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Fatty acid profile, chemical composition, and sensory effects of crude glycerin on the *longissimus dorsi* of crossbred Boer goat kids

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ABSTRACT - The objectives of this trial were to evaluate the fatty acid profile, chemical composition, and sensory effects of crude glycerin on the *longissimus dorsi* muscle of crossbred Boer goat kids. Twenty crossbred Boer goat kids (20.8±2.9 kg of BW at slaughter) were used in a completely randomized block design to determine the effect of partial replacement of corn by crude glycerin on chemical composition, *longissimus dorsi* muscle fatty acid profile, and sensory characteristics of meat. Kids were penned individually for 51 d and fed an isonitrogenous (140.0±2.0 g.kg^{-1} CP, DM basis) diet composed of 700 g.kg^{-1} concentrate and 300 g.kg^{-1} Tifton (*Cynodon* sp.) hay. Increasing levels of crude glycerin (80.0 g/100 g glycerol, DM basis) were 0, 40, 80 or 120 g.kg^{-1}. There was no effect on the moisture, protein, or total lipids in the *longissimus dorsi*; however, the ash content decreased linearly with glycerin addition. Linear decrease for linoleic acid (3.57, 2.84, 3.76, and 2.33) and ω6:ω3 ratio (10.61, 9.71, 7.26, and 7.18 for CG0, CG40, CG80 and CG120, respectively) was observed with crude glycerin inclusion. Saturated, monounsaturated, and polyunsaturated fatty acids were not affected by treatments. In the sensory assessment, crude glycerin changed the toughness, color intensity, and overall appreciation of the *longissimus dorsi* muscle. The partial replacement of corn by crude glycerin has a low impact on chemical composition and meat fatty acid profile. Based on the overall appreciation, it is recommended to include 80 g.kg^{-1} crude glycerin in the diet.

Key Words: glycerol, meat flavor, meat quality

Introduction

The increase in biodiesel production has led to increased stocks of glycerol with a subsequent price reduction, making glycerol a potential high-energy feed source for ruminants (Avila et al., 2011). Crude glycerin is a co-product of biodiesel production with a high concentration of glycerol. Due to the high production of biodiesel, there is a wide availability of crude glycerin, and it is becoming an interesting ingredient for animal nutrition.

A potential application for glycerin is as a gluconeogenic substrate for ruminants (Chung et al., 2007). Glycerol can be converted to glucose in the liver and can provide energy for cellular metabolism (Goff and Horst, 2001). Glycerol enters the gluconeogenic pathway at the level of dihydroxyacetone phosphate and 3-phosphoglyceraldehyde (Leng, 1970; Krehbiel, 2008). Therefore, glycerin could be used as an energetic ingredient in animal diets replacing cereals, which are usually more expensive.

Several researchers (Eiras et al., 2014; Lage et al., 2014) have estimated the energy value of crude glycerin in beef and lamb diets. However, to our knowledge, there are no studies that evaluate the effects on the meat quality of glycerin supplementation to finishing goat kids fed high-concentrate diets.

The objective of this study were to determine the effects of the partial replacement of corn by crude glycerin on the chemical composition, sensorial characteristics, and fatty acid profile of the *longissimus dorsi* muscle in goat kids.

Material and Methods

The experiment was conducted in Northeast Brazil (03°44'33" S, 43°21'21" W). Experimental protocols were approved by the Institutional Animal Care and Use Committee of Universidade Federal do Maranhão (Case no. 23115.003553/2012-74).

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Twenty male castrated crossbred Boer goat kids, with an initial body weight (BW) of 16.1±2.1 kg, at 90±18 days of age, were used in this study. The animals were housed in covered pens (1 animal/pen) with concrete floor and dimensions of 1.3 m × 3.5 m. All animals were dewormed with 1 g/kg moxidectin (Cydectin, Fort Dodge Animal Health, Campinas, SP, Brazil) at a dose of 1 mL/50 kg of BW.

Initially, kids were subjected to a period of 14 days of adaptation to the experimental diets. After this, animals were divided in a randomized complete block design with five blocks and four experimental treatments. The treatments were defined by four concentrations of crude glycerin (0, 40, 80, and 120 g/kg in the dietary DM) as a substitute of ground corn (Table 1). Experimental diets were composed of 300 g.kg^{-1} of Tifton 85 (*Cynodon* sp.) hay and 700 g.kg^{-1} of concentrate (DM basis). Diets were isonitrogenous (140 g.kg^{-1} CP, DM basis) and were formulated according to the National Research Council for a gain of 150 g.day^{-1} (NRC, 2007).

The crude glycerin used in this study originated from vegetable oils of castor bean, soybean, cottonseed, and sunflower and had 0.07 g/100 g of methanol and 80.0 g/100 g of glycerol. It was produced by methylic route and obtained from Petrobrás Biocombustíveis S.A, Quixadá-CE, Brazil.

Kids were fed once a day at 08.00 h. The amount of feed offered to animals were calculated according to the previous dry matter intake (DMI) and adjustments were made whenever necessary for the refused feed not to exceed 100 g.kg^{-1} of daily intake.

At the end of the 51 days of the feeding trial, the animals (20.8±2.9 kg BW) were subjected to solid fasting and free water for 16 h and slaughtered in accordance with the RIISPOA standards (Brasil, 1997).

Table 1 - Ingredients and chemical composition of experimental diets (g.kg^{-1}, DM basis)

Ingredient	Diet[1]			
	CG0	CG40	CG80	CG120
Tifton hay	300.0	300.0	300.0	300.0
Ground corn	530.0	470.0	420.0	378.0
Soybean meal	143.0	163.0	173.0	175.0
Crude glycerin	0.0	40.0	80.0	120.0
Limestone	12.0	12.0	12.0	12.0
Mineral premix[2]	15.0	15.0	15.0	15.0
Chemical analysis				
Dry matter, as-fed basis	856.4	856.3	857.2	858.1
Crude protein	136.0	140.9	140.9	139.3
Neutral detergent fiber	323.4	319.8	315.7	311.4

[1] CG0 - 0 g.kg^{-1}, no crude glycerin; CG40 - 40 g.kg^{-1} crude glycerin; CG80 - 80 g.kg^{-1} crude glycerin; CG120 - 120 g.kg^{-1} crude glycerin.
[2] Composition: Ca - 130.4 g.kg^{-1}; P - 70.5 g.kg^{-1}; Mg - 10 g.kg^{-1}; S - 70 g.kg^{-1}; Cl - 210.8 g.kg^{-1}; Na - 140.5 g.kg^{-1}; Mn - 1,100 mg.kg^{-1}; Fe - 500 mg.kg^{-1}; Zn - 4,600 mg.kg^{-1}; Cu - 300 mg.kg^{-1}; Co - 40 mg.kg^{-1}; I - 55 mg.kg^{-1}; Se - 30 mg.kg^{-1}.

The *longisimus dorsi* muscle was separated from the bone for quantitative assessments of chemical composition, lipid profile, and sensory characteristics. They were packed in plastic bags, and finally frozen in commercial freezers at –18 ºC for a period not exceeding two months, when the analyses were performed. All goat meat evaluations were performed in triplicate.

Longissimus dorsi muscle samples were thawed in a refrigerator for 24 h. Then, they were cleaned and ground in a blender until obtaining a homogeneous sample. The moisture content was determined by drying the meat in an oven at 105 ºC until constant weight (Method 985.41; AOAC, 2000); the ash content was obtained by burning the material in muffle at 550 ºC (Method 920.15; AOAC, 2000); and the nitrogen content was determined through the Kjeldahl method, using the factor of 6.38 to convert the total nitrogen into protein nitrogen (Method 928.08; AOAC, 2000). The total lipid concentrations were measured in accordance with the method of Folch et al. (1957), by subjecting the sample to extraction with a mixture of chloroform and methanol (2:1 ratio).

After extraction of total lipids, fatty acid methyl esters were prepared by direct esterificationof lipids in muscle tissue, according to Hartman and Lago (1973). The resulting fatty acid methyl esthers (FAME) were then analyzed by GLC using a VARIAN 430-GC (California, USA) equipped with a flame ionization detector. The separation occurred in a fused silica capillary column (CP WAX 52 CB, VARIAN), polar type, packed with Polyethylene, with dimensions of 60 m × 0.25 mm id × 0.2 µ of film thickness. Methyl esters samples (2 µL) were injected into a split/splitless type injector at 250 ºC. The chromatograms, with retention times and the percentages of areas of fatty acids, were recorded using the Galaxie Chromatography Data System software. Helium was used as the carrier gas (1 mL/min). The initial oven temperature was 100 °C, set to reach 240 °C, adding 2.5 °C per minute, standing for 20 min. The injector and detector temperatures were maintained at 250 °C and 260 °C, respectively. The fatty acids were identified by comparing retention times of the methyl esters of the standards samples (SupelcoME19-Kit - Fatty Acid Methyl Esters C6-C22).

The auxiliary gases were nitrogen (30 mL/min), hydrogen (30 mL/min), and synthetic air (300 mL/min). The fatty acids were identified by comparing the retention times of methyl esters of the samples with authentic standards (Merck, USA). The fatty acids results were expressed in g/100 g.

The day before sensory assessment, samples were thawed initially at room temperature (6 h) and then kept at 4 ºC overnight. In the morning of sensory assessment, the *longissimus dorsi* muscle sample was cut into 2.0 cm-thick steaks and a section from the lateral edge of the steak (the ''tail''), consisting mainly of fat with a small amount of muscle, was also removed.

Chops were placed on a domestic grill (Tricity Double Oven and Grill, Model 2142, Thorn Domestic Appliances, England, UK) and cooked, turning every 3 min, to an internal muscle temperature of 75 ºC as measured by a thermocouple probe (Comark, Model 9001, fitted with a K-type thermocouple, Stevenage, Hertfordshire SG12TA, UK) inserted into the approximate geometric center of each steak.

Ten trained taste panel members assessed lamb juiciness intensity, toughness, color intensity, aroma, flavor, and overall appreciation using a non-structured line scale measuring 9 cm. More details are in Sanudo et al. (1998).

Unsalted crackers and water at room temperature were used to dissipate residual flavors and particles among evaluations.

Data were analyzed as a completely randomized block using the SAS (Statistical Analysis System, version 9.0) software. The blocks were defined according to the initial BW of the kids. A check of homoscedasticity of variance was performed using Levene's test. The MIXED procedure was used to analyze the effects of treatment on meat quality traits, with the animal serving as the experimental unit according to the following statistical model: $Y = \mu + Bi + Dj + Eij$, in which: μ = overall mean; Bi = effect of block; Dj = effect of diet; and Eij = the residual error. Means were obtained using the LSMEANS command. The blocks and animals were included as random effects, while diet was included as fixed effect.

Orthogonal polynomials for treatment responses were determined by linear and quadratic responses to increasing concentrations of crude glycerin incorporation. Effects were declared significant at $P<0.05$ and trends were discussed between $P>0.05$ and $P<0.10$. For the sensory analysis, means were compared using the Ryan-Einot-Gabriel-Welsch test ($P<0.05$).

Results

The addition of crude glycerin did not change the moisture, protein, or total lipid concentrations of the meat (Table 2). However, the ash content (g/100 g) decreased linearly ($P<0.01$) with the increase of crude glycerin concentration in the diets.

There was no effect of crude glycerin on the total concentrations of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids of the meat (Table 3). Additionally, meat monounsaturated: saturated fatty acid (M:S) and polyunsaturated:saturated fatty acid (P:S) ratios and the (C18:0+C18:1)/C16:0 ratio were not affected by the experimental diets (Table 3). However, the linoleic concentrations and the ω6:ω3 ratio in the meat decreased linearly ($P<0.05$) with the inclusion of crude glycerin.

There were no significant differences in juiciness, flavor, and aroma among treatments, with the average values within the range of 4.59 to 5.44 for juiciness, 4.16 to 4.62 for flavor, and 4.19 to 4.85 for aroma (Table 4). However, diet affected toughness, color, and overall appreciation of the meat ($P<0.05$).

The toughness was lower when kids were fed 40 $g.kg^{-1}$ of crude glycerin in the diet in comparison with 80 $g.kg^{-1}$and 120 $g.kg^{-1}$ of crude glycerin. However, the value did not differ from the control diet.

The color was less expressive ($P<0.05$) when animals were fed diets with 80 $g.kg^{-1}$ of crude glycerin. The meat of kids fed the control diet and 40 $g.kg^{-1}$ of crude glycerin showed higher ($P<0.05$) overall appreciation when compared with those fed 120 $g.kg^{-1}$ of crude glycerin. However, the appreciation values did not differ ($P>0.05$) from the meat of kids fed 80 $g.kg^{-1}$ of crude glycerin.

Table 2 - Chemical composition of the *longissimus dorsi* from crossbred Boer goat kids fed glycerin

Item (g/100 g)	Diet[1]				SEM	Effect[2]	
	CG0	CG40	CG80	CG120		L	Q
Moisture	72.73	72.00	73.15	72.69	0.41	0.590	0.747
Protein	23.06	23.06	22.66	22.53	0.23	0.076	0.767
Total lipids	3.43	3.83	3.06	3.71	0.42	0.973	0.778
Ash	1.14	1.09	1.11	1.06	0.01	0.004	0.938

SEM - standard error of the mean.
[1] CG0 - 0 $g.kg^{-1}$, no crude glycerin; CG40 - 40 $g.kg^{-1}$ crude glycerin; CG80 - 80 $g.kg^{-1}$ crude glycerin; CG120 - 120 $g.kg^{-1}$ crude glycerin.
[2] Linear (L) and quadratic (Q) effects.

Table 3 - Fatty acid profile of the *longissimus dorsi* muscle from crossbred Boer goat kids fed crude glycerin

Item	Diet[1]				SEM	Effect[2]	
	CG0	CG40	CG80	CG120		L	Q
Saturated	42.69	42.47	41.35	42.97	1.61	0.952	0.386
C10:0 (Capric)	0.13	0.09	0.09	0.07	0.01	0.066	0.668
C12:0 (Lauric)	0.09	0.08	0.06	0.05	0.01	0.222	0.878
C14:0 (Miristic)	1.44	1.51	1.68	1.55	0.10	0.308	0.391
C16:0 (Palmitic)	20.45	20.72	20.18	20.55	0.52	0.923	0.921
C18:0 (Estearic)	16.14	15.88	14.69	16.46	1.19	0.963	0.357
Monounsaturated	52.42	53.45	53.07	53.37	1.96	0.578	0.697
C16:1 (Palmitoleic)	1.70	1.76	1.73	1.98	0.18	0.285	0.569
C18:1 ω-9 (Oleic)	48.00	48.68	47.59	47.84	1.66	0.670	0.793
C18:1 ω-9 trans (Elaidic)	2.02	1.73	1.93	1.80	0.12	0.404	0.507
C20:1 ω-9 cis (Eicosanoic)	0.11	0.11	0.12	0.13	0.01	0.054	0.386
Polyunsaturated	4.83	4.02	5.60	3.76	0.58	0.284	0.156
C18:2 ω-6 (Linoleic)	3.57	2.84	3.76	2.33	0.35	0.017	0.093
C20:2 ω-6 (Eicosadienoic)	0.60	0.59	0.81	0.60	0.10	0.669	0.322
C20:3 ω-6 (dihomo -γ- linolenic)	0.20	0.19	0.26	0.20	0.02	0.222	0.095
C20:5 ω3 (EPA)	0.40	0.40	0.58	0.40	0.06	0.437	0.265
Others	4.54	4.85	6.18	6.34	0.58	0.034	0.901
M:S	1.24	1.27	1.30	1.27	0.09	0.837	0.470
P:S	0.12	0.10	0.13	0.09	0.01	0.494	0.565
ω6:ω3	10.61	9.71	7.26	7.18	1.13	0.027	0.680
(C18:0+C18:1)/C16:0	3.16	3.14	3.10	3.08	0.11	0.591	0.988

SEM - standard error of the mean.
M:S - ratio between monounsaturated and saturated fatty acids; P:S - ratio between polyunsaturated and saturated fatty acids; ω6:ω3 - ratio between ω6 and ω3 fatty acids.
[1] CG0 - 0 g.kg^{-1}, no crude glycerin; CG40 - 40 g.kg^{-1} crude glycerin; CG80 - 80 g.kg^{-1} crude glycerin; CG120 - 120 g.kg^{-1} crude glycerin.
[2] Linear (L) and quadratic (Q) effects.

Table 4 - Sensory assessment of the *longissimus dorsi* from crossbred Boer goat kids fed crude glycerin

Item (points)	Diet[1]				SEM
	CG0	CG40	CG80	CG120	
Toughness	4.55ab	3.56a	4.75b	4.76b	0.25
Juiciness	5.23	4.59	5.44	4.99	0.22
Flavor	4.48	4.62	4.52	4.16	0.25
Color	5.24a	5.62a	4.35b	5.20a	0.23
Aroma	4.58	4.85	4.42	4.19	0.29
Overall appreciation	7.10a	6.86a	6.44ab	5.94b	0.19

Means in the same row followed by different letters differ.
SEM - standard error of the mean.
[1] CG0 - 0 g.kg^{-1}, no crude glycerin; CG40 - 40 g.kg^{-1} crude glycerin; CG80 - 80 g.kg^{-1} crude glycerin; CG120 - 120 g.kg^{-1} crude glycerin.

Discussion

In the present study, it was expected that the inclusion of crude glycerin in the diets would increase the intramuscular fat content because, according to Schoonmaker et al. (2004), the crude glycerin likely would decrease the acetate: propionate ratio in the rumen, possibly due to the increases in propionate, the precursor of glucose, which is the main carbon source for the deposition of fat tissue.

According to Smith and Crouse (1994), in research conducted with Angus cattle, the glucose that originated from the propionic acid is responsible for up to 10 g.kg^{-1} of the acetyl units in the subcutaneous fat tissue, and 500 to 750 g.kg^{-1} of the intramuscular fat tissue. However, the absence of an improvement in intramuscular fat accretion in his study seemingly refutes this hypothesis because the feeding of crude glycerin may result in the suppression of intramuscular fat accretion (Drouillard, 2012) or may tend to decrease the deposition of intramuscular fat within the *longissimus dorsi* muscle (Elam et al., 2008).

The ash content decreased linearly with crude glycerin supplementation. There was no biological explanation for this result because the ash content is usually affected by the age of the animal (Madruga et al., 1999) and not by diet effects. In this study, kids were slaughtered at similar ages (150±18 days old). The ash values of the meat were close to those found by Lage et al. (2014) in research with lambs fed diets composed of 300 g.kg^{-1} of corn silage and 700 g.kg^{-1} concentrate (DM basis).

There was no effect of crude glycerin on the total concentrations of saturated, monounsaturated, and polyunsaturated fatty acids in the meat (Table 3). The main

fatty acids found in the *longissimus dorsi* muscle were C18:1ω-9 cis (oleic), C16:0 (palmitic), C18:0 (stearic), and C18: 2ω-6 (linoleic) (Table 3). This is in agreement with the view by Banskalieva et al. (2000) who, after assessments in several studies, found same fatty acids as the main muscle lipids in goats.

Saturated and monounsaturated fatty acids represented approximately 42.4 g/100 g and 53.1 g/100 g of total fatty acids (FA) in the *longissimus* muscle (Table 3), respectively. Krueger et al. (2010) reported that glycerol has the potential to increase the amount of unsaturated fatty acids available to be incorporated in meat products, due to the inhibition of lipolysis in the rumen. However, in this study, the total concentrations of polyunsaturated fatty acids in the meat did not change with crude glycerin inclusion.

Linoleic acid (C18:2) decreased ($P<0.05$) with an increase in the crude glycerin levels. The lower content of this FA in the *longissimus* muscle of goat kids might be related to the replacement of corn by glycerin because the concentration of linoleic acid is higher in corn.

The PUFA:SFA ratio was not affected by the addition of crude glycerin and the values varied from 0.09 (0 $g.kg^{-1}$ of crude glycerin) to 0.12 (120 $g.kg^{-1}$ of crude glycerin). This ratio is below the recommended rate of 0.40, the minimum value that is considered beneficial to human health.

The ω6:ω3 ratio had an average of 8.7, which is not considered healthy and is above the rate of 4 recommended by Wood et al. (2003). This high ratio may be explained by the higher proportion of concentrate in the diets, which increases the total ω6 fatty acids in the diets.

The crude glycerin decreased linearly ($P<0.05$) the ω6:ω3 ratio (10.61 to 7.18 g/100 g), due to the addition of glycerin in replacement by corn, which is rich in linoleic fatty acid (ω6).

Considering that oleic fatty acid (C18:1) decreases the plasma cholesterol level in the blood, while palmitic fatty acid (C16:0) raises the level and stearic acid (C18:0) has no influence (Rhee et al., 2000), it is important to analyze the behavior of these three fatty acids. Therefore, the (C18:0+C18:1)/C16:0 ratio could possibly better describe the effects of different fatty acids on human health. The mean value found in this study for this ratio was 3.12, which is above the values found in the review carried out by Banskalieva et al. (2000), which are between 2 and 3 for goat meat.

The results for juiciness (5.06 points) were considered very good (Lepetit, 2008) (Table 4). Juiciness is related to moisture and intramuscular contents present in the cooked meat. Because the chemical composition of the meat, except the ash content, was not altered by the increases of crude glycerin concentration, there was also an absence of effect for juiciness.

Aroma is also an important attribute when purchasing a product. According to Madruga (1997), the aroma and taste of the meat are directly associated with the fat content in the muscle. In the present study, the treatments produced no changes in these qualities, which demonstrated that crude glycerin did not change the properties that alter the flavor and aroma on the meat of goat kids. Similar sensory attributes were found by Eiras et al. (2014), who evaluated the meat of cattle fed diets with up to 180 $g.kg^{-1}$ crude glycerin.

According to Mancini and Hunt (2005), meat color is an important commercial trait that influences consumer behavior. Although the treatment with 80 g kg^{-1} of crude glycerin produced a less expressive color (4.35) (Table 4), this value was considered satisfactory (Mancini and Hunt, 2005). This outcome confirmed that the substitution did not alter the color attribute of goat meat or cause rejection by the tasters and that the acceptance of color depends on several factors, among them regional consumption habits and customs (Pinheiro et al., 2010).

As previously mentioned, juiciness and flavor can influence the results for overall appreciation (Hocquette et al., 2012). However, consumers did not detect differences in these characteristics in the meat. Indeed, meat appreciation may be altered by toughness (Wood et al., 2008).

Conclusions

The inclusion of crude glycerin does not affect the chemical composition, except of ash, of the meat of goat kids and promotes improvement in the ω6:ω3 ratio. Based on its overall appreciation, it is recommended to include 80 $g.kg^{-1}$ crude glycerin in the diet.

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