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# **Effect of age and sexual condition on the fatty acid profile of intramuscular fat of cattle finished in feedlot**

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#### **Abstract**

The objective of this study was to evaluate the fatty acid (FA) profile of intramuscular fat of cattle of two categories (16 or 26 months old) and two sexual conditions (Bulls or Steers) finished in feedlot. A total of 32 castrated (n=16) or noncastrated  $(n=16)$  animals of the genetic groups ½Purunã + ½Canchim were used. The animals were finished in feedlot, receiving a diet with 50% roughage and 50% concentrated feed. After the slaughter, one *Longissimus dorsi* sample from each animal was sent to the laboratory for FA profile analyses. The FA content of C17:0 *iso* and C18:0 was higher for animals slaughtered at 26 months. Lignoceric acid (C24:0) showed a higher content for animals aged 16 months. Animals slaughtered at 16 months showed higher C18:1 *cis* 11, C18:1 *cis* 12, C18:1 *cis* 15, and lower C18:1 *trans* 16 than those slaughtered at 26 months. The C20:4 and C22:5 were lower as the animals' slaughter age increased. The unsaturated/saturated and polyunsaturated/saturated ratios showed no difference for slaughter age. The concentrations of C24:0, C18:1 *trans* 10, C18:1 *cis* 11, C18:1 *cis* 12, and C22:1 were greater in the meat of bulls than in steers. Bulls had a higher content of polyunsaturated FAs and polyunsaturated/saturated ratio than steers.

**Keywords:** canchim; saturated; unsaturated; polyunsaturated; Purunã.

**Practical Application:** Age does not affect the intramuscular fat quality, while castration decreases the amount of polyunsaturated fatty acids.

# **1 INTRODUCTION**

Producers and researchers of beef cattle agree that reducing the slaughter age from 26 to 16 months intensifies the production on the property, making it more efficient in quality food production and financial resource management (Kuss et al., 2009). While the meat of younger animals has great softness, the carcass of animals aged close to 24 months is desired by the final consumer due to its higher muscle/fat ratio, lower amount of lipids, and softness similar to that of 14-month-old animals (Pacheco et al., 2011).

Beef quality can be affected by several factors such as nutrition, slaughter age, body weight, sexual condition, pre-slaughter management, and post-slaughter management (Nogalski et al., 2014). In several countries, bull calves are commonly used for meat production (Missio et al., 2017; Pogorzelska-Przybyłek et al., 2018) than steers due to the higher performance presented by this category. However, steers present carcasses with a higher fat content than bulls, with greater acceptance by consumers of their sensory quality (Missio et al., 2017). On the contrary, the meat of bulls can be a healthier option since they are leaner than steers. Nevertheless, fat quality is more critical than fat content.

The fatty acid composition in the diet is very important for human nutrition. Literature has shown that dietary fatty acids with different degrees of saturation have different effects on human health (Lengyel et al., 2003). Beef is considered one of the meats with the most significant detrimental effect on human health due to its lipid composition, consisting mainly of saturated fatty acids (SFAs). However, it has been widely demonstrated that long-chain unsaturated fatty acids participate in several metabolic processes beneficial to human health (Cook et al., 2001; Varela et al., 2004). In addition, fats from the meat of ruminant animals are natural sources of some of those fatty acids, such as oleic acid and conjugated linoleic acid (CLA) isomers, respectively, C18:2 cis-9 and trans-11 (French et al., 2000).

The aim of the study was to evaluate whether slaughter age and sexual condition would affect the fatty acid profile of beef.

# **2 MATERIALS AND METHODS**

The Committee on Ethics of Animal Use (CEUA) of the Universidade Tecnológica Federal do Paraná, located in Dois Vizinhos, Paraná State, Brazil, approved all procedures involving animals in this study, protocol 2018-30.

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We used 16 bull calves and 16 steers of the genetic group ½Purunã + ½Canchim distributed according to slaughter age 16 or 26 months. The castrated animals underwent this procedure at 7 months old by the surgical method of bilateral orchiectomy (removal of testicles by surgical means and cord ligation by cauterization).

The experiment was conducted in a covered feedlot, where the animals remained in individual pens,  $10 \text{ m}^2$ , with wooden troughs and masonry drinkers. The animals were fed a diet with 11.2% crude protein and 3.07 Mcal of digestible energy/kg of dry matter (DM). The diet was 50% roughage (corn silage) and 50% concentrate feed with 73.0% corn grain, 25% soybean meal, 1% common salt, and 1% calcitic limestone, based on DM. The chemical analysis of the diet is shown in Table 1. More details on the animals' husbandry and performance can be observed in Cullmann et al. (2017).

The slaughter of the animals was pre-stipulated based on the body condition (between 3.5 and 4.0 points), aiming at a covering degree recommended by slaughterhouses (thickness between 3 and 6 mm). When the mean of the lots reached the recommended body score, the animals fasted on solids for 16 h. Then, they were weighed and transported in a cattle truck

**Table 1**. Chemical composition of feedstuff of the experimental diet.

	Feed				
<b>Parameters</b>	Corn silage	Corn grain	Soybean meal		
Dry matter (DM), %	35.84	89.37	89.54		
Crude protein, %	5.66	7.67	44.75		
Neutral detergent fiber, %	43.55	21.88	12.97		
Acid detergent fiber, %	26.84	13.08	4.40		
Ether extract, %	2.19	4.80	2.37		
Ash, %	3.06	1.24	5.98		
Non-nitrogen extract, %	67.68	75.83	43.38		
Organic matter, %	96.90	98.76	94.02		
Total digestible nutrients, %	60.48	81.65	69.75		
Digestible energy <sup>1</sup> , Mcal/kg DM	2.67	3.60	3.07		

1 DE: 4.409 \* TDN/100.

to a commercial slaughterhouse, where they were slaughtered after a minimum rest of 24 h, according to the normal slaughter flow of the establishment. The animals were stunned with a pneumatically powered penetrating captive bolt gun before slaughter to ensure they were unconscious during the sticking and bleeding procedures until death. The 26-month-old animals were slaughtered with 516.2 kg, while the 16-month-old ones were slaughtered with 470.5 kg. The steers were slaughtered with an average of 445.5 kg, while bulls were slaughtered with 541.3 kg. More information on the results regarding the carcass and meat quality (Table 2) of animals can be found in the studies by Kuss et al. (2009, 2010).

After 24 h of chilling, a muscle section of *Longissimus dorsi* between 11th and 13st ribs of each left half-carcass was taken and vacuum packed. The samples were identified, frozen in a freezing tunnel (-30 to -35 °C), and kept in a freezer (-20 °C). Then, the samples were sent to the Laboratory of Agro-industry, Food, and Nutrition of the Universidade de São Paulo (Esalq/ USP) for extraction and methylation analyses, quantitative determination of the lipid fraction, and qualitative determination of fatty acids. The steaks were ground in a food processor with dry ice to avoid heating and possible chemical changes.

Transmethylated samples were analyzed in a FINNIGAN Focus GC Gas Chromatograph, with flame ionization detector, capillary column CP-Sil 88 (Varian), 100 m long by 0.25 μm inner diameter, and 0.20 μm thick film. Hydrogen was used as a drag gas at a 1.8 mL/min flow rate. The starting oven temperature program was 70 °C, standby time 4 min, 175 °C (13 °C/ min) standby time 27 min, 215 °C (4 °C/min) standby time 9 min, and then increasing 7 °C/min up to 230 °C, remaining for 5 min, totaling 65 min. The vaporizer temperature was 250 °C, and the detector temperature was 300 °C.

An aliquot of 1 μL of the esterified extract was injected into the chromatograph. The fatty acid identification was made by comparing retention times and percentages of fatty acids obtained through the Chromquest 4.1 software (Thermo Electron, Italy).

The fatty acids were identified by comparing the retention times of the methyl esters samples with standards of fatty acids from butter reference BCR-CRM 164, Anhydrous Milk Fat-Producer (BCR Institute for Materials and Reference Measurements). After that, they were quantified by normalization

**Table 2**. Slaughter weight and fat deposition in the carcass and meat of steers and bulls finished in feedlot according to category and sexual condition.

	Slaughter age		<b>Sexual condition</b>		
	16	26	<b>Steers</b>	<b>Bulls</b>	
Slaughter weight, kg	$470.5 \pm 14.6$	$516.2 \pm 13.7$	$445.5 \pm 9.9$	$541.3 \pm 10.2$	
Fat thickness, mm	$4.9 \pm 0.4$	$3.8 \pm 0.4$	$4.6 \pm 0.3$	$4.0 \pm 0.3$	
Fat in carcass, %	$25.7 \pm 1.7$	$23.9 \pm 1.6$	$27.0 \pm 1.2a$	$22.6 \pm 1.2 b$	
Marbling†	$7.9 \pm 1.0$	$6.0 \pm 1.0$	8.20.7a	$5.7 \pm 0.7$ b	

†1 to 3: trace; 4 to 6: light; 7 to 9: small; 10 to 12: medium; 13 to 15: moderate; 16 to 18: abundant. Source: Adapted from Kuss et al. (2009; 2010).

of the methyl esters. The fatty acid results were expressed as a percentage of area (%).

The quantity of each fatty acid was used to calculate the indices of atherogenicity (AI) and thrombogenicity (TI), as proposed by Ulbricht and Southgate (1991), and the hypocholesterolemic/hypercholesterolaemic ratio (HH), as suggested by Santos-Silva et al. (2002) (Equations 1–3).

$$
AI = \frac{C12.0 \pm 4^{*} C14.0 \pm C16.0}{\sum \text{MUFA} + \sum n \cdot 6 \text{ PUFA} + \sum n \cdot 3 \text{ PUFA}} \tag{1}
$$

$$
TI = \frac{C14:0 \pm C16:0 \pm C18:0}{(0.5 \times \Sigma \text{ MUEA}) + (0.5 \times \Sigma n \cdot 6 \text{ PUEA}) + (3 \times \Sigma n \cdot 3 \text{ PUEA}) + (\Sigma n \cdot 3 \text{ PUEA} \times n \cdot 6 \text{ PUEA})}
$$
(2)

HH = C18:1 
$$
n-9 \pm C18:2 n-6 \pm C20:4 n-6 \pm C18:3 n-3 \pm C20:5 n-3 \pm C22:5 n-3
$$
  
C14:0 + C16:0  
(3)

The experimental design was completely randomized in a factorial arrangement of 2×2 (slaughter age and sexual condition) with 8 replicates per treatment, totaling 32 animals. One animal represented one experimental unit.

The statistical model is expressed as follows (Equation 4):

$$
Y_{ij} = \mu + C_i + S_j + C^* S_{ij} + E_{ij}
$$
 (4)

Where:

Y<sub>ii</sub>: dependent variables;

μ: general mean for all observations;

Ci : effect of the *i*th animal category;

*i*: young or super-young;

Sj : effect of the *j*th sexual condition;

*j*: non-castrated or castrated;

 $C^*S$ : effect of the animal category  $\times$  sexual condition interaction;

 $E_{ii}$ : random error for each observation.

Statistical analysis was performed through the SAS program. Means were compared using the least-squares means (LSMEANS) with a significance level of 5%, and the dependent variables were submitted to correlation analysis.

#### **3 RESULTS**

There was no interaction between category and sexual condition for any fatty acids. The fatty acid contents of C17:0 *iso* and C18:0 were higher for animals slaughtered at 26 months old. The content of lignoceric acid (C24:0) was higher (p≤0.01) in slaughtered animals aged 16 months (Table 3). Regarding sexual condition, a major (p≤0.01) concentration of C24:0 was observed in the meat of bulls.

The monounsaturated fatty acids (MUFAs) with the highest concentration in meat are the C18:1 with its isomers and the 16:1 *cis* 9 *n*-7, representing an average of 96.96% of the total MUFA in this study. Bulls had a higher content of C18:1 *trans* 10, C18: 1 *cis* 11, C18:1 *cis* 12, and C22:1 (Table 4). Animals slaughtered at 16 months showed higher C18:1 *cis* 11, C18:1 *cis* 12, C18:1 *cis* 15, and lower C18:1 *trans* 16 than those slaughtered at 26 months.

Arachidonic (C20:4) and eicosapentaenoic (C22:5) fatty acids were affected ( $p<0.01$ ) by slaughter age. The content of these compounds decreased when the slaughter age of the animals was prolonged (Table 5). There was no effect (p>0.05) of





a, b: different letters in the same effect represent significant differences (p<0.05).

<b>Fatty acid</b>	Slaughter age				<b>Sexual condition</b>	
	16 months	26 months	<b>SEM</b>	<b>Steers</b>	<b>Bulls</b>	<b>SEM</b>
C12:1	0.007	0.004	0.001	0.003	0.008	0.001
C14:1 cis 9	0.765	0.687	0.072	0.708	0.744	0.073
C16:1 $cis$ 9 (n-7)	4.170	4.120	0.218	4.203	4.086	0.219
C17:1	0.527	0.501	0.017	0.513	0.514	0.016
$C18:1$ trans 10	0.622	0.902	0.101	0.599 <sup>b</sup>	$0.926^{\circ}$	0.101
C18:1 trans 16	0.090 <sup>b</sup>	$0.142^{\rm a}$	0.012	0.114	0.118	0.012
C18:1 cis 9	39.926	39.457	0.873	40.004	39.379	0,876
C18:1 cis 11	$1.696^{\circ}$	1.390 <sup>b</sup>	0.065	1.417 <sup>b</sup>	1.669a	0.068
C18:1 cis 12	$0.133^{a}$	0.073 <sup>b</sup>	0.010	0.073 <sup>b</sup>	$0.134$ <sup>a</sup>	0.010
C18:1 cis 13	0.236	0.229	0.025	0.231	0.234	0.024
C18:1 cis 15	$0.085^{\rm b}$	$0.110^a$	0.005	0.102	0.093	0.005
C22:1	0.842	0.321	0.179	0.223 <sup>b</sup>	$0.941$ <sup>a</sup>	0.186

**Table 4**. Composition of MUFAs of the intramuscular fat of steers and bulls finished in feedlot according to category and sexual condition.

a, b: different letters in the same effect represent significant differences (p<0.05).





a, b: different letters in the same effect represent significant differences (p<0.05).

slaughter age and sexual condition on conjugated linoleic acid (cis9-trans11, CLA) in the muscle.

We did not observe any difference (p>0.05) in the total saturated, unsaturated, monounsaturated, and PUFAs, as their relations, for slaughter age (Table 6). Castration did not influence the sum of saturated, unsaturated, and MUFAs. On the contrary, the sum of PUFAs was higher in the meat of bulls than in the meat of steers.

The unsaturated/saturated (UFA/SFA) ratio was not influenced (p>0.05) by castration. The polyunsaturated/saturated (PUFA/SFA) ratio was higher in the meat of bulls due to these animals' higher content of PUFAs.

The human health indices (AI, TI, and HH ratios) were not influenced by slaughter age and sexual condition of the animals.

## **4 DISCUSSION**

SFAs are associated with increased obesity and cardiovascular disease, leading the medical community to recommend increasing the consumption of PUFAs. French et al. (2000) reported that the most undesirable fatty acid would be C14:0, which was on average 6.48% of the total SFA in this study. The C16:0 was mentioned as having the lowest hypercholesterolemic effect, and it had a share of 57.49% of the total SFA. The C18:0 corresponded to 32.17% of the total SFA in meat, having a null effect or even reducing cholesterol levels since, in the organism, it immediately transforms into oleic acid (C18:1 ω-9) (Kim et al., 2016). Therefore, it makes no sense to consider the sum of these three fatty acids, as it is usually done to limit beef in the diet.

Wang et al. (2021) found that the increase in age decreased the contents of C15:0, C18:0, and the sum of SFA and increased the proportions of C14:1 and C16:1. Regarding these fatty acids



**Table 6**. Total composition and relationships between fatty acids of the intramuscular fat of steers and bulls finished in feedlot according to category and sexual condition.

a, b: different letters in the same effect represent significant differences (P<0.05). HH: hypocholesterolemic/hypercholesterolemic ratio. Saturated: C10:0 + C12:0 + C13:0 *ant* + C14:0 + C14:0 iso + C15:0 iso + C15:0 ant + C15:0 + C16:0 iso + C16:0 + C16:0 + C17:0 iso + C17:0 + C18:0 + C24:0. Unsaturated: C12:1 + C14:1 cis 9 + C16:1 cis 9 (n-7) + C17:1 + C18:1 trans 10 + C18:1 trans 16 + C18:1 cis 9 + C18:1 cis 11 + C18:1 cis 12 + C18:1 cis 13 + C18:1 cis 15 + C22:1 + C18:2 trans 11 cis 15 + C18:2 cis 9,12 + C18:2 cis 9 trans 11 + C18:3 (n-3)  $\omega$ -3 + C20:3 + C20:4 + C20: 5 + C22:5. Monounsaturated (MUFA): C12:1 + C14:1 cis 9 + C16:1 cis 9 (n-7) + C17:1 + C18:1 trans 10 + C18:1 trans 16 + C18:1 cis 9 + C18:1 cis 1 + C18:1 cis 12 + C18:1 cis 12 + C18:1 cis 12 + C18:1 cis 13 + C18:1 *cis* 15 + C22:1. Polyunsaturated (PUFA): C18:2 *trans* 11 *cis* 15 + C18:2 *cis* 9,12 + C18:2 *cis* 9 *trans* 11 + C18:3 (*n*-3) ω-3 + C20:3 + C20:4 + C20:5 + C22:5.

in our study, only C18:0 showed an increase in its content with increasing slaughter age. Lengyel et al. (2003) reported that several studies have demonstrated the effect of increasing age on the increase in the sum of SFAs and decrease in polyunsaturated acids. However, these authors stated that the most significant changes in fatty acid content occur between 7 and 14 months old than at older ages due to two reasons. One of them would be that the proportion of neutral lipids and phospholipids, which influence the fatty acid composition of intramuscular fat (Zembayashi et al., 1995), shows different patterns at different ages. However, phospholipids are believed to be a small and constant proportion regardless of sex, breed, or age conditions (Malau-Aduli et al., 2000). Therefore, the difference in diet could provide another explanation for the change in fatty acid profile. During the feedlot period, the animals received the same type of feed. However, prior to the experiment, younger animals received a more energetic diet from weaning until the beginning of the feedlot. In contrast, the older ones received a diet with more fiber. Although it was not measured, according to Lengyel et al. (2003), who observed changes in the fatty acid profile in very young phases of animals, the fatty acid profile at the beginning of the finishing period could already be different.

According to Nürnberg et al. (1998), the amount of adipose tissue increases with age, causing changes such as the rapid growth of the diameter of the adipose tissue cell until 12 months old. After this period, a slight growth reduction occurs until 2 years old. The growth rate of adipocytes decreases over time, increasing the relative importance of lipid droplets to the membrane, where unsaturated fatty acids are concentrated (Duckett et al., 1993). Thus, the fatty acid profile becomes less unsaturated over time since PUFAs are related to the fraction of phospholipids, which also reduces over time (Duckett et al., 1993). In our study, the age difference between animals was probably not enough to cause changes in both adipocyte growth and lipid droplet deposition. Therefore, it did not affect the fatty acid profile.

Diets with high amounts of MUFAs, such as C18:1, have been shown to reduce plasma concentrations of cholesterol, LDL-cholesterol, and triacylglycerol in humans (Kris-Etherton et al., 1999). In addition, replacing SFA with *cis* unsaturated fatty acids, such as 18:1, can reduce the risk of coronary artery disease in humans (Mensink et al., 2003). The reduction in slaughter age provided meat with more C18:1 *cis* 11 and C18:1 *cis* 12 and less C18:1 *trans* 16 and C18:1 *cis* 15, so the sum of MUFA was not affected by the slaughter age of the animals.

Bulls showed higher PUFAs C18:2 *cis* 9,12 and C18:3 (*n*-3), which is reflected in the higher content of total PUFAs. Nian et al. (2018) also observed higher PUFA in bulls due to low intramuscular fat content. There was a negative relationship between PUFA and intramuscular fat, which was also observed by Sami et al. (2006). According to these authors, this negative relationship occurs due to the dilution of a relatively constant amount of phospholipids by increasing triglycerides with increasing marbling, reducing the amount of phospholipids extracted from the structural components of PUFA-rich muscle cells.

Generally, the percentage of SFA and PUFA is negatively related because PUFA is preferentially deposited in phospholipids, while SFA and MUFA are in neutral lipids, present mainly in triacylglycerols, and contain less than 4% of PUFA (Nian et al., 2018). Eichhorn et al. (1985) already pointed out that bulls have a lower triacylglycerol/phospholipid ratio and consequently a higher relative proportion of PUFA. This is because bulls have leaner muscle tissue and lower marbling than steers, which was verified in the present study.

Wood et al. (2008) reported that the amount of fatty acid C18:2 *n*-6 decreases with increased fat deposition because the phospholipid content decreases as muscle lipid content increases, while the proportion of lipids with a higher SFA and MUFA content increases. Steers presented a higher percentage of carcass fat and marbling content than bulls (Kuss et al., 2009). In humans, fatty acids C18:2 *n*-6 and C18:3 *n*-3 are necessary

to maintain cell membranes, brain functions, and the transmission of nerve impulses, and they are classified as essential for the organism.

Similar to our study, Nian et al. (2018) observed a higher proportion of linoleic acid (C18:2n6c) in bulls. These authors explain that the decrease in ruminal biohydrogenation is because of possible modifications in the ruminal microbial ecosystem due to behavioral dietary differences modifying intake, passage rate, salivary secretion, motility, or ruminal volume, indicating that all these factors should be better investigated.

According to Kim et al. (2016), CLA has properties that reduce the risk of breast tumors and inhibit the induction of skin tumors due to its anticarcinogenic properties, reduction of body fat, and prevention of diabetes. Thus, there is a constant search for an increase in this fatty acid in the meat of ruminants. However, our study did not observe any effect of age or sexual condition on CLA content. De la Torre et al. (2006) reported that factors intrinsic to animals, such as breed, sex, and age, can influence CLA content in ruminant animal products. These authors observed that the rate of CLA deposition does not depend on the final amount of body fat. However, it is influenced by other factors such as animal age, which was not observed in the present study, and the diet in particular.

The SFA is related to a higher amount of LDL in the blood, leading to hypercholesterolemia, which increases the lipid concentration in the blood. On the contrary, MUFA, such as 18:1c9, and PUFA, such as 18:2 *n*-6, 18:3 *n*-3, and 18:3 *n*-6, increase the number of hepatic LDL receptors and thus decrease LDL production, which consequently reduces circulating LDL and contributes to the improvement of human health, with reduction of cardiovascular diseases (Scollan et al., 2014). Regarding these fatty acids, only C18:3 *n*-3 showed a significant difference, in which bulls had higher values in the meat. This is good news for the Brazilian consumer since most of the animals slaughtered in Brazil are noncastrated. The *n*-3 fatty acids have been identified as potential benefits for health, such as the prevention of cardiovascular diseases, stimulation of the development of the cerebral cortex, and children's cognitive ability (Oppedisano et al., 2020).

As for nutritional indices, the AI) and TI can better characterize the health properties of foods of animal or plant origin than a simple approach based on the total SFA content or the PUFA/SFA ratio (Fehily et al., 1994). AI and TI relate hypercholesterolemic fatty acids (C12:0, C14:0, C16:0, and C18:0) with the sum of unsaturated fatty acids, which are antiatherogenic and antithrombogenic. Our results show that age and castration do not influence these characteristics. The ratio of HH fatty acids is calculated according to the knowledge of the effects of individual fatty acids on cholesterol metabolism (Dietschy, 1998).

For meat products, the value of 2 for the HH ratio was referenced (Silva et al., 2022). Values higher than 2 correspond to meats of superior nutritional value with an abundance of fatty acids that promote a reduction in plasma cholesterol (hypocholesterolemia) and, thus, a decrease in the risk of cardiovascular diseases (Zárate et al., 2016). The meat of our animals presented an HH ratio lower than recommended, regardless of age and sexual condition. This may have happened due to the feeding system that the animals were submitted (feedlot). Silva et al. (2022) observed that the meat of grass-fed buffaloes was healthier than that of animals finished in feedlot.

# **5 CONCLUSION**

Reducing the slaughter age from 26 to 16 months does not significantly influence the fatty acid profile of intramuscular fat.

The castration of cattle reduces the total PUFAs and the polyunsaturated/saturated ratio. Despite this, the nutritional index, AI, and TI were not influenced by the sexual condition of the animals.

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