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Dietary glycerin does not affect meat quality of Ile de France lambs

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ABSTRACT - The objective of this study was to evaluate the physicochemical and nutritional characteristics of meat from three muscles of Ile de France lambs fed diets containing different levels of glycerin as an alternative to corn. Thirty non-castrated feedlot Ile de France lambs weighing 15±0.2 kg were housed in individual pens and slaughtered at 32±0.2 kg. Treatments consisted of three diets: sugarcane + 0% glycerin; sugarcane + 10% glycerin; and sugarcane + 20% glycerin; and three muscles were evaluated: *triceps brachii*, *semimembranosus*, and *longissimus lumborum*. No interactions were observed among glycerin levels and muscle types for the quality characteristics evaluated. Muscles were similar in terms of physicochemical and nutritional characteristics, and only differed in sarcomere length, diameter of muscle fibers, and cholesterol content. Dietary glycerin did not affect pH, water holding capacity (24 h and 30 days after slaughter), weight loss by thawing and cooking, shear force, diameter of muscle fibers, sarcomere length, lipid oxidation, chemical composition, or meat cholesterol content. Dietary glycerin resulted in a linear decrease of muscle yellowness from 3.99 to 3.29. Dietary glycerin altered intramuscular fat fatty acid profile, increasing the concentration of margaric acid (1.47 to 2.61%). Up to 20% glycerin can be included in the diet of feedlot lambs without any harm to meat quality.

Key Words: co-products, fatty acids, glycerol, muscles, omega-3

Introduction

The demand for animal protein has increased worldwide in the past decades (Tilman and Clark, 2014). This recent development has pushed producers in the search for better and cheaper practices. Specifically in Brazil, the intensification of sheep farming and the introduction of genetically improved breeds have resulted in increased productivity. In parallel, farmers have sought diet formulations that meet high-performance animal needs. Better diets shorten time to slaughter and produce better-quality lambs for an ever-more demanding consumer market. This production system is costly, and the use of alternative feeds that do not affect meat quality is greatly needed. The growing Brazilian biodiesel industry has become a source of the alternative feed glycerin, a biodiesel co-product that can substitute corn and sorghum with the advantage of being sweet-tasting and well accepted by animals (Schröder and Südekum, 1999).

Several researchers have evaluated the effects of different levels of glycerin on feedlot lamb performance (Musselman et al., 2008; Gunn et al., 2010; Gomes et al., 2011; Lage et al., 2014a). However, few studies have focused on the potential changes in meat quality and nutritional value brought about by dietary glycerin (Françozo et al., 2013; Bartoñ et al., 2013; Lage et al., 2014b). A potential effect of dietary glycerin for ruminant meat quality is the increased availability of gluconeogenic compounds, which allow for a greater deposition of intramuscular fat (Mach et al., 2009). Krueger et al. (2010) demonstrated that the addition of glycerin to ruminant diets alters the meat fatty acid profile because glycerol suppresses ruminal lipolysis. More recently, a study using *in vitro* simulations further indicated that dietary glycerin reduces ruminal lipolysis without adversely affecting ruminal digestion, and this fact could indeed alter meat fatty acid profiles (Edwards et al., 2012).

Nevertheless, these findings remain controversial as other authors reported no differences in carcass and meat quality parameters (Terré et al., 2011; Bartoñ et al., 2013). Moreover, there has been little normalization among studies as to glycerin quality, dilution ratios, and maximum amounts. Thus, this study evaluated the qualitative aspects of meat

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from three muscles of Ile de France lambs fed diets containing different levels of glycerin as an alternative energy source to corn.

Material and Methods

The experiment was conducted in the city of Jaboticabal, SP, Brazil, with geographic coordinates 21º14'05" S latitude, 48º17'09" W longitude, 615.01 m altitude, atmospheric pressure of 944.3 hPa, average temperature 22.2 ºC, relative humidity of 72.5%, and precipitation of 1453.4 mm, and complied with the guidelines set by the Ethics Committee in Animal Use (case no. 005962/12). Thirty newly weaned 45-day-old uncastrated male Ile de France lambs with 15±0.2 kg body weight were used. The animals were individually identified, dewormed, and housed in 1.0 m^2 individual pens, installed in a shed, with suspended slatted floor and equipped with feeders and drinkers. These lambs were randomly distributed into three experimental groups with ten animals each. The following treatments were studied: sugarcane + 0% glycerin; sugarcane + 10% glycerin; and sugarcane + 20% glycerin, based on the dry matter of the diets.

The concentrate was composed of ground corn grain, soybean meal, urea, dicalcium phosphate, limestone, and mineral and vitamin supplements. The glycerin (83.90% glycerol, 12.31% moisture, and 3.79% salts) was weighed and mixed with the concentrate at the time of supplying the diets for lambs. The IAC 86-2480 sugarcane (developed by the Agronomical Institute of Campinas for use in animal feed due to the low amount of fiber compared with other varieties) was chopped to 1.0 cm pieces and supplied fresh. The resulting diets (Tables 1 and 2) had similar protein (16%) and energy (3.35 Mcal metabolizable energy/kg DM) contents and were prepared in accordance with the NRC (2007) recommended requirements for weaned lambs, for an estimated weight gain of 250 g/day. The roughage:concentrate ratio was 50:50 and the diets were fed *ad libitum* (at least 10% was left over) at 7.00 h and 17. 00 h.

The lambs were weighed fortnightly to evaluate the performance, and when they reached 32.0±0.2 kg of body weight (slaughter criteria) and 127±9 days of age they were fasted for solid foods for 16 h and slaughtered. The feedlot period lasted 91 days, with 14 days of adaptation and 77 of data collection.

The slaughter occurred after stunning with a concussion gun, model TEC PC 10. The jugular veins and carotid arteries were sectioned for bleeding, with subsequent

Table 1 - Percentage of ingredients and chemical composition of diets (DM basis)

Composition	Glycerin (% DM)		
	0	10	20
Ingredient, % DM			
Sugarcane	50.00	50.00	50.00
Glycerin[1]	0.00	10.00	20.00
Corn grain	25.43	13.69	1.04
Soybean meal	21.77	23.71	26.50
Dicalcium phosphate	0.28	0.22	0.18
Calcium carbonate	0.57	0.43	0.33
Mineral-vitamin supplements[2]	0.95	0.95	0.95
Urea	1.00	1.00	1.00
Chemical composition			
Dry matter[3]	40.89	41.04	40.97
Organic matter[4]	94.66	93.28	91.83
Mineral matter[4]	5.34	6.72	8.17
Crude protein[4]	16.53	16.74	16.90
Ether extract[4]	1.96	1.10	0.63
Neutral detergent fiber	29.37	29.53	29.04
Acid detergent fiber	12.34	13.17	12.05
Total carbohydrates	78.87	75.98	72.11
Non-fiber carbohydrates[5]	56.35	52.81	51.74
Gross energy (Mcal/kg DM)	3.88	3.99	3.72

DM - dry matter; NDF - neutral detergent fiber.
[1] Glycerin = 83.90% glycerol, 12.31% moisture, and 3.79% salts.
[2] Guaranteed levels per kg of product: 120 g Ca; 90 g Cl; 62 g Na; 54 g Mg; 50 g P; 34 g S; 1,600 mg Zn; 1,500 mg Mn; 1,064 mg Fe; 730 mg F (Max); 50 mg Cu; 25 mg I; 20 mg Se; 10 mg Co; 100,000 IU vitamin A; 40,000 IU vitamin D3; and 600 IU vitamin E.
[3] %.
[4] % DM.
[5] Non-fiber carbohydrates = 100 − (crude protein + ether extract + mineral matter + NDF).

Table 2 - Percentage composition of fatty acids in diets

Fatty acid (%)	Glycerin (% DM)		
	0	10	20
Saturated			
Capric (C10:0)	0.04	0.03	0.03
Lauric (C12:0)	0.11	0.10	0.17
Myristic (C14:0)	0.26	0.23	0.39
Pentadecanoic (C15:0)	0.11	0.07	0.10
Palmitic (C16:0)	17.21	19.08	22.98
Margaric (C17:0)	0.20	0.15	0.20
Stearic (C18:0)	4.36	4.68	4.27
Arachidic (C20:0)	0.87	0.77	0.73
Behenic (C22:0)	0.35	0.38	0.49
Tricosanoic (C23:0)	0.14	0.14	0.15
Lignoceric (C24:0)	0.60	0.54	0.54
Monounsaturated			
Palmitoleic (C16:1)	1.40	1.87	0.91
Heptadecenoic (C17:1)	0.07	0.08	0.11
Oleic (C18:1ω9)	31.61	31.19	30.10
Cis-vaccenic (C18:1 ω7)	0.98	0.88	0.93
Eicosenoic (C20:1 ω9)	0.39	0.37	0.38
Polyunsaturated			
Linoleic (C18:2 ω6)	38.82	36.65	35.07
α-linolenic (C18:3 ω3)	2.19	2.56	2.18
γ-linolenic (C18:3 ω6)	0.04	0.04	0.03
Eicosadienoic (C20:2)	0.21	0.16	0.21
Eicosatrienoic (C20:3 ω6)	0.04	0.03	0.03
Omega-3[1] (ω3)	2.19	2.56	2.18
Omega-6[2] (ω6)	38.90	39.72	35.13
Omega-6:Omega-3 (ω6:ω3)	17.76	15.51	16.11

DM - dry matter.
[1] ω3 = (C18:3 ω3).
[2] ω6 = (C18:2ω6 + C18:3ω6 + C20:3ω6).

evisceration and removal of the head and ends of the limbs, according to procedures characterizing humane slaughter. The carcasses were identified with the animal number and stored in a cold chamber (6 °C) for 24 h.

After 24 h, we measured the pH and color of the *triceps brachii* (early development), *semimembranosus* (intermediate), and *longissimus lumborum* (late) muscles. The pH was measured, in triplicate, with a digital pH meter, Testo 205, equipped with a penetrating electrode. The meat color was determined 30 min after sectioning the samples, to expose the myoglobin to oxygen, as described by Cañeque and Sañudo (2000), using a colorimeter, model Minolta CR-400, illuminant D65, evaluating the lightness (L*), redness (a*), and yellowness (b*). The color aspects were assessed by the CIELAB color system using 0º/45º.

The carcasses were divided longitudinally and the three muscles of the half-carcasses were marked, stored in plastic bags, vacuum-packed and frozen at −18 °C for later analysis. For the qualitative analysis, the muscles were thawed at 10 °C in a BOD incubator for 12 h, with the exception of the samples for determination of thawing loss (30 days after being frozen – TL30D), which remained in the BOD incubator at 5 °C for 16 h and were weighed before and after this period, according to the methodology described by Koohmaraie et al. (1998).

To determine the water holding capacity (WHC), 2 g of the samples were collected and subjected to 10 kg of pressure for 5 min. The WHC value was determined as the difference between the weight of the sample before and after pressure (Hamm, 1986). The results were expressed in percentage of water retained in relation to the initial weight of the sample. The cooking weight loss (CWL) was determined using thawed samples, as the difference between the weight of a steak before and after cooking in an oven pre-heated to 175 °C. A thermometer was used to monitor the internal temperature of the steak until the center reached 71 °C. Subsequently, each steak was conditioned to room temperature, and after temperature stabilization, the steak was weighed to obtain the CWL, in percentage. The shear force (SF) was obtained from the same samples used for the CWL. Six cylindrical samples per steak, with 1.27 cm diameter (Wheeler et al., 1995), free of visible fat and connective tissue, were obtained from the center of the cooked samples, with the direction of the muscle fibers parallel to the length. The cylinders were completely sheared perpendicularly to the muscle fibers with a Warner-Bratzler blade of 1.016 mm at a speed of 300 mm/min, using a Texture Analyser, model Brookfield CT3 10K.

Five-gram samples of muscle were taken for measurement of sarcomere length and diameter of the muscle fibers. The samples were placed in plastic containers with 10% buffered formalin (50 mL) and, after mounting, they were cleaved, dehydrated, cleared, embedded in paraffin, and sectioned in micrometers (Pika–Seiko), with a thickness of eight micra. Histological sections (longitudinal and transverse) were stained with Mallory's phosphotungstic acid haematoxylin, according to the technique recommended by Behmer et al. (1976). The slides were assessed under an optical microscope (model Olympus BX51) coupled to an Olympus DP72 model camera, with the methods being based on 10 sarcomere counts of five different myofibrils (50 sarcomeres) and 50 fiber diameters (random). Morphometric analysis of sarcomere lengths and diameters of the fibers was performed using the ImageJ computer program.

The lipid oxidation was determined in accordance with the methodologies proposed by Pikul et al. (1989).

Samples of the muscles were lyophilized for 72 h and then milled in a ball mill to obtain the laboratory samples. These samples were used to determine the chemical composition of the meat in terms of moisture (930.15), protein (954.01), fat (920.39), and ash (942.05), in accordance with the AOAC (1995) methods for meat.

Lipid extraction was performed as described by Bligh and Dyer (1959) to determine the fatty acid profile. Ten grams of raw meat were subjected to lipid extraction using chloroform and methanol in the proportion of 2:1. Following this, an aliquot of 5 mL of the chloroform extract was evaporated using gaseous nitrogen and was subjected to saponification using 12% potassium hydroxide solution in ethanol. The extracted lipids were converted to fatty acid methyl esters following the technique of Hartman and Lago (1973) and were analyzed using a Shimadzu 14B gas chromatograph, equipped with a flame ionization detector and fused silica capillary column (OMEGAWAX250, size: 30 m × 0.25 mm × 0.25 μm, cat. No. 24136-SUPELCO). The injector and detector temperatures were 250 °C and 280 °C, respectively, and the helium flow ratio was 1 mL/min. After the injection (1 μL), the column temperature was held at 100 °C for 2 min, and then increased to 220 °C at 4 °C/min. The temperature was then kept at 220 °C for 25 min. The peaks were identified by comparing retention times with the standards for fatty acid methyl esters from Sigma.

Determination of the cholesterol content of the muscles was performed by spectrophotometry (Spectronic® 20 - Genesys, Jakarta, Indonesia), as described by Bragagnolo and Rodrigues-Amaya (2001). The total lipids were measured by extraction from approximately 10 g samples of loin in 200 mL of a chloroform-methanol mixture (2:1). From this extract, 5 mL of sample were dried using nitrogen

gas, followed by addition of 10 mL of 12% KOH in 90% ethanol. The solution was then placed in a water bath at 80 °C and agitated for 15 min. At the end of this process, 5 mL of water were added, and after cooling, 10 mL of hexane were added and the solution was agitated by vortexing. After separation of the phases, a 10-mL sample was dried using nitrogen gas. Finally, 6 mL of acetic acid saturated with concentrated ferrous sulfate were added. Once cooled, the absorbance was determined at 538 nm.

The atherogenicity (AI) and thrombogenicity (TI) indices were calculated as described by Ulbricht and Southgate (1991), and the activity of the Δ9-desaturases (C16 and C18) and elongase enzymes, in accordance with Malau-Aduli et al. (1997).

The experimental design was completely randomized, with three treatments (three levels of glycerin) evaluated in three different muscles (*triceps brachii*, *semimembranosus*, and *longissimus lumborum*) with 10 replications each. The muscle factor was considered a longitudinal factor (repeated measures). The data were analyzed using the MIXED procedure of SAS (Statistical Analysis System, version 9.1) and subjected to analysis of variance. The glycerin content was broken down into linear or quadratic effects and muscle types were compared by Tukey's test. Significant differences were declared at P<0.05.

The mathematical model used was:

$$Y_{ijk} = \mu + TG_i + TM_j + (TG \times TM)_{ij} + e_{ijk},$$

in which Y_{ijk} = observed value of the variable that received glycerin content i, muscle j, and repetition k; μ = overall mean; TG_i = level of glycerin i, ranging between 1 and 3 (0, 10, and 20% inclusion of glycerin); TM_j = type of muscle j (*triceps brachii*, *semimembranosus*, and *longissimus lumborum*); $(TG \times TM)_{ij}$ = effect of interaction between the glycerin content i and muscle type j; and e_{ijk} = random error associated with each observation.

Results

No interactions were observed (P>0.05) among glycerin levels and muscle types for any of the physical and chemical variables evaluated (Table 3). The *triceps brachii*, *semimembranosus*, and *longissimus lumborum* muscles of the lambs were similar in terms of physical and chemical characteristics and only differed (P<0.05) in sarcomere length and diameter of muscle fibers (Table 3).

Final muscle pH, evaluated 24 h after slaughter, was not affected (P>0.05) by dietary glycerin. The final pH values observed in this study ranged from 5.63 to 5.66 (Table 3).

The diets did not affect (P>0.05) the lightness (39.20) and redness (13.43) of muscles. However, a linear decrease in yellowness from 3.99 (D0) to 3.29 (D20) occurred with increasing amounts of glycerin (Table 3).

There was no effect of glycerin (P>0.05) on meat water holding capacity 24 h (63.48%) and thirty days (54.44%) after slaughter (Table 3). The water holding capacity decreased in the three muscles between 24 h and 30 days after slaughter. The replacement of corn by glycerin did not affect (P>0.05) weight loss by thawing of muscles (6.10%) 30 days after slaughter (Table 3).

Weight losses during the cooking process (38.31%) and shear force (3.83 kgf) of meat were not affected (P>0.05) by dietary glycerin (Table 3). The lipid oxidation (1.70 mg of malonaldehyde/kg of meat) was also not significantly altered by the replacement of corn by glycerin in the diets (Table 3).

Table 3 - Chemical and physical characteristics of *triceps brachii* (TB), *semimembranosus* (SM), and *longissimus lumborum* (LL) muscles of feedlot lambs fed diets with different levels of glycerin (% DM)

Variable	Glycerin (% DM)			Muscle			SEM	P-value		
	0	10	20	TB	SM	LL		G	M	G × M
pH 24h	5.63	5.66	5.64	5.65	5.63	5.65	0.044	0.398	0.411	0.262
L*	39.91	39.05	39.32	39.58	39.65	39.37	1.157	0.747	0.102	0.238
a*	13.33	14.10	13.53	13.37	13.42	13.50	1.107	0.214	0.125	0.463
b*	3.99	3.71	3.29	3.63	3.68	3.54	0.713	0.011	0.122	0.137
WHC 24h (%)	64.76	62.51	63.19	63.34	63.71	63.41	3.540	0.399	0.352	0.735
WHC 30D (%)	56.22	54.56	52.56	54.58	54.20	54.57	3.332	0.089	0.130	0.830
TL 30D (%)	5.09	7.00	6.22	6.24	6.19	6.30	2.041	0.151	0.101	0.262
CWL (%)	38.22	38.60	38.10	38.49	38.57	38.46	3.078	0.091	0.083	0.554
SF (kgf)	3.93	3.68	3.88	3.99	3.93	3.87	0.391	0.183	0.117	0.693
Lipid oxidation[1]	1.62	1.40	2.07	1.68	1.72	1.70	0.730	0.222	0.449	0.913
SL (μm)	1.81	1.81	1.80	1.86A	1.84A	1.72B	0,653	0.991	<0.001	0.985
FD (μm)	30.85	30.61	30.43	34.67B	32.65B	27.69A	2.189	0.100	<0.001	0.378

Means in the same row with different letters for different types of muscle differ according to Tukey's test (P<0.05).
DM - dry matter; SEM - standard error of the mean; G - effect of glycerin; M - effect of muscle; G × M - interaction between the glycerin levels and types of muscle.
L* - lightness; a* - redness; b* - yellowness; WHC 24h - water holding capacity after 24 h; WHC 30D - water holding capacity after 30 days; TL30D - thawing loss after 30 days;
CWL - cooking weight loss; SF - shear force; SL - sarcomere length; FD - fiber diameter.
[1] mg of malonaldehyde/kg of meat.

Muscle fiber diameter (30.63 µm) and sarcomere length (1.81 µm) were not affected by dietary glycerin (P>0.05). However, differences were observed between the types of muscle for both variables (P<0.001). As there was no interaction between glycerin levels and muscle types, these differences are not related to dietary glycerin. Fiber diameter and sarcomere lengths did not differ between the *triceps brachii* (34.67 and 1.86 µm) and *semimembranosus* (32.65 and 1.84 µm) muscles and, in both cases, they were larger (P<0.05) than seen in the *longissimus lumborum* (27.69 and 1.72 µm).

Interactions were not observed (P>0.05) between glycerin levels and muscle types for any of the nutritional characteristics evaluated. The three muscles had similar nutritional characteristics and only differed (P<0.001) in cholesterol content (Table 4).

Despite the high glycerol content of the glycerin, no effects (P>0.05) of dietary glycerin were observed on muscle chemical composition (Table 4).

Muscle cholesterol levels (38.33 mg/100 g of meat) were not significantly altered by the replacement of corn by glycerin (Table 4). However, the amount of cholesterol

Table 4 - Chemical composition and cholesterol content of *triceps brachii* (TB), *semimembranosus* (SM), and *longissimus lumborum* (LL) muscles of feedlot lambs fed diets with different levels of glycerin (% DM)

Variable	Glycerin (% DM)			Muscle			SEM	P-value		
	0	10	20	TB	SM	LL		G	M	G × M
Moisture[1]	77.56	78.05	78.11	77.98	77.95	77.79	0.614	0.219	0.116	0.598
Protein[1]	19.15	18.70	18.82	18.66	18.96	18.92	0.547	0.182	0.079	0.961
Fat[1]	2.14	2.12	1.95	2.23	1.96	2.16	0.665	0.181	0.120	0.445
Ash[1]	1.15	1.13	1.12	1.13	1.13	1.13	0.076	0.118	0.974	0.972
Cholesterol[2]	39.16	40.50	35.49	28.89B	52.41A	33.85B	3.103	0.387	<0.001	0.509

DM - dry matter; SEM - standard error of the mean; G - effect of glycerin; M - effect of muscle; G × M - interaction between the glycerin levels and types of muscle.
Means in the same row with different letters for different types of muscle differ according to Tukey's test (P<0.05).
[1] g/100 g of meat.
[2] mg/100 g of meat.

Table 5 - Fatty acid profile of *triceps brachii* (TB), *semimembranosus* (SM), and *longissimus lumborum* (LL) muscles of feedlot lambs fed diets with different levels of glycerin (% DM)

Variable (%)	Glycerin (% DM)			Muscle			SEM	P-value		
	0	10	20	TB	SM	LL		G	M	G × M
Saturated										
Capric (C10:0)	0.13	0.15	0.15	0.16	0.15	0.18	0.067	0.116	0.069	0.849
Lauric (C12:0)	0.26	0.24	0.29	0.25	0.25	0.24	0.221	0.844	0.256	0.334
Myristic (C14:0)	3.48	3.32	3.35	3.30	3.26	3.27	1.245	0.981	0.212	0.619
Pentadecanoic (C15:0)	0.55	0.62	0.64	0.61	0.60	0.59	0.151	0.335	0.240	0.746
Palmitic (C16:0)	27.30	26.63	26.50	26.97	27.03	27.08	1.036	0.501	0.211	0.069
Margaric (C17:0)	1.47	2.23	2.61	2.15	2.17	2.20	0.438	<0.001	0.102	0.689
Stearic (C18:0)	17.37	17.08	16.63	16.66	16.63	16.71	2.112	0.554	0.184	0.986
Arachidic (C20:0)	0.09	0.09	0.08	0.08	0.08	0.09	0.011	0.306	0.551	0.901
Monounsaturated										
Myristoleic (C14:1)	0.16	0.21	0.22	0.21	0.20	0.21	0.091	0.497	0.407	0.894
Palmitoleic (C16:1)	1.74	1.90	1.83	1.84	1.83	1.82	0.268	0.559	0.226	0.568
Heptadecenoic (C17:1)	1.39	1.26	1.45	1.22	1.19	1.17	0.217	0.230	0.113	0.212
Oleic (C18:1 ω9)	40.07	40.33	40.25	40.54	40.50	40.47	1.382	0.935	0.201	0.982
Cis-vaccenic (C18:1 ω7)	1.76	1.81	1.73	1.74	1.75	1.77	0.184	0.301	0.394	0.703
Eicosenoic (C20:1 ω9)	0.08	0.08	0.08	0.08	0.08	0.08	0.009	0.938	0.889	0.754
Nervonic (C24:1 ω9)	0.06	0.04	0.07	0.05	0.07	0.06	0.027	0.441	0.098	0.586
Polyunsaturated										
Linoleic (C18:2 ω6)	2.97	2.95	2.98	2.97	3.00	2.96	0.714	0.353	0.267	0.239
α-linolenic (C18:3 ω3)	0.11	0.11	0.13	0.12	0.12	0.12	0.025	0.084	0.891	0.322
γ-linolenic (C18:3 ω6)	0.11	0.11	0.14	0.12	0.13	0.12	0.021	0.123	0.281	0.678
CLA (C18:2 c9,t11)	0.27	0.26	0.23	0.29	0.27	0.26	0.068	0.583	0.199	0.429
Eicosadienoic (C20:2)	0.02	0.03	0.03	0.03	0.03	0.02	0.009	0.580	0.298	0.102
Eicosatrienoic (C20:3 ω6)	0.07	0.07	0.07	0.06	0.07	0.07	0.029	0.832	0.209	0.745
Arachidonic (C20:4 ω6)	0.46	0.43	0.44	0.48	0.50	0.53	0.315	0.618	0.387	0.619
Eicosapentaenoic (C20:5 ω3)	0.01	0.01	0.02	0.01	0.02	0.01	0.012	0.163	0.185	0.717
Docosatetraenoic (C22:4 ω6)	0.06	0.04	0.07	0.06	0.06	0.06	0.037	0.214	0.886	0.854
Docosahexaenoic (C22:6 ω3)	0.01	0.00	0.01	0.00	0.01	0.01	0.010	0.229	0.115	0.521

DM - dry matter; SEM - standard error of the mean; G - effect of glycerin; M - effect of muscle; G × M - interaction between the glycerin levels and types of muscle.

differed (P<0.05) among muscles, with similar values for the *triceps brachii* (28.89 mg/100 g of meat) and *longissimus lumborum* (33.85 mg/100 g of meat) muscles and higher value for the *semimembranosus* muscle (52.41 mg/100 g of meat).

There were no interactions (P>0.05) between glycerin levels and muscle types for any of the variables that comprise the fatty acid profile (Table 5). Irrespective of the treatment, the most abundant fatty acids in the evaluated muscles were the saturated myristic (3.40%), palmitic (26.80%), and stearic (17.00%) acids; the monounsaturated oleic acid (40.2%) and the polyunsaturated linoleic acid (2.90%), altogether constituting 90.30% of the total meat fatty acids. Increasing dietary glycerin did not affect individual fatty acid profile (P>0.05), except for a linear increase (1.47 to 2.61) in margaric fatty acid.

Total concentrations of saturated (50.42%), unsaturated (49.58%), monounsaturated (45.51%) and polyunsaturated (4.07%) fatty acids were not affected by the dietary glycerin inclusion and did not differ (P>0.05) among muscles (Table 6). The unsatured:satured (0.98), monounsaturated: saturated (0.90), and polyunsaturated:saturated (0.08) fatty acid ratios were not changed by dietary glycerin (P>0.05, Table 6), showing that the increase in margaric fatty acid did not alter the ratios.

The concentrations of omega-3 (0.14) and omega-6 (3.66) fatty acids were not affected (P<0.05) by the dietary glycerin. Consequently, the omega-6:omega-3 (27.12)

Table 6 - Concentrations (%) and ratios of fatty acids in *triceps brachii* (TB), *semimembranosus* (SM), and *longissimus lumborum* (LL) muscles of feedlot lambs fed diets with different levels of glycerin (% DM)

Variable	Glycerin (% DM)			Muscle			SEM	P-value		
	0	10	20	TB	SM	LL		G	M	G × M
SFA[1]	50.65	50.36	50.25	50.18	50.17	50.26	1.605	0.861	0.057	0.874
UFA[2]	49.35	49.64	49.75	49.82	49.83	49.74	1.605	0.861	0.057	0.874
MUFA[3]	45.26	45.63	45.63	45.68	45.62	45.58	1.395	0.658	0.203	0.990
PUFA[4]	4.09	4.01	4.12	4.14	4.21	4.16	1.132	0.414	0.099	0.870
UFA:SFA	0.97	0.99	0.99	0.99	0.99	0.99	0.071	0.781	0.898	0.869
MUFA:SFA	0.89	0.91	0.91	0.91	0.91	0.91	0.539	0.611	0.929	0.948
PUFA:SFA	0.08	0.08	0.08	0.08	0.08	0.08	0.021	0.890	0.932	0.836
Omega-3[5]	0.13	0.12	0.16	0.14	0.14	0.14	0.053	0.359	0.930	0.689
Omega-6[6]	3.67	3.60	3.70	3.69	3.76	3.74	0.763	0.176	0.353	0.911
n6:n3	28.23	30.00	23.13	26.36	26.86	26.71	4.215	0.701	0.081	0.649

DM - dry matter; SEM - standard error of the mean; G - effect of glycerin; M - effect of muscle; G × M - interaction between the glycerin levels and types of muscle.
SFA - saturated fatty acids; UFA - unsaturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids.
[1] SFA = (C10:0+C12:0+C14:0+C15:0+C16:0+C17:0+C18:0+C20:0).
[2] UFA = (C14:1+C16:1+C17:1+C18:1ω9+C18:1ω7+C20:1ω9+C24:1ω9+C18:2ω6+C18:3ω3+C18:23ω6+C18:2c9.t11+C20:2+C20:3ω6+C20:4ω6+C20:5ω3+C22:4ω6+C22:6ω3).
[3] MUFA = (C14:1+C16:1+C17:1+C18:1ω9+C18:1ω7+C20:1ω9+C24:1ω9).
[4] PUFA = (C18:2ω6+C18:3ω3+C18:23ω6+C18:2c9.t11+C20:2+C20:3ω6+C20:4ω6+C20:5ω3+C22:4ω6+C22:6ω3).
[5] Omega 3 = (C18:3ω3+C20:5ω3+C22:6ω3).
[6] Omega 6 = (C18:2ω6+C18:3ω6+C20:3ω6+C20:4ω6+C22:4ω6).

Table 7 - Indices, sums, and ratios of fatty acids in *triceps brachii* (TB), *semimembranosus* (SM), and *longissimus lumborum* (LL) muscles of feedlot lambs fed diets with different levels of glycerin (% DM)

Variable	Glycerin (% DM)			Muscle			SEM	P-value		
	0	10	20	TB	SM	LL		G	M	G × M
Δ^9 desaturase 16[1]	5.99	6.66	6.46	6.39	6.34	6.30	0.814	0.239	0.199	0.868
Δ^9 desaturase 18[2]	69.76	70.25	70.76	70.87	70.89	70.78	2.117	0.681	0.107	0.918
Elongase[3]	66.42	66.80	66.75	66.50	66.44	66.43	1.345	0.805	0.314	0.903
Atherogenicity[4]	0.84	0.81	0.81	0.81	0.81	0.81	0.092	0.921	0.975	0.975
Thrombogenicity[5]	1.93	1.88	1.85	1.87	1.86	1.87	0.506	0.253	0.748	0.931
Hypocholesterolemic (h)[6]	43.61	43.82	43.80	44.11	44.12	44.08	1.474	0.881	0.381	0.940
Hypercholesterolemic (H)[7]	30.78	29.95	29.85	30.27	30.29	30.35	1.786	0.368	0.359	0.935
h:H	1.42	1.46	1.47	1.46	1.46	1.45	0.122	0.643	0.708	0.900
Neutrals[8]	17.50	17.23	16.78	16.82	16.78	17.89	2.091	0.678	0.117	0.898

DM - dry matter; SEM - standard error of the mean; G - effect of glycerin; M - effect of muscle; G × M - interaction between the glycerin levels and types of muscle.
[1] Δ^9 desaturase 16 = 100[(C16:1cis-9)/(C16:1cis-9+C16:0)].
[2] Δ^9 desaturase 18 = 100[(C18:1cis-9)/(C18:1cis-9+C18:0)].
[3] Elongase = 100[(C18:0+C18:1cis9)/(C16:0+C16:1cis9+C18:0+C18:1cis9)].
[4] Atherogenicity = [(C12:0+(4 × C14:0)+C16:0)]/UFA.
[5] Thrombogenicity = [(C14:0+C16:0+C18:0)/[(0.5×ΣMUFA)+(0.5 × Σω6+(3 × Σω3)+(Σω3/Σω6)].
[6] h = (C18:1cis9+C18:2ω6+C18:3ω3+C20:4ω6).
[7] H = (C14:0+C16:0).
[8] Neutrals = C10:0+C18:0.

ratio was also unchanged (P>0.05) by the dietary glycerin (Table 6).

The activities of desaturases (6.37 and 70.25) and elongase (66.65) enzymes, the atherogenicity index (0.82), and the thrombogenicity index (1.88) were not affected by dietary glycerin (P<0.05). The amounts of hypocholesterolemic (43.74) and hypercholesterolemic (30.19) fatty acids and the h:H ratio (1.45) were not affected (P>0.05, Table 7) by dietary glycerin.

Discussion

According to Duarte et al. (2011) in unstressed animals, the values of muscle final pH usually range from 5.50 to 5.80. The final pH values observed in this study ranged from 5.63 to 5.66, resting within the normal range for ovine meat and indicating that an adequate process of *rigor mortis* occurred (Devine et al., 1993).

The replacement of corn by glycerin reduces the amount of dietary carotenoid pigments, and may have caused this decrease in yellowness that occurred uniformly in the three muscles. Despite the changes in yellowness, coloration variables remained within previously reported normal ranges of 30.03 to 49.47 for L*, 8.24 to 23.53 for a*, and 3.30 to 11.10 for b* (Sañudo et al., 2008).

The water holding capacity decrease in the three muscles between 24 h and 30 days after slaughter was expected. As previously described for fish meat, this decrease may result from protein denaturation during the freezing process or storage, which alters interfibrilar spaces, causes structural changes, and expels water (Shenouda, 1980).

The results for weight loss by thawing of muscles 30 days after slaughter differ from those reported by Lage et al. (2014a), who analyzed the *longissimus dorsi* of feedlot Santa Ines lambs and reported thawing losses differences related to dietary glycerin. However, these authors used very low-grade glycerin with only 36.2% glycerol, which could explain several discrepancies in their results.

Considering the results of shear force, the meat from the three muscles could be considered tender, as a previous study showed that an shear force below 5 kgf is considered tender (Tatum et al.,1999). Agreeing with this study, San Vito et al. (2014) found no difference in the shear force of *longissimus* muscle of Nellore bulls fed diets containing up to 28% glycerin.

The non-interference of the dietary glycerin in lipid oxidation can be aligned with the lack of change in muscle unsaturated fatty acids, as it is known that muscle lipid oxidation is associated with unsaturated fat content (Narciso-Gaytán et al., 2010).

The differences in sarcomere length were expected, as functional demands define muscle phenotype. Thus, the fatigue-resistant and red-fiber rich *longissimus lumborum* should indeed have a smaller fiber diameter and sarcomere length (Du et al., 2010).

The chemical composition values were within the recommended range of 75% moisture, 20% protein, 3% fat, and 2% non-protein substances including minerals, vitamins, and carbohydrates (Tornberg, 2005). Because propionate is the main substrate for gluconeogenesis, this alteration could result in a higher glucose uptake by animals, reducing amino acid and triglyceride mobilization for gluconeogenesis. Thus, increases in dietary glycerol may improve protein and fat deposition in lambs.

The difference in the cholesterol level was not expected because no differences were found in total fat content, and concentrations of hypo- and hypercholesterolemic fatty acids and these variables are highly associated (Costa et al., 2002). Nevertheless, the cholesterol level of the three muscles is considered low (<90 mg/100 g), even when compared with results (44.27 mg/100 g) from Almeida et al. (2014), who evaluated meat from Ile de France lambs slaughtered with similar weight. The meat cholesterol content has become an important issue to consumers. Several studies indicated that in the adult man, the serum cholesterol level is essentially independent of the cholesterol intake (Chizzolini et al., 1999).

Odd-chain fatty acids are formed through *de novo* synthesis from propionic acid, produced during rumen fermentation (Christie, 1981). Thus, the increase in the amount of C17:0 may result from the increase in rumen propionate induced by glycerol-rich diets (Drouillard, 2008). An increase in fat C17:0 content has previously been found in Arcott Canadian lambs fed diets containing up to 21% glycerin (Avila-Stagno et al., 2013). This increase in C17:0 does not pose hazards to human health (Scollan et al., 2006).

The PUFA:SFA ratio of 0.08 was lower than recommended for a healthy diet (Wood et al., 2003). However, this ratio is usually low in ruminant meat, and nutritional manipulation does not improve it because of ruminal bio-hydrogenation (Scollan et al., 2006). In relation to omega fatty acids, even without an increase in quantity and being present in small concentrations, the omega-3 fatty acid family benefits the human health, especially by reducing circulating LDL values (Harris et al., 2008).

Enzyme activity is maintained in parallel with stable levels of the substrates palmitic and stearic acids. On the other hand, AI and TI are related to pro- and anti-atherogenic acids, and low index values correspond to higher levels of anti-atherogenic fatty acids in fat, and, consequently, a greater preventive potential against coronary heart diseases

(Ulbright and Southgate, 1991). A previous study reported a similar maintenance of desaturase and elongase activities in bulls fed 18% glycerin, although the AI was reduced with increasing dietary glycerin (Carvalho et al., 2014).

The h:H ratio values are lower than those previously reported for Ile de France lambs feeding on diets containing alternative ingredients such as sunflower seeds and vitamin E (Almeida et al., 2014). As there are no recommended values, it is assumed that higher ratios equal healthier animal fat.

Conclusions

The replacement of corn with up to 20% dietary glycerin does not affect the physicochemical and nutritional characteristics of Ile de France lamb meat derived from three different muscles. Glycerin can be included in the diet of feedlot lambs without any harm to meat quality.

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