

TISSUE COMPOSITION, QUALITY TRAITS AND FATTY ACID PROFILE OF *LONGISSIMUS* MUSCLE OF LAMBS FED INCREASING LEVELS OF CRUDE GLYCERIN¹

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ABSTRACT: The objective of this study was to evaluate the tissue composition, quality traits and fatty acid profile of *longissimus* muscle of lambs fed increasing levels of crude glycerin. Twenty-four intact male lambs were allocated in a completely randomized design with four treatments and six replicates. The treatments consisted of the replacement of corn with 0, 2.5, 5.0 and 7.5% crude glycerin (dry matter basis) in the diet. The animals were slaughtered when they reached a body condition score of 2.5 to 3.0. After cooling the carcasses, the meat was removed for the determination of tissue composition and *longissimus* muscle for the evaluation of quality characteristics. The different crude glycerin levels did not affect tissue weight or yield, or the qualitative characteristics of *longissimus* muscle. An increasing linear effect was observed for ether extract. There was a quadratic effect on C17:0 fatty acids, and C18:1 n-9c and C18:3 n-3 fatty acids decreased linearly. A quadratic effect was observed for total unsaturated fatty acids and a linear decreasing effect for n-3 fatty acids. The unsaturated:saturated fatty acid ratio decreased linearly and there was an increasing linear effect on the n6:n3 ratio. Crude glycerin can replace corn without compromising tissue composition and quality traits of *longissimus* muscle. The inclusion of up to 7.5% crude glycerin (dry matter basis) in the diet for Pantaneiro lambs improves the nutritional aspects of fat.

Keywords: glycerol, meat quality, sheep.

COMPOSIÇÃO TECIDUAL, CARACTERÍSTICAS QUALITATIVAS E PERFIL DE ÁCIDOS GRAXOS DO MÚSCULO *LONGISSIMUS* DE CORDEIROS ALIMENTADOS COM TEORES CRESCENTES DE GLICERINA BRUTA

RESUMO: O objetivo foi avaliar a composição tecidual, as características qualitativas e o perfil de ácidos graxos do *Longissimus* de cordeiro alimentados com teores crescentes de glicerina bruta (GB) na dieta. Foram utilizados 24 cordeiros machos, não castrados, distribuídos em delineamento inteiramente casualizado, com quatro tratamentos e seis repetições. Os tratamentos testados foram 0; 2,5; 5,0 e 7,5% de inclusão de GB na matéria seca da dieta em substituição ao milho. Os animais foram abatidos quando atingiram escore de condição corporal entre 2,5 e 3,0. Após o resfriamento das carcaças, o lombo foi retirado e utilizado para a determinação da composição tecidual, e o músculo *Longissimus* utilizado para determinação das características qualitativas. Os diferentes teores de glicerina bruta não influenciaram os pesos e rendimentos dos tecidos, e as características qualitativas do *Longissimus*. O teor de extrato etéreo apresentou efeito linear crescente. Houve efeito quadrático para o ácido graxo C17:0. O ácido graxo C18:1 n-9c e C18:3 n-3 diminuiu linearmente. Houve efeito quadrático para o total de ácidos graxos insaturados e efeito linear decrescente para os ácidos graxos n-3. A relação ácidos graxos insaturados:saturados apresentou efeito linear decrescente e houve efeito linear crescente para a relação n6:n3. A glicerina bruta pode ser utilizada em substituição ao milho na dieta de cordeiros sem ocasionar prejuízos na composição tecidual e características qualitativas do *Longissimus*. A inclusão de glicerina bruta até 7,5% da matéria seca na dieta para cordeiros Pantaneiros melhora os aspectos nutricionais de gordura.

Palavras-chave: glicerol, qualidade da carne, ovinos.

INTRODUCTION

Crude glycerin is generated from biodiesel production. However, this co-product has been a matter of concern for the agroindustry because of its environment pollution potential (PELLEGRIN *et al.*, 2012). Glycerol is the main component of glycerin and has been studied as feed for ruminants to replace energetic compounds (MACH *et al.*, 2009). The fermentation of glycerol increases the levels of propionate in the rumen, which is a precursor of gluconeogenesis. Furthermore, higher fat deposition in meat is observed, affecting quality traits such as juiciness and tenderness (KREHBIEL, 2008).

According to KRUEGER *et al.* (2010), the inclusion of crude glycerin in ruminant diets can modify the fatty acid composition of meat. In an *in vitro* study, the addition of 2% and 20% glycerol inhibited lipolysis by ruminal microorganisms, increasing free fatty acids in the rumen from 48 to 77%. Thus, glycerol supplementation could promote the passage of intact lipids, providing higher levels of unsaturated fatty acids to be incorporated into meat and milk. Studies using crude glycerin found no deleterious effects on the performance of animals and their products (BARROS *et al.*, 2015; LAGE *et al.*, 2014; REGO *et al.*, 2015; RODRIGUES and RONDINA, 2013).

The genetic group of Pantaneiro sheep is adapted to the conditions of the Pantanal, state of Mato Grosso do Sul, Brazil. These animals can be used as a genetic resource to improve meat and milk production in this state (CRISPIM *et al.*, 2013; VARGAS JR. *et al.*, 2014). In this respect, the objective of the present study was to assess the proximate composition, qualitative traits and fatty acid composition of the loin of Pantaneiro lambs fed a diet in which corn was replaced with increasing levels of crude glycerin.

MATERIAL AND METHODS

All procedures were approved by the Ethics Committee on Animal Use of the Federal University of Grande Dourados (Protocol No. 013/2012). The experiment was conducted at the Federal University of Grande Dourados, Dourados, MS, Brazil.

Twenty-four intact male lambs with an average age of 172 days and average final weight of 36.99 ± 2.95 kg were used. The animals belong to a naturalized sheep breed of the state of Mato Grosso do Sul, called "Pantaneiro". The animals were housed in a covered area in individual pens (2 m²)

equipped with a feed bunk and nipple drinker. Prior to the beginning of the experiment, the animals were numbered and weighed and an anthelmintic (1% ivermectin) was administered. The animals were kept confined for 10 days for adaptation to the facilities and diets.

The experiment was conducted in a completely randomized design consisting of four treatments and six replicates. The treatments tested were the replacement of corn grain with increasing levels of crude glycerin (0.0, 2.5, 5.0 and 7.5% of dry matter, DM). The crude glycerin used in this experiment was composed of 39.3% glycerol, 47.3% fatty acids, and 12.1 mg/kg sodium. The diets were formulated to provide an average daily gain of 200 g/day following the nutritional requirements of the NRC (2007). Oat hay was used as forage and the concentrate consisted of ground corn and/or crude glycerin, soybean meal, ground soybean and mineral mix (Table 1). The forage:concentrate ratio was 25:75. The diets were provided to the animals in three daily meals at 08:00, 11:00 and 16:00 h. The food was offered *ad libitum*, allowing 10 to 20% of leftovers.

The body condition score (BCS) was used as a slaughter criterion. The animals were slaughtered when they reached a BCS of 2.5 (slightly lean) to 3.0 (normal) using the BCS system: 1 (excessively lean) to 5 (over-fat) at intervals of 0.5 (OSÓRIO and OSÓRIO, 2005). Prior to slaughter, the animals were fasted from solids for 12 h. The animals were slaughtered according to the guidelines of the Industrial and Sanitary Inspection Regulation of Animal Products - RIISPOA (BRASIL, 1952).

After slaughter, the carcasses were chilled for 24 h at 4°C and the final pH was measured. The carcasses were divided into two half-carcasses. The left half-carcass was divided into eight cuts, adapted from SÁNCHEZ and SÁNCHEZ (1988) and cited by OSÓRIO and OSÓRIO (2005). From these cuts, the tenderloin was obtained from the region between the 13th thoracic and first sacral vertebra. The tenderloin was dissected and used for quantification of tissue composition. The *longissimus* muscle was removed and stored for subsequent qualitative analysis.

The dissection procedure was performed according to the method described by OSÓRIO and OSÓRIO (2005). The following tissues were obtained: subcutaneous fat (located beneath the skin), intermuscular fat (located beneath the deep fascia, associated with muscles), muscle, bone (free from any other tissue), and others (unidentified tissue comprising tendons, glands, nerves, and blood vessels).

Meat color was determined 30 min after cross-

Table 1. Proportion (%) of ingredients and chemical composition of the experimental diets

| Composition | Crude glycerin (% DM) | | | |
|-----------------------------------|-----------------------|-------|-------|-------|
| | 0.0 | 2.5 | 5.0 | 7.5 |
| Ingredients (%DM) | | | | |
| Oat hay | 24.33 | 24.33 | 24.33 | 24.33 |
| Soybean meal | 11.06 | 11.06 | 11.06 | 11.06 |
| Ground soybean | 4.42 | 4.42 | 4.42 | 4.42 |
| Crude glycerin | - | 2.5 | 5.0 | 7.5 |
| Ground corn | 58.62 | 56.12 | 53.62 | 51.12 |
| Limestone | 1.11 | 1.11 | 1.11 | 1.11 |
| Salt | 0.46 | 0.46 | 0.46 | 0.46 |
| Chemical composition | | | | |
| Dry matter (%) | 87.89 | 88.34 | 89.21 | 89.28 |
| Neutral detergent fiber (% DM) | 24.92 | 24.69 | 24.47 | 24.24 |
| Acid detergent fiber (% DM) | 14.54 | 14.44 | 14.34 | 14.24 |
| Ash (% DM) | 6.06 | 5.75 | 6.24 | 6.72 |
| Crude protein (% DM) | 16.15 | 15.90 | 15.65 | 15.40 |
| Ether extract (% DM) | 3.41 | 4.72 | 5.26 | 6.83 |
| Metabolizable energy (Mcal/kg DM) | 2.91 | 2.86 | 2.86 | 2.87 |

cut of the muscle to expose myoglobin to oxygen (COSTA *et al.*, 2011). A digital colorimeter (CR-400 Konica Minolta, Tokyo, Japan) was used to evaluate lightness (L^*), redness (a^*), and yellowness (b^*). After color evaluation, approximately 2 g of meat was collected to determine its water-holding capacity, defined as the difference between the weights of the sample before and after it was subjected to a pressure of 2.25 kg for 5 min as described by CAÑEQUE and SAÑUDO (2000).

For the analysis of cooking losses (CL), the meat samples were cooked in an electric oven at 175 °C until a temperature of 70 °C was reached in their geometric centers measured with a digital thermometer. The weights of the steaks before and after cooking were measured to calculate CL. After cooling, cylindrical samples were removed from the steaks using a drawn punch measuring 1.3 cm in diameter. A texture analyzer coupled to a 1-mm thick Warner-Bratzler blade was used to measure the force necessary to transversally cut each cylinder as described by OSÓRIO *et al.* (1998).

The methods of the AOAC (1995) were used for centesimal analysis: moisture (31.1.02); crude protein (31.1.08); ether extract (31.4.02), and ash (31.1.04). Fatty acids were analyzed by extraction of total lipids from 4.0 g of wet samples with chloroform/methanol (2/1, v/v) for 10 min, according to FOLCH *et al.* (1957). One microliter of fatty acid methyl

esters pipetted from the supernatant was quantified by gas chromatography (Shimadzu, GC 17A, Kyoto, Japan) using a flame ionization detector, a split/splitless injector, and a DB-Wax capillary column (60 m × 0.25 mm; Agilent Technologies, J&W Scientific, Santa Clara, CA, USA.). Methanol, chloroform, and potassium chloride (0.88%) were used for the saponification process.

The initial column temperature was set at 80°C for 2 min at a rate of 3°C/min. The temperature was then increased to 180°C at a rate of 30°C/min, kept at this temperature for 30 min, and increased to 200°C at a rate of 3°C/min, remaining at this temperature for 108 min. The detector temperature was 240°C. Helium was used as the carrier gas at a flow rate of 8.0 mL/min (splitter ratio 1:50). For the identification of fatty acids, the retention times were compared to methyl ester standards (Sigma-Aldrich), and the measurement was carried out by area normalization expressing the result as a percentage area of each acid on the total area fatty acids (%), according to the method of HARTMAN and LAGO (1973).

Based on the profile of the fatty acids identified, total saturated fatty acids (SFA), unsaturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids (PUFA) were calculated, as well as the ratios of PUFA:SFA and n-6:n-3. The ratio of hypocholesterolemic fatty acids and

hypercholesterolemic (h:H) = (C18:1cis9 + C18:2 n-6 + C20:4 n-6 + C18:3 n-3 + C20:5 n-3 + C22:5 n-3)/(C14:0 + C16:0), according to SANTOS-SILVA *et al.* (2002), were also computed.

The statistical analyses were performed using the SAS (SAS Institute, Cary, NC, USA). The Shapiro-Wilk test was used to verify the normality of residuals and the Bartlett test to evaluate the homogeneity of variance. The data were submitted to regression analysis, adopting a level of significance of 5%.

RESULTS AND DISCUSSION

The increasing levels of crude glycerin did not affect ($P>0.05$) the weight or yield of *longissimus* tissue composition (Table 2). This result can be attributed to the slaughter criterion (body condition), in which animals of the same genetic group with similar body condition at slaughter have a similar tissue composition (SILVA SOBRINHO *et al.*, 2008). We therefore assume that the muscles of the different treatments were similar, although we did not study the tissue composition of loin cut.

The different levels of crude glycerin did

not affect ($P>0.05$) the physical characteristics of *longissimus* muscle (Table 3). POLIZEL (2013) evaluated animals of the same genetic group and similar age and found no difference between treatments, indicating that crude glycerin does not affect the physical characteristics of meat.

The final pH was 5.48, a value slightly below the range of 5.5 to 5.8 considered normal for lamb meat (SAÑUDO *et al.*, 1992). Under similar conditions of carcass storage and subcutaneous fat thickness, animals fed different diets may have suitable final pH (OLIVEIRA, 2013) (Table 2).

Animals fed largest crude glycerin levels in the diet may have lower losses due to muscle exudate glycerol capacity to retain water in the muscle (LAGE *et al.*, 2014). The effect of dietary inclusion of glycerol on the water retention of muscle has been reported by PARKER *et al.* (2007), who evaluated the effect of glycerol inclusion in beef cattle diets during the transportation period and found that treatment with glycerol resulted in hyperhydration of the animal, improving meat quality.

The experimental diets did not affect shear force (SF) (Table 3), which ranged from 3.24 to 3.97 kg. According to DUCKETT (2003), meat cuts with SF

Table 2. Tissue composition of *longissimus* muscle of lambs fed increasing levels of crude glycerin

| Characteristics | Crude glycerin (%DM) | | | | ¹ SEM | P value | |
|--------------------|----------------------|-------|-------|-------|------------------|----------------|----------------|
| | 0.0 | 2.5 | 5.0 | 7.5 | | ² L | ³ Q |
| | Weight (kg) | | | | | | |
| <i>Longissimus</i> | 1.15 | 1.10 | 1.11 | 1.16 | 0.3695 | 0.3981 | 0.3525 |
| Muscle | 0.60 | 0.58 | 0.57 | 0.58 | 0.2843 | 0.6679 | 0.7536 |
| Subcutaneous fat | 0.15 | 0.13 | 0.15 | 0.17 | 0.2586 | 0.6819 | 0.5307 |
| Intermuscular fat | 0.21 | 0.18 | 0.15 | 0.19 | 0.2800 | 0.2487 | 0.2958 |
| Total fat | 0.35 | 0.31 | 0.30 | 0.36 | 0.2784 | 0.1338 | 0.1169 |
| Bone | 0.12 | 0.13 | 0.15 | 0.14 | 0.2440 | 0.5926 | 0.6901 |
| Other | 0.07 | 0.04 | 0.06 | 0.04 | 0.1865 | 0.5016 | 0.6436 |
| | Yield (%) | | | | | | |
| Muscle:fat | 1.82 | 1.86 | 2.04 | 1.66 | 0.6903 | 0.3571 | 0.2886 |
| Muscle:bone | 5.83 | 6.07 | 4.09 | 4.87 | 1.5393 | 0.5629 | 0.7840 |
| Loin | 13.06 | 13.20 | 13.27 | 13.80 | 1.4394 | 0.9607 | 0.8182 |
| Muscle | 65.41 | 67.07 | 64.45 | 61.34 | 3.0120 | 0.7222 | 0.5271 |
| Subcutaneous fat | 15.62 | 15.53 | 16.30 | 18.30 | 2.6651 | 0.8799 | 0.7210 |
| Intermuscular fat | 22.27 | 20.87 | 16.51 | 19.96 | 2.8251 | 0.3608 | 0.4650 |
| Total fat | 37.89 | 36.40 | 32.81 | 38.26 | 2.5741 | 0.2123 | 0.2130 |
| Bone | 12.73 | 14.00 | 16.51 | 14.33 | 2.3048 | 0.3405 | 0.4366 |
| Other | 7.13 | 5.06 | 6.41 | 4.83 | 1.9334 | 0.7021 | 0.8736 |

¹SEM: standard error of the mean. ²L: linear effect. ³Q: quadratic effect.

Table 3. Quality traits of *longissimus* muscle of lambs fed increasing levels of crude glycerin

| ¹ Characteristics | Crude glycerin (%DM) | | | | ² SEM | P value | |
|------------------------------|----------------------|-------|-------|-------|------------------|----------------|----------------|
| | 0.0 | 2.5 | 5.0 | 7.5 | | ³ L | ⁴ Q |
| pH | 5.51 | 5.47 | 5.52 | 5.43 | 0.2655 | 0.7172 | 0.4265 |
| WHC (%) | 82.92 | 84.15 | 85.42 | 86.46 | 1.8169 | 0.5352 | 0.9459 |
| CL (%) | 26.62 | 26.62 | 33.23 | 24.15 | 2.3731 | 0.1082 | 0.0868 |
| SF (kg) | 3.28 | 3.24 | 3.97 | 3.58 | 1.0186 | 0.5061 | 0.6504 |
| L* | 40.47 | 41.90 | 41.95 | 40.42 | 1.4308 | 0.1069 | 0.0920 |
| a* | 22.94 | 23.92 | 22.90 | 22.89 | 0.9434 | 0.2776 | 0.1833 |
| b* | 5.98 | 7.26 | 6.43 | 6.77 | 0.7997 | 0.0513 | 0.0817 |

¹WHC: water-holding capacity; CL: cooking losses; SF: shear force; L*: lightness; a*: redness; b*: yellowness. ²SEM: standard error of the mean. ³L: linear effect. ⁴Q: quadratic effect.

values less than 4.5 kg can be considered tender. The values obtained in this experiment were lower than those reported by LAGE *et al.* (2014), who found a SF of 4.44 kg when lambs were fed a diet containing increasing levels of crude glycerin (3, 6, 9 and 12%). One factor that may explain this result was that the animals of the present study had the same BCS at slaughter and 16.4% of subcutaneous fat in the carcass. Subcutaneous fat acts as an insulator and can reduce the effects of cold shortening (SAÑUDO *et al.*, 1996). Slaughtering animals with the same BCS can standardize the final product, regardless of treatment.

Lightness (L*) was not affected by crude glycerin level in the diet (P>0.05). The value of L* is related to final pH and kinetic properties of *rigor mortis* (LEDWARD *et al.*, 1986; ALBERTI *et al.*, 2005). There were no differences in pH or water-holding capacity. Redness (a*) or yellowness (b*) was also not affected by the diet (P>0.05). These results might be related to the age of the animals at slaughter since redness is influenced by age. RENERRE (1990) reported an increase in myoglobin concentration with increasing age of the animal.

There was no effect (P>0.05) of increasing levels of crude glycerin on moisture, crude

protein or mineral content of *longissimus* muscle (Table 4). Ether extract content increased linearly (P<0.0001). The increasing levels of crude glycerin may have led to an increase in the proportion of propionate in the rumen, which is a precursor of glucose, consequently increasing intramuscular fat. According to HOOD *et al.* (1972), marbling requires glucose as a carbon source.

Saturated C16:0 (24.34%) and C18:0 (20.77%), monounsaturated C18:1 n-9c (34.43%) and polyunsaturated C18:2 n-6c (6.15%) fatty acids were the most abundant in Pantaneiro lamb meat (Table 5). The concentrations of C14:0 (myristic), C16:0 (palmitic) and C18:0 (stearic) fatty acids were not affected (P>0.05) by increasing levels of crude glycerin in the diet (Table 5). The importance of these results is related to the function of these fatty acids in human cholesterol metabolism, in which C14:0 and C16:0 are hypercholesterolemic and C18:0 is a neutral fatty acid (ULBRICHT and SOUTHGATE, 1991).

A quadratic effect was observed for C17:0 fatty acid (P<0.05) and the highest level was found in the control diet. Odd chain fatty acids such as margaric acid can be synthesized by rumen microbes from propionate and acetate (SAUVANT and BAS, 2001). TERRÉ *et al.* (2011) observed higher margaric acid

Table 4. Centesimal composition of *longissimus* muscle of lambs fed increasing levels of crude glycerin

| Characteristics | Crude glycerin (%DM) | | | | ¹ SEM | P value | |
|-------------------|----------------------|-------|-------|-------|------------------|----------------------|----------------|
| | 0.0 | 2.5 | 5.0 | 7.5 | | ² L | ³ Q |
| Moisture (%) | 76.23 | 75.72 | 74.99 | 74.61 | 0.9684 | 0.3093 | 0.8877 |
| Crude protein (%) | 18.66 | 19.85 | 18.62 | 18.84 | 0.8684 | 0.2358 | 0.1813 |
| Ether extract (%) | 3.70 | 4.54 | 5.92 | 6.70 | 1.0257 | ⁴ <0.0001 | - |
| Ash (%) | 1.41 | 1.42 | 1.51 | 1.61 | 0.5039 | 0.9925 | 0.6655 |

¹SEM: standard error of the mean. ²L: linear effect. ³Q: quadratic effect. ⁴ $\hat{y}=3.67+0.41x$ (R²=0.5871).

Table 5. Fatty acid composition of *longissimus* muscle of lambs fed increasing levels of crude glycerin

| Fatty acids (% of total fatty acids) | Crude glycerin (%DM) | | | | ¹ SEM | P value | |
|---|----------------------|-------|-------|-------|------------------|---------------------|---------------------|
| | 0.0 | 2.5 | 5.0 | 7.5 | | ² L | ³ Q |
| Saturated | | | | | | | |
| C 6:0 | 0.05 | 0.04 | 0.03 | 0.04 | 0.1489 | 0.5326 | 0.7255 |
| C 10:0 | 0.15 | 0.17 | 0.15 | 0.17 | 0.1841 | 0.7964 | 0.9560 |
| C 14:0 | 2.36 | 1.90 | 2.39 | 2.67 | 0.8572 | 0.3854 | 0.2293 |
| C 16:0 | 24.06 | 24.00 | 24.60 | 24.69 | 0.9741 | 0.8221 | 0.8459 |
| C 17:0 | 1.59 | 1.38 | 1.40 | 1.52 | 0.4328 | 0.0367 | ⁴ 0.0424 |
| C 18:0 | 19.37 | 21.26 | 21.98 | 20.46 | 1.7137 | 0.1297 | 0.1696 |
| C 20:0 | 0.20 | 0.15 | 0.20 | 0.22 | 0.2181 | 0.2036 | 0.1009 |
| C 21:0 | 0.11 | 0.15 | 0.10 | 0.14 | 0.2041 | 0.7001 | 0.5990 |
| Monounsaturated | | | | | | | |
| C 15:1 | 0.01 | 0.02 | 0.01 | 0.02 | 0.0752 | 0.3577 | 0.3583 |
| C 16:1 | 2.41 | 2.01 | 2.22 | 2.30 | 0.6253 | 0.1588 | 0.1540 |
| C 18:1 n-9c | 36.15 | 33.51 | 33.61 | 34.45 | 1.4874 | ⁵ 0.0392 | - |
| C 18:1 n-9t | 0.33 | 0.33 | 0.28 | 0.36 | 0.3040 | 0.4370 | 0.3669 |
| C 20:1 | 0.46 | 0.48 | 0.47 | 0.45 | 0.3282 | 0.7328 | 0.6563 |
| Polyunsaturated | | | | | | | |
| C 18:2 n-6c | 5.39 | 6.51 | 5.98 | 6.72 | 0.9775 | 0.3089 | 0.6261 |
| C 18:2 n-6t | 0.11 | 0.07 | 0.11 | 0.11 | 0.2229 | 0.5102 | 0.4276 |
| C 18:3 n-3 | 0.38 | 0.35 | 0.35 | 0.28 | 0.2877 | ⁶ 0.0434 | - |
| C 18:3 n-6 | 0.16 | 0.19 | 0.15 | 0.18 | 0.2137 | 0.9940 | 0.9361 |
| C 20:2 | 0.06 | 0.04 | 0.04 | 0.06 | 0.1438 | 0.0525 | ⁷ 0.0467 |
| C 20:3 n-3 | 0.13 | 0.16 | 0.11 | 0.14 | 0.1997 | 0.9957 | 0.9456 |
| C 20:3 n-6 | 1.88 | 1.96 | 1.68 | 1.75 | 0.6501 | 0.8341 | 0.9736 |
| C 20:4 n-6 | 1.71 | 2.08 | 1.86 | 2.15 | 0.8275 | 0.6936 | 0.8769 |
| C 20:5 n-3 | 0.43 | 0.38 | 0.34 | 0.36 | 0.2922 | 0.1528 | 0.2906 |

¹SEM: standard error of the mean. ²L: linear effect. ³Q: quadratic effect. ⁴ $\hat{y}=1.58-0.11x+0.01x^2$ ($R^2=0.1919$). ⁵ $\hat{y}=36.05-1.24x+0.14x^2$ ($R^2=0.1999$). ⁶ $\hat{y}=0.39-0.01x$ ($R^2=0.1727$). ⁷ $\hat{y}=0.06-0.01x+0.001x^2$ ($R^2=0.2134$).

levels when they fed animals 5 and 10% crude glycerin.

The C18:1 n-9c (oleic) and C18:3 n-3 (linolenic) fatty acids decreased linearly with increasing levels of crude glycerin. These results differ from those reported by AVILA-STAGNO *et al.* (2013), who observed an increase of 3.3% in oleic acid levels in animals fed diets containing 21% glycerol. However, these authors added higher levels of glycerin with 99.5% purity (7%, 14% and 21% glycerol). Higher proportions of oleic and linolenic acids are desirable in intramuscular fat because of their LDL-lowering capacity, contributing to a reduction in obesity, cancer and cardiovascular disease in humans (PEREZ *et al.*, 2002).

Increasing levels of crude glycerin exerted a quadratic effect on the sum of unsaturated fatty acids ($P<0.05$) (Table 6). According to KRUEGER *et al.* (2010), the inclusion of 2% and 20% glycerol on *in vitro* studies for rumen microbes, could inhibit the ruminal lipolysis by increasing free fatty acids on rumen. Then, the glycerol supplementation could inhibit fat degradation by ruminal microbes, allowing the passage of the intact lipids, increasing the amount of unsaturated fatty acids.

Then-3 fatty acid levels and unsaturated:saturated ratio decreased linearly ($P<0.05$), while the n-6:n-3 ratio increased linearly ($P<0.05$) (Table 6). These results are directly related to the linear decrease in

Table 6. Total levels and ratios of saturated, unsaturated, monounsaturated and polyunsaturated fatty acids in *longissimus* muscle of lambs fed increasing levels of crude glycerin

| Fatty acids | Crude glycerin (%DM) | | | | ¹ SEM | P value | |
|---------------------------|----------------------|-------|-------|-------|------------------|---------------------|---------------------|
| | 0.0 | 2.5 | 5.0 | 7.5 | | ² L | ³ Q |
| Saturated | 48.67 | 49.85 | 51.04 | 48.89 | 1.6404 | 0.1341 | 0.1452 |
| Unsaturated | 50.69 | 48.34 | 47.75 | 50.48 | 1.5769 | 0.0218 | ⁵ 0.0206 |
| Monounsaturated | 40.24 | 36.58 | 37.08 | 36.35 | 1.7413 | 0.1020 | 0.2495 |
| Polyunsaturated | 10.45 | 11.76 | 10.66 | 12.43 | 1.5107 | 0.9235 | 0.8093 |
| n-6 | 9.22 | 10.79 | 9.77 | 11.49 | 1.5246 | 0.7613 | 0.9339 |
| n-3 | 1.00 | 0.92 | 0.82 | 0.79 | 0.3631 | ⁶ 0.0068 | - |
| Hypercholesterolemic | 26.93 | 26.55 | 27.00 | 26.96 | 1.0808 | 0.8373 | 0.7609 |
| Unsaturated:saturated | 1.05 | 0.97 | 0.94 | 1.00 | 0.3017 | ⁷ 0.0485 | - |
| Polyunsaturated:saturated | 0.22 | 0.24 | 0.21 | 0.23 | 0.2026 | 0.9053 | 0.9182 |
| Monounsaturated:saturated | 0.83 | 0.74 | 0.73 | 0.73 | 0.3230 | 0.1230 | 0.2482 |
| n-6:n-3 | 10.05 | 13.10 | 11.84 | 12.94 | 1.2583 | ⁸ 0.0320 | - |
| ⁴ h:H | 1.67 | 1.65 | 1.56 | 1.61 | 0.2031 | 0.0268 | ⁹ 0.0447 |

¹SEM: standard error of the mean. ²L: linear effect. ³Q: quadratic effect. ⁴h:H: ratio of hypocholesterolemic fatty acids and hypercholesterolemic. ⁵ $\hat{y}=50.77-1.57x+0.20x^2$ ($R^2=0.2320$). ⁶ $\hat{y}=0.99-0.03x$ ($R^2=0.2889$). ⁷ $\hat{y}=1.05-0.05x+0.005x^2$ ($R^2=0.1828$). ⁸ $\hat{y}=10.86+0.29x$ ($R^2=0.22$). ⁹ $\hat{y}=0.50+0.03x-0.003x^2$ ($R^2=0.2398$).

C18:3 n-3 and C18:1 n-9c fatty acids, respectively (Table 5). According to WOOD *et al.* (2003), a suitable n-6:n-3 ratio should be below 4. However, this ratio ranged from 10.05 to 13.10 in this study. The n-6:n-3 ratio can be influenced by diet, with this ratio ranging from 1.4 to 2.0 in animals finished on pasture and from 6.0 to 10.0 in animals finished with concentrate since forages are rich in C18:3 n-3 and grains are rich in C18:2 n-6 (BOUFAIED *et al.*, 2003). This result was confirmed in the present study in which the n6:n3 ratio was influenced by the inclusion of crude glycerin in the diet of Pantaneiro feedlot-finished lambs.

A quadratic effect was found for the hyper/hypocholesterolemic ratio ($P<0.005$). The lowest ratios were observed for the treatments without (0.51) and with 7.5% crude glycerin (0.53). Lower ratios are considered beneficial because of the higher amounts of monounsaturated fatty acids and PUFA, which are hypercholesterolemic and contribute to lower LDL-cholesterol in humans (VALSTA *et al.*, 2005).

CONCLUSION

Crude glycerin can be used to replace corn without compromising tissue composition and quality traits of *longissimus* muscle. The inclusion of up to 7.5% crude glycerin (dry matter basis) in the

diet for Pantaneiro lambs improves the nutritional aspects of fat.

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