriva<sup>®</sup>) groups.

Fatty acid (%)	Treatment groups		p Value
	Intact	Immunocastrated	
Saturated	41.33 ± 5.19	42.68 ± 2.98	NS
Monounsaturated	41.69 ± 4.70	47.70 ± 3.90	*
Polyunsaturated	16.99 ± 9.38	9.62 ± 4.98	*
n-3	4.72 ± 3.53	2.80 ± 1.40	NS
n-6	12.27 ± 5.98	6.82 ± 3.65	*
PUFA/SFA	0.44 ± 0.34	0.23 ± 0.13	NS
n-6/n-3	2.86 ± 0.47	2.45 ± 0.75	NS

NS – not significant differences between treatments ( $p > 0.05$ ).

\* Significant difference at 5% probability ( $p < 0.05$ ).

**Table 4**

Correlation of the fatty acids with the back fat thickness and beef ether extract from the immunocastrated bulls (Bopriva®).

Fatty acids	Back fat thickness (mm)		Ether extract (%)	
	r	p Value	r	p Value
<i>Saturated</i>				
C14:0	0.13	NS	0.54	*
C15:0	0.04	NS	0.23	NS
C16:0	0.17	NS	0.44	*
C17:0	0.22	NS	0.36	*
C18:0	0.31	NS	0.12	NS
C21:0	−0.01	NS	0.25	NS
<i>Monounsaturated</i>				
C14:1	−0.13	NS	0.48	*
C15:1	−0.37	*	0.13	NS
C16:1 n7	0.09	NS	0.08	NS
C16:1 n9	0.00	NS	0.67	*
C17:1	0.27	NS	0.24	NS
C18:1 n9	0.20	NS	0.74	*
<i>Polyunsaturated</i>				
C18:2 n6	−0.24	NS	−0.66	*
C18:3 n3	−0.22	NS	−0.34	NS
C20:3 n6	0.03	NS	−0.50	*
C20:4 n6	−0.18	NS	−0.72	*
C20:5 n3	−0.04	NS	−0.49	*
C22:6 n3	−0.06	NS	−0.52	*
<i>Activity indices of desaturases enzymes</i>				
Delta C 16	−0.00	NS	0.61	*
Delta C 18	−0.16	NS	0.36	NS
Saturated	0.27	NS	0.39	*
Monounsaturated	0.16	NS	0.74	*
Polyunsaturated	−0.24	NS	−0.67	*
n−3	−0.25	NS	−0.55	*
n−6	−0.23	NS	−0.71	*
PUFA: SFA	−0.24	NS	−0.59	*
n−6/n−3	0.04	NS	−0.20	NS

NS – not significant differences between treatments ( $p > 0.05$ ).

\* Significant difference at 5% probability ( $p < 0.05$ ).

As the percentage of beef ether extract increased there was an increase in the amount of saturated and monounsaturated fatty acids, however, inverse results were observed for total polyunsaturated fatty acids, omega 3, omega 6 and PUFA/SFA ratio, which decreased with increasing ether extract.

#### 4. Conclusion

Immunocastration of bulls using Bopriva® improved carcass back fat thickness and ether extract when compared to intact group. Furthermore, immunocastration produced no differences in the concentrations of saturated fatty acids in beef, i.e., those considered harmful to health. By contrast, immunocastration increased the percentage of monounsaturated fatty acids (mainly oleic acid) in the beef, which may be beneficial to human health. Because of this relationship, immunocastration may be an alternative to produce meat with the same or better fatty acid profile than that found in intact group.

The study was approved by the Ethics Committee on Animal Use of the State University of Londrina, under protocol number 10370.2012.49.

#### References

A.O.A.C. Association of Official Agricultural Chemists (1990). (15th ed.). *Official Methods of the Association of the Agricultural Chemists, Vol. 2*.

Adams, T. E., & Adams, B. M. (1992). Feedlot performance of steers and bulls actively immunized against gonadotropin-releasing hormone. *Journal of Animal Science*, 70(6), 1691–1698.

Amatayakul-Chantler, S., Jackson, J. A., Stegner, J., King, V., Rubio, L. M. S., Howard, R., ... Walker, J. (2012). Immunocastration of *Bos indicus* × brown swiss bulls in feedlot with gonadotropin-releasing hormone vaccine bopriva provides improved

performance and meat quality. *Journal of Animal Science*, 90(11), 3718–3728. <http://dx.doi.org/10.2527/jas.2011-4826>.

Amatayakul-Chantler, S., Hoe, F., Jackson, J. A., Roca, R. O., Stegner, J. E., King, V., ... Walker, J. (2013). Effects on performance and carcass and meat quality attributes following immunocastration with the gonadotropin releasing factor vaccine bopriva or surgical castration of *Bos indicus* bulls raised on pasture in Brazil. *Meat Science*, 95(1), 78–84. <http://dx.doi.org/10.1016/j.meatsci.2013.04.008>.

Belk, K. E., Scanga, J. A., Smith, G. C., & Grandin, T. (2002). The relationship between good handling/stunning and meat quality in beef, pork, and lamb. *American Meat Institute Foundation, Animal Handling And Stunning Conference on February 21–22*.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), 911–917.

Bonneau, M., & Enright, W. J. (1995). Immunocastration in cattle and pigs. *Livestock Production Science*, 42(2–3), 193–200. [http://dx.doi.org/10.1016/0301-6226\(95\)00020-L](http://dx.doi.org/10.1016/0301-6226(95)00020-L).

Brasil. Ministério da Agricultura, Pecuária e Abastecimento (1968.). *Departamento de Defesa e Inspeção Agropecuária. Regulamento de inspeção industrial e sanitária de produtos de origem animal*. São Paulo: Inspeção do SIPAMA (346 pp.).

Chambers, P., & Grandin, T. (2001). Guidelines for humane handling, transport and slaughter of livestock. *RAP publication (FAO)* (pp. 1–94) (Retrieved from <http://agris.fao.org/agris-search/search/display.do?f=2004/XF/XF04065.xml;XF2003405491>).

Choat, W. T., Paterson, J. A., Rainey, B. M., King, M. C., Smith, G. C., Belk, K. E., & Lipsey, R. J. (2006). The effects of cattle sex on carcass characteristics and longissimus muscle palatability. *Journal of Animal Science*, 84(7), 1820–1826. <http://dx.doi.org/10.2527/jas.2004-418>.

Chow, C. K. (2007). *Fatty acids in foods and their health implications* (3rd ed.). Boca Raton: CRC Press.

Clandinin, M. T., Cook, S. L., Konard, S. D., & French, M. A. (2000). The effect of palmitic acid on lipoprotein cholesterol levels. *International Journal of Food Sciences and Nutrition*, 51, S61–S71 (Suppl.).

De Freitas, V. M., Leão, K. M., Araujo, Neto, De, F. R., Marques, T. C., Ferreira, R. M., ... De Oliveira, E. B. (2015). Effects of surgical castration, immunocastration and homeopathy on the performance, carcass characteristics and behaviour of feedlot-finished crossbred bulls. *Semina: Ciências Agrárias*, 36(3), 1725. <http://dx.doi.org/10.5433/1679-0359.2015v36n3p1725>.

De Lacruz, J. P., Villalobos, M. A., Carmona, J. A., Romero, M., Agreda, J. M., & De Lacuesta, F. (2000). Antithrombotic potential of olive oil administration in rabbits with elevated cholesterol. *Thrombosis Research*, 100(4), 305–315.

De Smet, S., Raes, K., & Demeyer, D. (2004). Meat fatty acid composition as affected by fatness and genetic factors: a review. *Animal Research*, 53(1), 81–98.

Dolezal, H. G., Smith, G. C., Savell, J. W., & Carpenter, Z. L. (1982). Comparison of subcutaneous fat thickness, marbling and quality grade for predicting palatability of beef. *Journal of Food Science*, 47(2), 397–401.

Du Plessis, I., & Hoffman, L. C. (2007). Effect of slaughter age and breed on the carcass traits and meat quality of beef steers finished on natural pastures in the arid subtropics of South Africa. *South African Journal of Animal Science*, 37(3), 143–153. <http://dx.doi.org/10.4314/sajas.v37i3.4084>.

Elmore, J. S., Mottram, D. S., Enser, M., & Wood, J. D. (1997). Novel thiazoles and 3-thiazolines in cooked beef aroma. *Journal of Agricultural and Food Chemistry*, 45, 3603–3607.

Frankel, E. N. (1998). *Lipid oxidation*. Dundee: Oily Press.

Galbraith, H., Demspeter, D. G., & Miller, T. B. (1978). A note on the effect of castration on the growth performance and concentrations of some blood metabolites and hormones in British Friesian male cattle. *Animal Production*, 26(3), 339–342.

Gilmore, L. A., Walzem, R. L., Crouse, S. F., Smith, D. R., Adams, T. H., Vaidyanathan, V., ... Smith, S. B. (2011). Consumption of high-oleic acid ground beef increases hdl-cholesterol concentration but both high- and low-oleic acid ground beef decrease HDL particle diameter in normocholesterolemic men. *The Journal of Nutrition*, 141, 1188–1194.

Grundy, S. M., & Denke, M. A. (1990). Dietary influences on serum lipids and lipoproteins. *Journal of Lipid Research*, 31(7), 1149–1172.

Hata, A. N., & Breyer, R. M. (2004). Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. *Pharmacology and Therapeutics*, 103(2), 147–166. <http://dx.doi.org/10.1016/j.pharmthera.2004.06.003>.

Hennessy, D. (2008). *Improvac® mode of action*. *Technical bulletin, Apr*.

Horrobin, D. F., Jenkins, K., Bennett, C. N., & Christie, W. W. (2002). Eicosapentaenoic acid and arachidonic acid: collaboration and not antagonism is the key to biological understanding. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, 66(1), 83–90. <http://dx.doi.org/10.1054/plef.2001.0338>.

Huxsoll, C. C., Price, E. O., & Adams, T. E. (1998). Testis function, carcass traits, and aggressive behavior of beef bulls actively immunized against gonadotropin-releasing hormone. *Journal of Animal Science*, 76(7), 1760–1766.

ISO. International Organization for Standardization (1978). *Animal and vegetable fats and oils preparation of methyl esters of fatty acids — method ISO 5509*. Geneva, 1–6.

Jago, J. G., Bass, J. J., & Matthews, L. R. (1997). Evaluation of a vaccine to control bull behaviour. *Proceedings of the New Zealand Society of Animal Production*, 57(Cv), 91–95.

Janett, F., Gerig, T., Tschuor, A. C., Amatayakul-Chantler, S., Walker, J., Howard, R., ... Thun, R. (2012). Vaccination against gonadotropin-releasing factor (GnRF) with Bopriva significantly decreases testicular development, serum testosterone levels and physical activity in pubertal bulls. *Theriogenology*, 78(1), 182–188. <http://dx.doi.org/10.1016/j.theriogenology.2012.01.035>.

Jansen, S., López-Miranda, J., Castro, P., López-Segura, F., Marín, C., Ordóñez, J. M., ... Pérez-Jiménez, F. (2000). Low-fat and high-monounsaturated fatty acid diets decrease plasma cholesterol ester transfer protein concentrations in young, healthy, normolipemic men. *American Journal of Clinical Nutrition*, 72(1), 36–41.

- John, L. C. St., Lunt, D. K., & Smith, S. B. (1991). Fatty acid elongation and desaturation enzyme activities of bovine liver and subcutaneous adipose tissue microsomes. *Journal of Animal Science*, *69*(3), 1064–1073.
- Jonnalagadda, S. S., Egan, K., Heimbach, J. T., Harris, S. S., & Kris-Etherton, P. M. (1995). Fatty acid consumption pattern of americans: 1987–1988 USDA nationwide food consumption survey. *Nutrition Research*, *15*(12), 1767–1781.
- Kahveci, D., & Xu, X. (2011). Repeated hydrolysis process is effective for enrichment of omega 3 polyunsaturated fatty acids in salmon oil by *Candida rugosa* lipase. *Food Chemistry*, *129*(4), 1552–1558. <http://dx.doi.org/10.1016/j.foodchem.2011.05.142>.
- Kazala, E. C., Lozeman, F. J., Mir, P. S., Laroche, A., Bailey, D. R., Weselake, R. J., ... Weselake, R. J. (1999). Relationship of fatty acid composition to intramuscular fat content in beef from crossbred Wagyu cattle. *Journal of Animal Science*, *77*(7), 1717–1725.
- Lawrie, R. A. (2006). *Lawrie's Meat Science*, iv. <http://dx.doi.org/10.1016/B978-1-84569-159-2.50014-0>.
- Leat, W. M. F. (1975). Fatty acid composition of adipose tissue of Jersey cattle during growth and development. *Journal of Agricultural Science*, *85*, 551–558.
- Lichtenstein, A. H., Appel, L. J., Brands, M., Carnethon, M., Daniels, S., Franch, H. A., ... Wylie-Rosett, J. (2006). Diet and lifestyle recommendations revision: a scientific statement from the American Heart Association Nutrition Committee. *Circulation*, *114*(1), 82–96.
- Lopez-Huertas, E. (2010). Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. *Pharmacological Research*, *61*(3), 200–207. <http://dx.doi.org/10.1016/j.phrs.2009.10.007>.
- Malau-Aduli, A. E. O., Siebert, B. D., Bottema, C. D. K., & Pitchford, W. S. (1997). A comparison of the fatty acid composition of triacylglycerols in adipose tissue from Limousin and Jersey cattle. *Australian Journal of Agricultural Research*, *48*(5), 715–722.
- Marmer, W. N., Maxwell, R. J., & Williams, J. E. (1984). Effects of dietary regimen and tissue site on bovine fatty acid profiles. *Journal of Animal Science*, *59*(1), 109–121.
- Monteiro, A. C. G., Bessa, R. J. B., Navas, D. R., & Lemos, J. P. C. (2013). Fatty acid profile and lipids nutritional value of intramuscular fat from Alentejana bulls and steers. *Revista Portuguesa de Ciências Veterinárias*, *108*, 120–126.
- Mottram, D. S., & Edwards, R. A. (1983). The role of triglycerides and phospholipids in the aroma of cooked beef. *Journal of the Science of Food and Agriculture*, *34*(5), 517–522.
- Padre, R. D. G., Aricetti, J. A., Moreira, F. B., Mizubuti, I. Y., do Prado, I. N., Visentainer, J. V., ... Matsushita, M. (2006). Fatty acid profile, and chemical composition of longissimus muscle of bovine steers and bulls finished in pasture system. *Meat Science*, *74*(2), 242–248. <http://dx.doi.org/10.1016/j.meatsci.2006.02.012>.
- Pitchford, W., Deland, M. P. B., Siebert, B. D., Malau-Aduli, A. E. O., & Bottema, C. D. K. (2002). Genetic variation in fatness and fatty acid composition of crossbred cattle. *Journal of Animal Science*, *80*(1), 2825–2832 (Retrieved from <http://www.journalofanimalscience.org/content/80/11/2825.short>).
- Price, E. O., Adams, T. E., Huxsoll, C. C., & Borgwardt, R. E. (2003). Aggressive behavior is reduced in bulls actively immunized against gonadotropin-releasing hormone. *Journal of Animal Science*, *81*(2), 411–415.
- Prior, R. L., Smith, S. B., Schanbacher, B. D., & Mersmann, H. J. (1983). Lipid metabolism in finishing bulls and steers implanted with oestradiol-17  $\beta$ -dipropionate. *Animal Production*, *37*(1), 81–88.
- Robertson, I. S., Wilson, J. C., & Fraser, H. M. (1979). Immunological castration in male cattle. *Veterinary Record*, *105*(24), 556–557.
- Ruiz, M., Matsushita, M., Visentainer, J., Hernandez, J., De, A., Ribeiro, E., Shimokomaki, M., ... De Souza, N. (2005). Proximate chemical composition and fatty acid profiles of longissimus proximate chemical composition and fatty acid profiles of longissimus thoracis from pasture fed LHRH immunocastrated, castrated and intact *Bos indicus* bulls. *South African Journal of Animal Science*, *35*(1), 13–18. <http://dx.doi.org/10.4314/sajas.v35i1.4044>.
- Seideman, S. C., Cross, H. R., Oltjen, R. R., & Schanbacher, B. D. (1982). Utilization of the intact male for red meat production: a review. *Journal of Animal Science*, *55*, 826–840.
- Silva, D. J., & Queiroz, A. C. (2002). *Análise de alimentos: métodos químicos e biológicos*. Viçosa, MG: Editora UFV (235 pp.).
- Sinn, N., Milte, C. M., Street, S. J., Buckley, J. D., Coates, A. M., Petkov, J., & Howe, P. R. C. (2012). Effects of n-3 fatty acids, EPA v. DHA, on depressive symptoms, quality of life, memory and executive function in older adults with mild cognitive impairment: a 6-month randomised controlled trial. *British Journal of Nutrition*, *107*(11), 1682–1693. <http://dx.doi.org/10.1017/S0007114511004788>.
- Smith, G. C., & Carpenter, Z. L. (1973). Postmortem shrinkage of lamb carcasses. *Meat Science*, *36*(5), 862–867.
- UFV. Universidade Federal de Viçosa (2000). *Sistema de análises estatísticas e genéticas 401 – SAEG. Versão 8.0*. (Viçosa, MG, 142 pp.).
- Wood, J. D., Brown, S. N., Nute, G. R., Whittington, F. M., Perry, A. M., Johnson, S. P., & Enser, M. (1996). Effects of breed, feed level and conditioning time on the tenderness of pork. *Meat Science*, *44*(1–2), 105–112. [http://dx.doi.org/10.1016/S0309-1740\(96\)00044-7](http://dx.doi.org/10.1016/S0309-1740(96)00044-7).
- Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., ... Enser, M. (2003). Effects of fatty acids on meat quality: a review. *Meat Science*, *66*(1), 21–32. [http://dx.doi.org/10.1016/S0309-1740\(03\)00022-6](http://dx.doi.org/10.1016/S0309-1740(03)00022-6).
- Woutersen, R. A., Appel, M. J., Van Garderen-Hoetmer, A., & Wijnands, M. V. W. (1999). Dietary fat and carcinogenesis. *Mutation Research*, *443*(1–2), 111–127.