













# Fatty acids profile, atherogenic and thrombogenic health lipid indices in the meat of lambs that received canola grain

## *Perfil de ácidos graxos, índices de lipídios aterogênicos e trombogênicos em carne de cordeiros alimentados com grãos de canola*

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### ABSTRACT

This study evaluated the fatty acid composition and qualitative characteristics of meat from lambs fed in feedlot with increasing levels of canola grain. Meat obtained from 27 lambs fed 0, 8 and 16% inclusion of canola grain were analyzed. There was a reduction in the content of saturated fatty acids (SFA): lauric, heptadecanoic, and stearic; and unsaturated fatty acids (UFA): palmitoleic, oleic, and eicosatrienoic, with the increasing levels of canola grain. There was a linear reduction for the  $\omega 3$  acid and the  $\omega 3$ :  $\omega 6$  ratio, while the  $\omega 6$ :  $\omega 3$  ratio increased. But the values observed for this ratio ( $\omega 6$ :  $\omega 3$ ) were lower than 4, which is considered a satisfactory value. There was a slight increase (~2%) for the thrombogenicity index and atherogenicity index with the inclusion of canola grain. There was a reduction in the hypocholesterolemic: hypercholesterolemic ratio, with mean values of 2.09 for the diet with 0% inclusion, and 2.06 for 8 and 16% inclusion of canola. Although the inclusion of canola grain for lambs decreased the content of some UFA, reflecting alterations in the correlated nutritional properties, there is a reduction of SFA. The results for instrumental analysis, proximate composition, and sensory acceptance of the lamb meat were similar among the treatments.

**Keywords:** Hypercholesterolemic fatty acids. Hypocholesterolemic fatty acids. Meat quality. Unsaturated fatty acids. Oilseeds.

### RESUMO

Objetivou-se avaliar a composição dos ácidos graxos e as características qualitativas da carne de cordeiros terminados com níveis crescentes de canola grão na dieta. Foram estudadas amostras de carne provenientes de 27 cordeiros, confinados por 45 dias e alimentados com 00, 08 e 16% de inclusão de canola grão. Houve efeito para os ácidos graxos saturados (AGS): laurico, heptadecanoico e esteárico; ácidos graxos monoinsaturados (AGMI): palmitoleico e oleico; e ácidos graxos polinsaturados (AGPI): eicosatrienoico. Estes ácidos reduziram à medida que os níveis da canola grão aumentaram. Para os AGMI, houve efeito com maior concentração no músculo dos cordeiros alimentados sem a canola grão (49,80%). Houve uma redução linear para o ácido  $\omega 3$  e para a relação  $\omega 3$ : $\omega 6$ , enquanto que a relação  $\omega 6$ : $\omega 3$  aumentou. A canola grão influenciou o índice de aterogenicidade, com média de 0,57 para o tratamento com 0% de inclusão e 0,58 para os tratamentos com 8% e 16% de inclusão. Observou-se aumento linear para o índice de trombogenicidade com a inclusão da canola grão, cujas médias foram, respectivamente, 0,16, 0,17 e 0,18 para os tratamentos 0%, 8% e 16% de inclusão. Houve redução para a relação hipocolesterolêmicos-hipercolesterolêmicos, com médias de 2,09 para a dieta com 0% de inclusão, e 2,06 para 8% e 16% de inclusão da canola em grão na dieta. A canola grão não influenciou a análise instrumental, composição centesimal e atributos sensoriais da carne.

**Palavras-chave:** Ácidos graxos hipercolesterolêmicos. Ácidos graxos hipocolesterolêmicos. Ácidos graxos insaturados. Qualidade de carne. Oleaginosas.

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## Introduction

The consumer market values carcasses of young animals because, under these conditions, the product will have higher quality and probably bring health benefits. The feedlot finishing of lambs can provide early slaughter, resulting in carcasses that meet market requirements, ensuring a faster return on invested capital. However, this system often presents unfavorable economic balance, mainly due to the food, responsible for much of the production costs. Therefore, the replacement of traditional foods with alternative sources is extremely important when the goal is to reduce the production cost (Yamamoto et al., 2013).

Ingredients and diet composition influence the fatty acid composition of meat from ruminants because the fatty acids that reach the duodenum are, at least in part, of dietary origin as well as the result of rumen microbial biohydrogenation of lipids (Buccioni et al., 2012). The type of fat added can influence the unsaturated fatty acid biohydrogenation in the rumen and fatty acid profiles in ruminant tissues (Kišidayová et al., 2014)

Canola is rich in unsaturated fatty acids, such as oleic (C18: 1), linoleic (C18: 2 $\omega$ 6), and linolenic (C18: 3 $\omega$ 3) acids (Wada et al., 2008). Polyunsaturated fatty acids (PUFA) are called *good fats*, because they are important to every cell in the human body, and perform essential functions in the body. There are two main groups of PUFA: omega-3 ( $\omega$ 3) and omega-6 ( $\omega$ 6). Both are considered essential as a human cannot synthesize them, and without them, the body does not function properly. Other polyunsaturated fats also have important functions, but they are not considered essential because the organisms of most people can produce them

from linoleic and linolenic acids (Saldanha & Gonzales, 2012).

However, the increase of some  $\omega$ 6 or  $\omega$ 3 acids, or the change in the ratio between them, can trigger the production of thromboxanes and leukotrienes, which, in excess, is associated with diseases such as thrombosis, arrhythmias, arthritis, asthma, and psoriasis (Rocha, 2008). PUFA may promote or prevent the onset of atherosclerosis and coronary thrombosis, based on their effects on serum cholesterol and low-density lipoprotein cholesterol. Therefore, the evaluation of the fatty acid (FA) profile is extremely important. Based on scientific studies, indices were developed to help in the nutritional assessment of food, highlighting the indices of atherogenicity and thrombogenicity, both related to the lipid fraction (Ulbricht & Southgate, 1991), and blood cholesterol levels, which is related to the cardiovascular diseases in the human population (Oliveira et al., 2008).

Typically, the ratio of polyunsaturated and saturated fatty acids is used as a major index to evaluate the nutritional value of the fat (Bentes et al., 2009; Caldeira et al., 2010). However, this ratio is based on the chemical structure of FA and may not be the best way for this purpose, since it considers that all saturated fatty acids induce an increase in cholesterol and ignores the effects of monounsaturated fatty acids. Thus, the best method to assess the nutritional value of fat is the use of ratios based on the functional effects of FA, such as, for example, the ratio of hypocholesterolemic and hypercholesterolemic fatty acids (Bruker Advance III 700 MHz, Billerica, MA, USA h: H) (Caldeira et al., 2010).

In addition, other characteristics of meat quality traits can be affected by the type of feed administered to animals, such as color, water-holding capacity, pH, composition, and tenderness (Quiñones et al., 2019). The inclusion of canola meal did not affect the meat quality of Mutton Merino lambs and pigs, respectively (Little et al., 2015; Sekali et al., 2016). The production system can alter the FA composition, especially PUFA, that affects meat sensory properties (Sañudo et al., 2000). However, works about the effects of diets with canola grains inclusion in the lamb meat properties are scarce, especially about sensorial attributes.

Based on the context, this study aimed to evaluate the quality traits, fatty acid profile, and sensory properties of meat from feedlot lambs receiving canola grain in the diet.

## Material and Methods

The experiment was conducted in the Ovine Sector of the Experimental Station in the Animal Nutrition Laboratory and Meat Technology Laboratory, Federal University of Grande Dourados/UFGD, and at the Laboratory for

the evaluation of oilseed by-products, at the Center of Research Laboratories in Agroenergy and Environmental Conservation (LAPAC/FINEP), located in the municipality of Dourados, state of Mato Grosso do Sul, between October and December 2012. This experiment was conducted following the rules of the Ethics Committee on Animal Experimentation in this institution, according to the opinion 021/2012 - CEUA/UFGD.

Whole Santa Ines male sheep (n=27) with an initial weight of  $19.33 \pm 1.39$  kg and age of 5 months were randomly and individually housed and identified. Then, animals were subjected to 14 days of adaptation to the diet, management, and facilities for later data collection.

The treatments consisted of three levels of inclusion of canola grain, on a dry matter basis (% DM): 00, 08, and 16%. Diet was weighed and supplied as a complete feed. The mixture was made at the time of supply (silage + concentrate), individually *ad libitum*, in two daily meals (08:00 h am and 16:00 h), allowing 10% leftovers of the amount provided by the calculated margin of the previous day and water *ad libitum*. Corn silage was the forage used and the concentrates were balanced according to NRC (National Research Council, 2007) to be isonitrogenous (Table 1) to provide gains of 200 g/day. The forage: concentrate ratio was 10:90 in DM.

The length of the experimental period (45 days), and the criterion for slaughtering animals, were defined by the time required to reach body condition three (3.0), on a scale of one (1.0) to five (5.0), for standardization of the degree of carcass finishing, as described by Osório et al. (2012). Lamb weight was determined at the beginning (initial body weight-BWi), every 14 days, and at the end

(final body weight (BWf) of the experimental period. Body score was determined during animal weighing by palpation of the lumbar region and insertion of the tail. The final body weight was  $26.46 \pm 1.6$  kg and carcass weight of  $11.87 \pm 0.72$  kg.

A total of 27 left hind limbs was used. With the aid of a scalpel, *Semimembranosus* muscles were separated for instrumental analysis and fatty acid profile analysis. *Semitendinosus* muscles were used for proximate composition analysis and *Biceps femoris* muscles for sensory properties. All the samples were frozen at  $-20^{\circ}\text{C}$ . The steaks were vacuum packaged and transferred to the lab.

The pH and temperature (T  $^{\circ}\text{C}$ ) were determined immediately after slaughter (pH and T $^{\circ}\text{C}$  45 min) using a portable digital pH meter (Testo 205), with the introduction of the electrode directly into the *Semimembranosus* muscle. Carcasses were transferred to cold storage at  $4^{\circ}\text{C}$  for 24 h, and pH and T $^{\circ}\text{C}$  24 h were again determined.

For proximate composition analysis, the *Semitendinosus* muscle was homogenized in a food processor to obtain a homogeneous mass. The crude protein (CP) (# 990.03), total lipids (#945.16), moisture (#935.29), and ash (#942.05) were determined according to the Association of Official Analytical Chemists (2000).

On the day of the evaluation, the samples were thawed in the refrigerator ( $10^{\circ}\text{C}$ ) and cut into 2.5 cm-thick steaks using a standardized scale. Muscle color was determined by using a Minolta Chroma Meter CR-400 calibrated to a white tile standard, using the CIE system (L\*, a\*, b\*), in which L\* is brightness, a\* is redness and b\* is yellowness. After color evaluation, 2.0 g of sample was taken and subjected to a weight of  $25 \text{ kg min}^{-1}$  for determining the water holding capacity (WHC), according to the methodology described by Cañeque & Sañudo (2000).

Cooking loss was determined using an electric oven (Philco) preheated at  $170^{\circ}\text{C}$ . Raw meat samples were weighed and placed on trays with an iron grate. They were then transferred to an oven, where they remained until the internal temperature of the sample center was  $75^{\circ}\text{C}$ , this being determined temperature with a portable skewer type digital thermometer (Akso). After cooling to room temperature, samples were again weighed to calculate the percentage of loss during cooking. The shear force of the *Semimembranosus* was determined using five cylinders of 1.3 cm in diameter in the longitudinal direction of muscle fibers, and subjected to cutting in the transverse direction of the muscle fibers, using a Warner-Bratzler blade coupled to a Texture Analyzer TA-XT2i. The values were expressed in kilogram-force (kgf).

The fatty acid profile of canola grain (Table 2) and lamb meat was determined according to the recommendations of

Table 1 – Proximate and chemical composition of experimental diets, on a dry matter basis

Ingredient (%)	Level of canola grain (%)		
	00	08	16
Corn silage	10.00	10.00	10.00
Corn grain	67.73	63.44	59.15
Soybean meal	20.27	16.56	12.85
Canola grain	0.00	8.00	16.00
Mineral mix <sup>1</sup>	2.00	2.00	2.00
<b>Chemical composition (%)</b>			
Dry matter	78.87	79.44	79.27
Crude protein	16.69	15.82	16.52
Ether extract	3.49	6.18	8.87
Neutral detergent fiber	42.26	34.10	42.88
Acid detergent fiber	10.75	12.51	11.56
Mineral matter	4.73	4.34	4.82
Total carbohydrates	75.09	73.66	69.79
Total digestible nutrients	66.87	71.53	66.51

<sup>1</sup>Mineral Supplement (nutrients per kilogram of product): phosphorus 80g; calcium 140g; magnesium 7g; sulfur 12g; sodium 133g; 4,200 mg zinc; 300 mg copper; 800 mg manganese; 1500 mg iron; 100 mg cobalt; 150 mg iodine; selenium 15 mg; fluoride (max) 800 mg, phosphorus solubility of citric acid 2% (min) 95%.



Folch et al. (1957). The lipids were extracted from lamb meat samples using a mixture of chloroform-methanol (2:1, v/v). Transesterification of triglyceride acids was achieved using a solution of n-heptane and KOH/methanol. The grease material (200 mg) was transferred to a 10 mL test tube with a screw lid, to which 2.0 mL of n-heptane was added. The material was agitated until complete solubilization of the fatty matter. Then, 2.0 mL of 2 mol/L KOH in methanol were added and the solution was mixed vigorously for 5 min. The test tubes were tightly closed, protected from light, and stored at -18°C for further chromatographic analysis.

Fatty acid analyses were carried out a gas chromatography using a flame ionization detector gas chromatograph. A fused silica capillary column of 100 m x 0.25 mm x 0.20 µm was used for elution. The oven temperature was programmed as follows: initial temperature 100°C, initial hold, 1 min, and then raised to 170°C at 6.5°C min<sup>-1</sup>. Subsequently, there was another rise from 170 to 215°C at 2.7°C min<sup>-1</sup> and hold for 30 min. Finally, there was a rise from 215 to 230°C at 4°C min<sup>-1</sup>. The temperatures of the injector and detector were 270 and 280°C, respectively. Samples of 0.5 µL were injected in split mode using nitrogen as a carrier gas at a drift velocity of 1 mL min<sup>-1</sup>. Chromatograph peaks of fatty acids were identified by comparison with retention time using a mixture of Sigma (St Louis, MO, USA) standards,

and the nonadecanoic acid (19:0) was used as an internal standard. Quantification of fatty acids was performed using correction factors for peak areas and internal standard-based calculations, and the results were expressed in mg/g of tissue.

We also determined the atherogenicity index (AI) = [(C12:0+(4×C14:0)+C16:0)]/(Σ AGMI+Σ ω6+Σ ω3) and the thrombogenicity index (TI) = (C14:0+C16:0+C18:0)/[(0,5×Σ AGMI)+(0,5×Σ ω6+(3×Σ ω3)+(Σ ω3/Σ ω6)] according to Ulbricht & Southgate (1991), and the ratio of hypocholesterolemic and hypercholesterolemic fatty acids (h:H) = (C18:1cis9+C18:2ω6+20:4ω6+C18:3ω3+C20:5ω3+C22:5ω3+C22:6ω3)/(C14:0+C16:0) by the method of Santos-Silva et al. (2002).

Sensory evaluations were conducted in the muscle *Biceps femoris* (devoid of subcutaneous fat), which was roasted at 170°C, until reaching an internal of 75°C and then rested at room temperature for 10 min. According to the methodology described by Campo (2005), 80 untrained tasters of different ages, sheep meat lovers, were invited to participate in the study. Samples were placed by the different treatments on plates identified with tags, but so that the tasters did not know the treatments. In the descriptive test, we applied a 5 point-hedonic scale considering the attributes odor, flavor, tenderness, overall evaluation, and purchase intent. The five points of the scale were: 1 – like extremely; 2 - like moderately; 3 – neither like nor dislike; 4 - dislike moderately and 5 - dislike extremely.

All statistical analyses were run using PROC UNIVARIATE and PROC MIXED of Statistical Analysis System 9.2, at 0.05 probability. The statistical design was completely randomized (DIC). The statistical model is shown below:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij} \quad (1)$$

in which  $Y_{ij}$  represents the observation of canola grain level  $i$  in animal  $j$ ;  $\alpha_i$  represents the fixed effect of canola grain  $i$  ( $i = 1, 2, 3$ ), and  $\epsilon_{ij}$  represents the random error.

Sensory data were analyzed using a Kruskal-Wallis nonparametric test, at 0.05 probability.

## Results and Discussion

The inclusion of canola grain in the diet did not influence the initial and final pH, initial and final temperature, cooking loss, shear force, water holding capacity, lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) (Table 3). The same effect was reported by Smeti et al. (2018), which investigated the effect of dose and administration form of rosemary essential oils on lamb meat quality.

The initial (45 min) and final (24 h) pH values of the leg were 6.78 and 5.76, respectively (Table 3). This result is due to the nutritional status of animals and because slaughtering

Table 2 – Lipid profile of canola grain

Fatty acid	Canola grain (%)
C8:0 (Caprylic)	0.438
C10:0 (Capric)	0.227
C11:0 (Undecanoic)	0.002
C14:0 (Myristic)	2.466
C16:0 (Palmitic)	18.524
C17:0 (Heptadecanoic)	2.304
C18:0 (Stearic)	27.818
C20:0 (Arachidic)	1.101
C22:0 (Benic)	0.029
C24:0 (Lignoceric)	0.516
C15:1 (Pentadecenoic)	0.002
C16:1 (Palmitoleic)	3.262
C17:1 (Cis-10-Heptadecanoic)	0.013
C18:1 (Oleic)	26.868
C22:1 ω9 (Erucic)	6.265
C18:2 ω6t (Linolelaidic)	1.191
C18:2 ω6c (Linoleic)	8.918
C18:3 ω6 (γ-linolenic)	0.002
C20:3 ω6 (Dihomo-γ-linolenic)	0.008
C20:3 ω3 (Eicosatrienoic)	0.014
C20:4 ω6 (Arachidonic)	0.029
C20:5 ω3 (Icosapentaenoic)	0.002
C22:6 ω3 (Docosahexaenoic)	0.001

Table 3 – Fatty acid profile in the *Semimembranosus* muscle of lambs finished with increasing levels of canola grain in the diet

Fatty acid	Level of canola grain (%)			SEM	P-valor
	00	08	16		
<b>Saturated fatty acids</b>					
C10:0 (Capric)	0.12	0.11	0.11	0.0025	0.7900
C12:0 (Lauric)	0.12	0.12	0.11	0.0020	0.0039* <sup>1</sup>
C14:0 (Myristic)	2.19	2.22	2.21	0.0120	0.3255
C15:0 (Pentadecanoic)	0.23	0.21	0.23	0.0069	0.4535
C16:0 (Palmitic)	22.83	23.09	23.02	0.0342	0.6545
C17:0 (Heptadecanoic)	1.66	1.61	1.60	0.0077	0.0005* <sup>2</sup>
C18:0 (Stearic)	15.23	15.22	15.11	0.0190	0.0051* <sup>3</sup>
C20:0 (Arachidic)	0.11	0.11	0.11	0.0021	0.2345
<b>Monounsaturated fatty acids</b>					
C14:1 (Myristoleic)	0.10	0.11	0.11	0.0022	0.4553
C16:1 (Palmitoleic)	1.69	1.66	1.63	0.0088	0.0071* <sup>4</sup>
C18:1 (Oleic)	47.90	47.88	47.74	0.0323	0.0387* <sup>5</sup>
C20:1 (Eicosenoic)	0.11	0.10	0.10	0.0015	0.1343
<b>Polyunsaturated fatty acids</b>					
C18:2 ω6 (Linoleic)	3.98	3.99	3.97	0.0199	0.1603
C18:3 ω3 (α-linolenic)	0.20	0.19	0.20	0.0040	0.2904
C18:2 CLA (cis9-trans 11)	0.50	0.50	0.54	0.0113	0.7762
C20:2 (Eicosadienoic)	0.10	0.11	0.11	0.0012	0.1232
C20:3 ω3 (Eicosatrienoic)	1.51	1.46	1.33	0.0200	0.0000* <sup>6</sup>
C20:3 ω6 (Dihomo-γ-linolenic)	0.11	0.11	0.10	0.0016	0.1602
C20:4 (Arachidonic)	0.21	0.19	0.22	0.0083	0.1000
C20:5 ω3 (Eicosapentaenoic)	0.11	0.11	0.11	0.0029	0.1570
Saturated	42.48	42.68	42.50	0.0321	0.2343
Monounsaturated	49.80	49.75	49.58	0.0343	0.0059* <sup>7</sup>
Polyunsaturated	6.71	6.63	6.58	0.0360	0.3343
Conjugated linoleic acid	0.50	0.50	0.54	0.0113	0.1342
Omega 3	1.81	1.75	1.64	0.0193	0.0000* <sup>8</sup>
Omega 6	4.09	4.10	4.07	0.0203	0.3453
Omega 6: Omega 3	2.26	2.34	2.48	0.0287	0.0002* <sup>9</sup>
Polyunsaturated: Saturated	0.16	0.16	0.15	0.0009	0.1601
Atherogenicity index	0.57	0.58	0.58	0.0055	0.0187* <sup>10</sup>
Thrombogenicity index	1.16	1.17	1.18	0.0075	0.0001* <sup>11</sup>
h:H	2.09	2.06	2.06	0.0139	0.0097* <sup>12</sup>

\* = significant at 5% probability; SEM = standard error of the mean.

<sup>1</sup>Y = 0.128974 - 0.00678205X (r<sup>2</sup> = 0.46); <sup>2</sup>Y = 1.68051 - 0.0293590X (r<sup>2</sup> = 0.59); <sup>3</sup>Y = 15.3129 - 0.0625641X (r<sup>2</sup> = 0.44); <sup>4</sup>Y = 1.71744 - 0.0282051X (r<sup>2</sup> = 0.41); <sup>5</sup>Y = 48.0083 - 0.0833333X (r<sup>2</sup> = 0.27); <sup>6</sup>Y = 1.61538 - 0.0907692X (r<sup>2</sup> = 0.84); <sup>7</sup>Y = 49.9340 - 0.111282X (r<sup>2</sup> = 0.43); <sup>8</sup>Y = 1.90718 - 0.0860256X (r<sup>2</sup> = 0.81); <sup>9</sup>Y = 2.13098 + 0.114068X (r<sup>2</sup> = 0.64); <sup>10</sup>Y = 0.465651 - 0.0204129X (r<sup>2</sup> = 0.64); <sup>11</sup>Y = 0.56 + 0.0039X (r<sup>2</sup> = 0.75); <sup>12</sup>Y = 1.15 + 0.0078X (r<sup>2</sup> = 0.67); <sup>12</sup>Y = 2.09 - 0.01086X (r<sup>2</sup> = 0.75).

was carried out at the place of the experiment. This greatly reduced the pre-slaughter stress, an important factor for the final pH. The values for final pH were close to the reported by Ripoll et al. (2012), 5.6, which is considered acceptable for lamb meat. For Bouton et al. (1971), pH is one of the

main factors that act in the conversion of muscle into meat and plays a key role in food safety and meat quality.

Initial (45 min) and final (24 h) temperatures of the hind limbs were, respectively, 37.50°C and 8.02°C (Table 3). The correct drop in temperature and pH during the cooling process indicates that other quality parameters, such as water holding capacity, cooking loss, shear force, and color, will have satisfactory results, as they are influenced by temperature and pH (Bouton et al., 1971).

The proximate composition of meat from lambs finished with canola grain was not affected, with mean values of 77.17% for moisture; 1.05% for ash; 17.52% for protein; and 3.81% for lipids (Table 3). According to Leão et al. (2012), the proximate composition of lamb meat is approximately 75% moisture, 19% protein, 4% fat, and 1.1% mineral matter and can be influenced by diet. These values are close to those reported herein.

Among the components of meat, water is the major constituent, and its content is inversely proportional to fat (Pinheiro et al., 2012), i.e., the higher the water content, the lower the fat. Santos et al. (2009) evaluated the use of 8% of canola in grain and by-products of canola (rapeseed meal and rapeseed cake) in the total diet of Santa Ines sheep and found no significant differences between treatments for the characteristic chemical composition of the flesh. The average observed for treatment with canola in grain were: 70.95% moisture; 2.40% ash; 16.14% crude protein, and 9.72% fat.

The mean value found for cooking loss was 45.41%, which is higher than that observed by Yamamoto et al. (2013), who worked with sunflower seeds in the diet for lambs and reported a mean value of 37.07% in the *Semimembranosus* muscle. This parameter is important for evaluating meat quality in the preparation for consumption (Costa et al., 2011), being a characteristic influenced by the water holding capacity (WHC) of the meat (Monte et al., 2012).

WHC in the *Semimembranosus* muscle presented a mean value of 68.75%, higher than that recorded by Yamamoto et al. (2013) - 57.76%. The WHC is technically and gastronomically important, as it conserves flavor, improves the cooking process, and is also related to tenderness and juiciness, important properties for consumer acceptance (Quiñones et al., 2019). Also, meats with higher WHC have lower losses of nutrients through exudate, because, along with the water, soluble proteins are lost, as well as lipids, vitamins, and minerals (Pinheiro et al., 2010; Zeola et al., 2007).

The mean values obtained for shear force ranged from 3.47 to 3.49 kgf, indicating that the meat of lambs in this study can be considered tender, according to values presented by Cezar & Souza (2007). This variable can be

influenced by many reasons, for example, level of nutrition, management practices before slaughter, faster onset of rigor mortis, pH at post-mortem, pre-slaughter temperature, onset and extension of glycolysis, used muscle, post-slaughter management, packaging conditions, and methodology for the determinations, such as temperature and time during the cooking process (Bonacina et al., 2011, Mu et al., 2020).

The mean values of lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) were 41.14; 22.97; 9.56, respectively. Pinheiro et al. (2009) worked with sheep of different categories kept on Tifton 85 pasture in a rotational stocking, supplying concentrate daily, and found values of 40.10 for  $L^*$ ; 14.10 for  $a^*$ , and 2.61 for  $b^*$  for the *Semimembranosus* muscle. These values are far from those observed in this study, except for the  $L^*$  value that was similar. Nevertheless, sheep meat usually shows variations from 30.03 to 49.47 for  $L^*$ ; from 8.24 to 23.53 for  $a^*$ , and from 3.38 to 11.10 for  $b^*$  (Sañudo et al., 2000). In this way, the results obtained in this study are within these ranges.

The fatty acid (FA) profile of the *Semimembranosus* muscle of lambs identified 20 FA, including eight saturated fatty acids (SFA), four monounsaturated fatty acids (MUFA), and eight polyunsaturated fatty acids (PUFA) (Table 4). The prevalence of oleic, palmitic, and stearic FA was per the commonly accepted values for lambs (Smeti et al., 2018).

Regarding SFA, there was a linear effect for the lauric acid (C12:0), which was reduced with the inclusion of canola grain. This is probably related to the lipid profile of the grain that did not contain this FA. Lauric (C12:0) and myristic

(C14:0) acids promote hypercholesterolemia and are related to increased low-density lipoprotein (LDL) in blood serum. This results in the incidence of cardiovascular diseases and sequelae, together with increasing levels of serum cholesterol, leading to arteriosclerosis (Lira et al., 2005).

The content of heptadecanoic (C17:0) and stearic (C18:0) acids also decreased with the inclusion of canola grain in the fed diet. According to Gallo et al. (2007), diets with high ether extract content have a lower percentage of C17:0 acid. This may have occurred in this study, because the diets with 0%, 8%, and 16% inclusion canola grain showed increasing concentrations of lipids in the diet, with 3.49%, 6.18%, and 8.87%, respectively, and thus the concentrations of the C17:0 acid in sheep meat reduced. In ruminants, the half-life of free PUFA is relatively short due to their rapid hydrogenation by rumen microbes into the corresponding saturated configuration, having as final product the stearic acid (C18:0), if the biohydrogenation is complete (Buccioni et al., 2012; Kim et al., 2009). However, the reduction presented in the concentration of C18:0 acid indicates that the biohydrogenation of linoleic acid (C18:2) may have been incomplete (Loor et al., 2004). Meanwhile, stearic acid does not influence blood cholesterol levels (Madruga et al., 2006).

The concentration of palmitoleic MUFA (C16:1) was reduced (~3.5%) with canola grain in the fed diet. These acids are responsible for the metabolism of lipids, which may help balance the levels of HDL (good cholesterol) and LDL (bad cholesterol), reduce the blood sugar level, and promote the decrease of fat tissue surrounding the liver and heart (Radmann & Costa, 2008).

Another MUFA that was also decreased (~0.4%) was the oleic acid (C18:1). Even so, it was predominant in the muscle of lambs. In agreement with Sañudo et al. (2000), a high concentration of C18:1 acid in the composition of intramuscular fat of ruminants has been reported in the literature. While the C18:1 acid helps to reduce blood cholesterol level, the palmitic FA (C16:0) is the main acid responsible for the increase in serum cholesterol (Madruga et al., 2006) and may cause a higher incidence of cardiovascular events. However, we didn't find an effect for C16:0 in lamb meat feeding with canola grain.

Among the PUFAs, only eicosatrienoic acid (C20:3 $\omega$ 3) was influenced by canola grain levels, which linearly decreased, as shown in Table 4. This is probably explained by the low concentration of these FA in canola grain (Table 1). Fatty acids with unsaturation in carbon  $\omega$ 3 are considered beneficial to human health (Pelegrini et al., 2007), and some studies report benefits against diseases, such as cancer. It is

Table 4 – Chemical composition and instrumental analyses in the *Semitendinosus* muscle of lambs finished with increasing levels of canola grain in the diet

Variable	Level of canola grain (%)			SEM	Pr > F
	00	08	16		
<b>Chemical composition</b>					
Moisture	74.42	78.03	79.07	1.175	0.1600
Ash	1.17	1.02	0.95	0.056	0.1000
Protein	19.36	17.15	16.04	0.847	0.1340
Lipid	4.44	3.52	3.48	0.445	0.1111
<b>Instrumental Analysis</b>					
Initial pH	6.79	6.72	6.82	0.056	0.1234
Final pH	5.44	5.98	5.83	0.115	0.3458
Initial temperature (T °C)	37.75	38.25	36.58	0.353	0.1777
Final temperature (T °C)	6.88	8.67	8.43	0.660	0.2342
Cooking loss (%)	46.30	44.38	45.55	0.724	0.1222
Shear force (kgf)	3.47	3.49	3.48	0.145	0.1345
Water holding capacity (%)	66.97	71.19	68.10	0.831	0.1777
Lightness ( $L^*$ )	41.12	40.67	41.64	0.678	0.2134
Redness ( $a^*$ )	22.52	22.63	23.76	0.331	0.1334
Yellowness ( $b^*$ )	9.29	9.73	9.65	0.271	0.1335



believed that different mechanisms like transport through the membrane and immune functions contribute alone or in combination to improve the pathological condition (Yang et al., 2013).

In this study, the mean levels of SFA (43.55%) and PUFA (6.64%) were similar among the treatments ( $p>0.05$ ) and lower than those observed by Madruga et al. (2006), which evaluated the fatty acids profile in muscles of Santa Ines lambs and verified values of 44.47% for SFA and 12.33% for PUFA. For MUFA, there was a higher concentration ( $\sim 0.5\%$ ) in the meat of lambs that were not fed with canola grain than the treatments with 8 or 16% of grain inclusion in the diet (Table 4). Lamb meat is rich in saturated and monounsaturated fatty acids, with small amounts of polyunsaturated fatty acids (Leão et al., 2012), confirming the results obtained herein for SFA, MUFA, and PUFA.

There was a linear reduction in  $\omega 3$  acid and  $\omega 3$ :  $\omega 6$  ratio. Hence, the relationship  $\omega 6$ :  $\omega 3$  increased with the inclusion of canola grain (Table 4). The  $\omega 6$ :  $\omega 3$  ratio has been used as one of the criteria for assessing the quality of fat (Bentes et al., 2009), which should be lower than 4 (Pelegrini et al., 2007; Smeti et al., 2018). Thus, this index was considered satisfactory for all the treatments. The high intake of PUFA  $\omega 6$  associated with low intake of PUFA  $\omega 3$  causes physiological changes that trigger pro-inflammatory, pro-thrombotic states with increased vasospasm, vasoconstriction, and blood viscosity, favoring the onset of diseases (Patterson et al., 2012). The  $\omega 6$  and  $\omega 3$  FA have different roles in the human organism. Whereas the metabolic products of  $\omega 6$  FA promote inflammation and tumors,  $\omega 3$  FA acts in the opposite direction. Therefore, it is important to keep a dietary balance between the two types of FA, since they work together, promoting health and organic balance (Oliveira et al., 2013).

Statistical difference was detected for atherogenicity index (AI) (Table 4). The increase in the AI values ( $\sim 2\%$ ) may be due to reduction of MUFA and PUFA  $\omega 3$ , despite the reduction of C12:0 acid with the inclusion levels, since this index indicates the ratio of the sum of the main saturated fatty acids (C12:0, C14:0, and C16:0) and the sum of the main unsaturated (MUFA and PUFA,  $\omega 3$  and  $\omega 6$ ) (Ulbricht & Southgate, 1991). The observed values for AI were 0.57 for the treatment with 0% inclusion and 0.58 for treatments with 8% and 16% inclusion of canola grain. This index remained within the normal range, according to Bobe et al. (2004), who stated that the fat of the meat has values between 0.5 and 1.0.

Additionally, there was a linear increase for the thrombogenicity index (TI), according to the inclusion

levels of canola grain in the diet (Table 4). TI considers myristic (C14:0), palmitic (C16:0), and stearic (18:0) as thrombogenic acids and PUFA  $\omega 6$  and  $\omega 3$  and MUFA, as anti-thrombogenic acids (Ulbricht & Southgate 1991). As previously mentioned, the acids C14:0 decreased with the inclusion of canola grains, as also occurred for  $\omega 3$  and MUFA, thus causing an increase in TI ( $\sim 2\%$ ).

AI and TI indicate the stimulus potential of platelet aggregation. That is, the lower the values of AI and TI, the greater the amount of antiatherogenic FA present in certain fat or oil. Therefore, the higher the platelet aggregation, the greater the potential to prevent coronary heart disease (Turan et al., 2007).

For Santos-Silva et al. (2002), the best way to assess the nutritional value of the fatty acid profile is the use of ratios based on functional effects of FA, for example, the ratio of hypocholesterolemic (h) / hypercholesterolemic (H) FA. This ratio is an index that considers the functional activity of FA on the metabolism of lipoprotein transporting plasma cholesterol, whose type and quantity are related to increased or decreased risk of incidence of cardiovascular disease.

There was a reduction in the hypocholesterolemic: hypercholesterolemic ratio, with mean values of 2.09 for a diet with 0% inclusion, and 2.06 for 8% and 16% inclusion of canola grain in the diet (Table 4). The reference for meat products is the value of 2.0 for the h: H ratio (Santos-Silva et al., 2002). Values higher than 2.0 represent products with a desirable FA composition in the nutritional aspect, as they mostly consist of hypocholesterolemic FA and, thus, reduce the risk of cardiovascular disease (Frota et al., 2010). In this way, despite the reduction ( $\sim 1\%$ ) in the h: H ratio with the inclusion levels of canola grains, the observed values were satisfactory for all the treatments, which were above 2.0.

Regarding the sensory properties, there were no differences ( $p>0.05$ ) for the attributes evaluated, whose mean values were 2.12 for odor; 2.09 for flavor; 1.60 for tenderness, and 1.93 for overall evaluation (Table 5). These values correspond to 'like moderately' in the hedonic scale, which is indicative of good acceptability of the meat, even when the canola grain was added in high amounts in the fed diet. For purchase intention, the mean value ( $\sim 2$ ) corroborates this affirmation, which means that the testers will 'probably buy' the meat products. Sekali et al. (2016) also found that the inclusion of canola meal in the fed diet of Mutton Merino lambs did not affect the sensory acceptance of the meat.

The mean value for tenderness (1.60) in the subjective evaluation (sensory analysis) is per the shear force (3.48 kgf) checked in objective technique (instrumental analysis),

Table 5 – Sensory properties of the *Biceps femoris* muscle of lambs finished with increasing levels of canola grain in the diet

Variable	Level of canola grain (%)			Pr > F
	00	08	16	
Odor	2.72	1.82	1.83	0.1034
Flavor	2.23	2.00	2.03	0.1358
Tenderness	1.41	1.72	1.66	0.1077
Overall evaluation	2.05	1.97	1.76	0.1242
Purchase intent	2.14	2.00	1.90	0.1434

indicating that the addition of canola seed promoted satisfactory sensory characteristics. Sensory analysis is an important tool for assessing meat quality. However, the simultaneous application of instrumental techniques can specify more effectively the acceptance of the product on the market (Monte et al., 2012). Meat tenderness is a major attribute in the overall impression of the consumer and it can be defined as the facility to chew the meat. This parameter may be composed of three sensations perceived by the consumer: an initial sensation of ease of penetration of the meat by the teeth, another sensation, more prolonged, which is the resistance imposed by the meat to rupture during chewing, and the final sensation referring to the amount of residue remaining in the mouth (Monte et al., 2012).

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## Conclusion

Overall, although several differences in fatty acid concentration were found, the absolute changes were rather small and likely related to the high efficiency of PUFA biohydrogenation in the rumen.

## Conflicts of Interest

None.

## Ethics Statement

The research was conducted under the approval of the Ethics Committee on Animal Experimentation (021/2012), of Federal University of Grande Dourados.

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