Effects of crude glycerin from biodiesel on the diets of lambs: intake, digestibility, performance, feeding behavior, and serum metabolites

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ABSTRACT: This study was conducted to test the hypothesis that the inclusion of crude glycerin of up to 21% of DM in the diets of lambs will not compromise variables related to animal production or health. Forty-four uncastrated Santa Inês crossbreed lambs with an average age of 5 mo and a BW average of 23.9 ± 0.34 kg (mean ± SD) were distributed into four treatment groups (11 replicates per group) including 0%, 7%, 14%, or 21% crude glycerin on a DM basis. The inclusion of crude glycerin in the diets promoted a quadratic increase in DM ($P = 0.018$), CP ($P = 0.004$), and TDN ($P = 0.017$). There was a linear decrease ($P < 0.001$) in NDF and nonfibrous carbohydrate (**NFC**) intake caused by glycerin in the diets. There was a linear increase ($P < 0.001$) in ether extract (**EE**) intake. There was a linear reduction in NDF ($P = 0.011$) and NFC ($P < 0.001$) for effective consumption of the diets. There was a linear increase ($P < 0.001$) in EE effectively consumed by the lambs, and there were no differences in the CP that was effectively consumed ($P = 0.267$) by glycerin. Digestibility coefficients of DM, CP, NDF, NFC, and TDN presented a linear decrease in crude glycerin inclusion. The EE digestibility presented a linear increase. The inclusion of crude glycerin in the diets promoted a quadratic increase in final BW ($P = 0.015$), ADG ($P < 0.001$), and G:F ratio ($P < 0.001$). There was no effect ($P > 0.05$) of crude glycerin inclusion in the diets on time spent (%); number of events per day; duration of events (minutes) for feeding, rumination, and idling; number of chews per bolus; or total chewing time for the lambs. The inclusion of crude glycerin in the diets improved feeding efficiency of DM ($P = 0.005$) and NDF ($P = 0.004$). The rumination efficiency of DM ($P < 0.001$) and NDF ($P < 0.001$) presented a linear decrease. The total protein, albumin, globulin, albumin:globulin ratio, triglycerides, alanine aminotransferase levels, aspartate aminotransferase, and gamma glutamyl transferase serum concentrations did not differ ($P > 0.05$) through the addition of crude glycerin to the diets of the lambs. However, the crude glycerin in the diets led to a linear decrease in urea-N ($P = 0.004$) and glucose ($P < 0.001$), as well as a linear increase in the cholesterol ($P = 0.043$) serum concentrations of the lambs. The recommended inclusion of crude glycerin is up to a 4.7% DM level because of improved performance growth without compromising feeding behavior and blood metabolites.

Key words: byproduct, energy, feeding, glucose, propionate

J. Anim. Sci. 2018.96:1952–1961
doi: 10.1093/jas/sky075

INTRODUCTION

Biodiesel production from vegetal oils is a sustainable way to meet worldwide energy demands since these oils are derived from renewable

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Received October 19, 2017.
Accepted March 2, 2018.

sources. The chemical process of biodiesel production is often based on a transesterification reaction with an alkaline reagent that results in a byproduct called crude glycerin, which is mainly composed of glycerol and other components, such as lipids, alcohol, and minerals (Chanjula et al., 2014). The use of glycerin in animal feed has been studied in the past. However, the recent increase in biodiesel production and subsequent availability of crude glycerin has increased interest in using this byproduct in the diets of ruminants since it constitutes an energy-rich product (4.320 kcal of crude energy/kg pure glycerol) with the goal of reducing production costs (Lage et al., 2010; Chanjula et al., 2016a). Glycerol, after being ingested by a ruminant animal, can be directly absorbed by the ruminal epithelium (Redmond et al., 1993) and converted into glucose in the liver (Trabue et al., 2007), or it can be used as a substrate for the ruminal microbial population to produce VFA (Roger et al., 1992). The most important VFA produced is propionate, which is an important gluconeogenic compound for ruminants (Hales et al., 2013; van Cleef et al., 2016). Studies have been conducted to evaluate the use of glycerin in the diets of bulls (Chanjula et al., 2016b; Moreira et al., 2016; van Cleef et al., 2017), dairy cows (Fisher et al., 1973; Donkin et al., 2009), goats (Chanjula et al., 2015; Dias et al., 2015), and lambs (Gunn et al., 2010a, 2010b; Barros et al., 2015; Borghi et al., 2016; Cunha et al., 2016). Those studies mostly observed that inclusion of crude glycerin in levels greater than 15% DM in the diets modifies the ruminal parameters and subsequently affects the microbial fermentation pattern, feeding intake, digestibility, feeding behavior and performance growth, as observed in a review from Beserra et al. (2016).

However, this study aimed to test the hypothesis that inclusion of crude glycerin at greater concentrations (up to 21% of DM) in the diets of lambs will not compromise the variables related to animal production or health. Thus, we conducted a trial to evaluate the feed intake and digestibility of nutrients, performance growth, feeding behavior, and blood serum metabolites.

MATERIAL AND METHODS

Ethical Considerations

This study was conducted according to the recommendations in the Guide for the National Council for the Control of Animal Experimentation (**CONCEA**). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Federal University of Bahia, Bahia State, Brazil (Permit Number: 02-2014).

Animals and Trial Duration

Forty-four uncastrated Santa Inês crossbreed lambs with an average age of 5 mo and an average BW of 23.9 ± 0.34 kg (mean ± SD) were distributed into four treatment groups (11 replicates per group). The animals were housed in a covered shed in individual 1.6-m^2 pens with ad libitum access to water, feed, and minerals. The experiment lasted 75 d with 14 d of adaptation to the installation, location, and diet. Early in the adjustment period, the animals were identified and treated to control internal and external parasites.

Experimental Design, Diets, and Chemical Analyses

The experimental design was completely randomized with four treatments (diets) and 11 replicates (animals) per treatment. The crude glycerin (Table 1), used in the diets, was obtained from a biodiesel industry, located in the city of Iraquara, Bahia, Brazil. The diets were formulated based on the NRC (2007) requirements for an ADG of 200 g. The concentrated feed was composed of corn bran; soybean meal; mineral premix (Table 2); and 0%, 7%, 14%, or 21% crude glycerin on a DM basis (Table 3). The forage was *Cynodon dactylon* hay chopped into approximately 5-cm pieces.

Samples (triplicate) of crude glycerin were analyzed in the beginning of the trial to determine the chemical composition. Water content was determined by the Karl Fischer method, ASTM method D 6304 07, according the American Society for Testing and Materials (2007), the ash content was determined according the Hautfenne (1980), by the method 3.135, the glycerol and alcohol content was

Table 1. Chemical composition of crude glycerin used in the diets fed to crossbred Santa Inês lambs

Item	Value
Glycerol (% of mass)	55.1
Water (% of mass)	8.80
Alcohol (% of mass)	2.60
Total fatty acids (% of mass)	29.9
Ash (% of mass)	3.60
Sodium (mg/kg)	13,465
Potassium (mg/kg)	406
Calcium (mg/kg)	36.6
Magnesium (mg/kg)	14.3
Phosphorus (mg/kg)	1,623

Table 2. Chemical composition of ingredients used in the diets fed to the crossbred Santa Inês lambs

Chemical composition (g/kg)	*Cynodon* sp. hay	Corn bran	Soybean meal
DM (g/kg as fed)	912	892	931
Ash	60.8	12.2	64.3
CP	51.4	65.8	500
Ether extract	11.3	43.9	18.7
NDF*	752	131	156
ADF	380	37.3	77.2
NIDN[£]	386	213	111
ADIN[†]	68.6	102	59.7
ADL	53.6	10.8	8.5
Cellulose	327	26.5	68.7
Hemicellulose	372	93.7	79.2
Nonfiber carbohydrates	125	747	260

*Corrected for ash and protein.

[†]g/kg CP.

Table 3. Content proportions and chemical compositions of the experimental diets with crude glycerin fed to the crossbred Santa Inês lambs

	Crude glycerin (% DM total)			
Item	0	7	14	21
Ingredient (g/kg DM)				
Corn bran	250	167	83.0	0.00
Crude glycerin	0.00	70.0	140	210
Soybean meal	235	248	262	275
Mineral Premix*	15.0	15.0	15.0	15.0
Cynodon sp. hay	500	500	500	500
Chemical composition (g/kg DM)				
DM (g/kg as fed)	923	924	924	925
Ash	63.6	65.9	68.3	70.6
CP	160	161	162	163
Ether extract	21.0	38.5	56.0	73.5
NDF[†]	446	437	428	419
ADF	218	216	214	212
Nonfiber carbohydrates	310	298	286	274
Total digestible nutrients[‡]	673	694	716	738

*Assurance levels (per kilogram of active elements): 120 g of calcium, 87 g of phosphorus, 147 g of sodium, 18 g of sulfur, 590 mg of copper, 40 mg of cobalt, 20 mg of chromium, 1,800 mg of iron, 80 mg of iodine, 1,300 mg of mansganese, 15 mg of selenium, 3,800 mg of zinc, 300 mg of molybdenum, and maximum 870 mg of fluoride.

[†]Corrected for ash and protein.

[‡]Estimated values based on the NRC (2001).

determined according to the Brazilian National Sanitary Inspection Agency Guidelines (Brasil 2010), and the mineral composition was determined by the technique of inductively coupled plasma optical emission spectrometry, ASTM method D 5708:2005, according the American Society for Testing and Materials (2005).

Samples (triplicate) of the ingredients, the remaining feed, and fecal samples were pre-dried at 55°C for 72 h and ground with a Willey mill (Tecnal, Piracicaba City, São Paulo State, Brazil) using a 1-mm sieve then stored in airtight plastic containers (ASS, Ribeirão Preto City, São Paulo State, Brazil) and sealed properly until needed for a laboratory analysis to determine the chemical composition (in triplicate) according to the Association of Official Analytical Chemists (AOAC 2012). The DM (method 967.03), ash (method 942.05), CP (method 981.10), and ether extract (**EE**; method 920.29) were determined.

The NDF and ADF were determined according to the methods of Van Soest et al. (1991) with modifications that were proposed in the Ankom device manual (Ankom Technology Corporation, Macedon, New York, United States). NDF was corrected for ash and protein. The NDF residue was incinerated in an oven at 600°C for 4 h, and the protein correction was determined by subtracting the neutral detergent-insoluble protein (**NDIP**). The ADL was determined according to AOAC (2012) method 973.18, in which the ADF residue was treated with 72% sulfuric acid.

The nonfibrous carbohydrates (**NFC**) were calculated according to Mertens (1997) using the value of NDF corrected for ash and protein. The NDIP and acid detergent-insoluble protein (**ADIP**) contents were determined according to the methods of Licitra et al. (1996).

The TDN contents were calculated according to estimation formulas of digestibility for each analytical fraction (NRC 2001):

$$\mathrm{DNFC} = 0.98 \times (\mathrm{NFC})$$
$$\mathrm{DCP} = \mathrm{CP} \times \left[1 - (0.4 \times \mathrm{ADIN}/\mathrm{CP})\right]$$
$$\mathrm{DEE} = \mathrm{EE} - 1$$

$$\mathrm{DNDF} = 0.75 \times (\mathrm{NDFap} - \mathrm{ADL}) \times [1 - (\mathrm{ADL}/\mathrm{NDFap}) \times 0.667] - 7,$$

where DNFC is the digestible NFC, **DCP** indicates digestible CP, **DEE** is the digestible EE, **DNDF** is the digestible NDF, and **NDFap** is the NDF corrected for protein.

Nutrient Intake and Digestibility

Nutrient intake was determined by subtracting the amount of each nutrient contained in the refused feed from the total of each nutrient in the feed that was offered.

The digestibility assay was performed from 42 d to 49 d of the feedlot trial. The feces and orts from each animal were collected and quantified during this period (total collection). The animals were fitted with appropriate canvas bags for total fecal collection. After a period of 4 days of adaptation to the canvas bags, two daily fecal collections in the morning (8:00 h) and afternoon (16:00 h) were performed for seven consecutive days. Every day, a 30% aliquot of the total amount of the feces was collected and stored in plastic bags.

Composite samples of the feces and orts of each animal were prepared throughout the collection period and stored at –20°C until analysis. The samples were then thawed, dried in a forced-air oven at 55°C for 72 h, ground using a Willey cutting mill equipped with a 1-mm mesh sieve, and used for chemical analysis.

The digestibility coefficients (**DC**s) of DM, CP, NDF, and EE were calculated as follows: DC = [(kg of the portion ingested − kg of the portion excreted)/(kg of the portion ingested)] × 100.

The intake of TDN was calculated according to Sniffen et al. (1992) using the equation ITDN = [(ICP − CPf) + 2.25 (IEE − EEf) + ITC − TCf], in which ICP, IEE, and ITC represent the intake of CP, EE, and TC, respectively, and CPf, EEf, and TCf refer to the excretion of CP, EE, and TC, respectively, in the feces. Concentrations of TDN were obtained from the following equation: TDN = (TDN intake/DM intake) × 100.

The animals were individually weighed at the beginning of the experiment and again every 21 d to determine the performance of the lambs and their ADG. The weight measurements were performed in the morning before the first daily feeding and after a continuous fasting period of approximately 16 h.

Feed efficiency (G/F) was determined using the average daily weight gain divided by the DMI average of the goats fed the different diets, and ADG was calculated based on the differences in the initial and final BWs of the animals divided by the number of days in the trial period. Values were expressed as g/g.

Ingestive Behavior

Individual observations of the animals were performed on days 16, 31, and 46 for 24 h at 5-min intervals to evaluate ingestive behavior according to the methods of Johnson and Combs (1991). Two observers for each animal were positioned to interfere as little as possible with the animal behavior, and they recorded data on behavioral activities (feeding, rumination, and idling). The observers took turns every 3 h, and nighttime observations were conducted using artificial lighting. The observers counted both the number of ruminating chews and number of boluses ruminated per day. Additionally, the time and number of chews for each ruminal bolus per animal were recorded.

The feeding efficiency (**FE**), rumination efficiency (**RE**), and total chewing time (**TCT**, h/d), as the sum of the feeding time and ruminating (FT + RT), were determined according to the methods of Bürger et al. (2000). The results for the feeding behavior parameters were obtained using the following equations:

$$NRB = RT / NC;$$
$$NC = NRB \times NC;$$
$$FEDM = DMI / FT;\ FENDF = NDFI / FT;$$
$$REDM = DMI / RT;\ RENDF = NDFI / RT;\ \text{and}$$
$$TCT = FT + RT,$$

where **NRB** = number of ruminal boli, **NC** = daily number of chews, **FEDM** = feed efficiency of DM (g DM intake/h), **FENDF** = feed efficiency of NDF (g NDF intake/h), **DMI** (g) = daily DM intake, **NDFI** (g) = daily NDF intake, **FT** = time spent feeding daily, **REDM** = RE of DM (g of ruminated DM/h), **RENDF** = RE of NDF (ruminated NDF/h), **RT** (h/d) = rumination time, and TCT = total chewing time (h/d). For each animal, three measurements were obtained (using 3 days of ingestive behavior analysis and two observers) for each parameter. Based on these three measurements, the mean of each parameter was calculated for each animal for statistical analysis.

Blood Sample Collection and Parameters Analyzed

Before feeding, blood samples (10 mL) were collected by puncturing the jugular vein of all lambs and storing the samples in non–anti-coagulant vacutainers (BD Vacutainer, Franklin Lakes, NJ, United States) on the 60th day. Samples were centrifuged at 350 × *g* for 15 min, and the serum was frozen at −20°C until it was analyzed for total protein, albumin, globulin, A:G ratio, urea-nitrogen, glucose, cholesterol, triglycerides, alanine aminotransferase levels (**ALT**), aspartate aminotransferase (**AST**), and gamma glutamyl transferase (**GGT**) serum concentrations. These constituents were assayed in duplicate using colorimetric commercial kits from the Labtest (Labtest, São Paulo, Brazil) brand.

Statistical Analyses

To determine the relationship between the level of crude glycerin inclusion and each lamb that was evaluated, a polynomial contrast was used to determine the linear and quadratic relationships using PROC GLM from SAS (SAS Inst. Inc., Cary, NC). The initial BW was used in the statistical model as a covariate when it was significant.

For temporal analysis (urea-N and glucose), a mixed model was generated (the PROC MIXED) with treatments (3 degrees of freedom), postprandial time (3 degrees of freedom), and their interactions (9 degrees of freedom) as fixed effects. A first-order autoregressive "AR(1)" error structure was used by selecting a low Bayesian Information Criterion (**BIC**) value. Significance was declared when $P < 0.05$.

RESULTS

The inclusion of crude glycerin in the diets promoted a quadratic increase in DM ($P = 0.018$), CP ($P = 0.004$), and TDN ($P = 0.017$) with the regression equation indicating that the 4.52% crude glycerin inclusion led to greater DM intake (Table 4). There was a linear decrease ($P < 0.001$) in NDF and NFC intake when glycerin was included in the diets of lambs. However, there was a linear increase ($P < 0.001$) in EE intake.

There was a linear reduction in NDF ($P = 0.011$) and NFC ($P < 0.001$) for effective consumption. However, there was a linear increase ($P < 0.001$) in EE effectively consumed by the lambs, and the effectively consumed CP did not show any differences ($P = 0.267$) with glycerin inclusion in the diets of lambs.

The DCs of DM, CP, NDF, NFC, and TDN presented a linear decrease with the inclusion of crude glycerin (Table 4). For each 1% of crude glycerin that was added, there was a reduction of 0.87, 0.84, 1.62, and 0.39% in the DCs of DM, CP, NDF, and NFC, respectively. However, for the EE DC, there was a linear increase, with 0.31% more for each 1% of glycerin that was added.

The inclusion of crude glycerin in the diets promoted a quadratic effect on final BW ($P = 0.015$) and ADG ($P < 0.001$). Feed efficiency presented a linear increase ($P < 0.001$) through inclusion of crude glycerin in the diets of lambs. According

Table 4. Daily nutrient intake, diet consumed, digestibility coefficient, performance growth of the crossbred Santa Inês lambs fed diets with crude glycerin

	Crude glycerin (% DM)					*P* value*	
Item	0	7	14	21	SEM	Linear	Quadratic
Nutrient intake (g/d)							
DM	1,112	1,194	999	863	42.9	<0.001	0.018
CP	188	204	173	140	7.91	<0.001	0.004
Ether extract	24.8	48.1	59.3	68.5	1.97	<0.001	<0.001
NDF	457	485	388	341	18.3	<0.001	0.058
Nonfiber carbohydrates	373	369	296	238	13.2	<0.001	0.057
Total digestible nutrients	868	932	780	674	33.5	<0.001	0.017
Effectively consumed diet composition (g/d)							
CP	169	171	173	163	0.23	0.267	0.085
Ether extract	24.0	40.3	59.4	79.5	0.03	<0.001	0.002
NDF	410	407	388	395	0.45	0.011	0.322
Nonfiber carbohydrates	336	308	296	276	0.30	<0.001	0.258
Digestibility coefficient (%)							
DM	79.2	77.5	72.4	60.5	3.26	0.003	0.202
CP	79.2	76.5	73.2	60.7	3.47	0.006	0.263
Ether extract	86.2	92.5	93.1	93.3	1.30	0.014	0.084
NDF	71.4	68.8	61.8	35.8	6.28	0.006	0.153
Nonfiber carbohydrates	93.5	91.8	87.7	85.6	0.82	<0.001	0.839
Total digestible nutrients	78.1	78.1	74.8	65.1	3.01	0.019	0.203
Performance growth							
Initial BW (kg)	23.0	24.0	23.6	24.3	—	—	—
Final BW (kg)	37.9	40.4	36.0	32.3	1.27	<0.001	0.015
ADG (g/d)	191	200	158	103	8.85	<0.001	<0.001
G:F (g/g)	0.17	0.17	0.16	0.12	0.01	<0.001	0.017

*Significant at $P \leq 0.05$.

to the regression equation, the inclusion of crude glycerin of up to a 5.80% DM basis promoted an increase in BW gain, and the maximum weight observed was 39.3 kg. The same effect was observed for the ADG, which increased up to a 3.83% inclusion of crude glycerin.

There was no effect ($P > 0.05$) of crude glycerin inclusion in the diets on time spent (%); number of events per day; or duration of events (minutes) for feeding, rumination and idling performed by the lambs (Table 5). The number of chews per bolus and total time chewing were not affected ($P > 0.05$) by the tested levels of crude glycerin. The inclusion of crude glycerin in the diets affected the FE of DM ($P = 0.005$) and NDF ($P = 0.004$). According to the regression equations, for each 1% of crude glycerin added to the diets, there was a reduction of 14.7 g/h and 6.20 7 g/h in the FE for DM and NDF, respectively.

The RE of DM ($P < 0.001$) and NDF ($P < 0.001$) presented a linear decrease, and for each 1% of crude glycerin, there was a reduction of 3.72 g/h and 1.59 g/h, respectively.

The total protein, albumin, globulin, albumin:globulin ratio, triglycerides, ALT, AST, and GGT serum concentrations were not affected ($P > 0.05$) by the addition of crude glycerin to the diets of the lambs (Table 6). However, the crude glycerin in the diets led to a linear decrease in urea-N ($P = 0.004$) and glucose ($P < 0.001$), as well as a linear increase in the cholesterol ($P = 0.043$) serum concentrations of the lambs. Usually the serum proteins are affected (negatively) only in starvation conditions. The slight reduction in protein absorption without starvation were not able to change serum proteins, because of its homeostatics functions (as blood osmotic regulation). Serum proteins do not represent protein storage.

DISCUSSION

The liver of ruminant animals has great propionyl CoA synthetase activity (Ricks and Cook 1981), which is required for activation and subsequent metabolism of propionate. As a result, propionate is extensively metabolized by the ruminant liver during meals, which increases ATP production because of its use for glucose production and satiety signaling (Reynolds 1995). As for how rumen propionate from glycerol increased, there was likely a greater contribution of propionate to the liver, which may have contributed to satiety and, consequently, to the lowest DM intake by the animals. The increase in propionate production, the presence of lipids in the diet, and the reduction in the digestibility of NDFap together contributed to the

Table 5. Ingestive behavior of the Santa Inês crossbred lambs fed diets with crude glycerin

	Crude glycerin (% DM)					*P* value*	
Item	0	7	14	21	SEM	Linear	Quadratic
Daily spent time (%)							
Rumination	33.7	35.9	34.0	29.8	1.89	0.128	0.116
Feeding	14.4	16.4	17.5	13.5	1.64	0.831	0.080
Idling	51.8	47.6	48.5	56.7	2.93	0.271	0.052
Number of events/day							
Rumination	23.3	24.2	23.1	20.6	1.57	0.209	0.315
Feeding	15.4	17.1	16.7	14.4	1.63	0.656	0.235
Idling	36.1	38.3	36.9	32.2	2.19	0.185	0.121
Average duration of events (min)							
Rumination	21.1	22.2	22.5	21.5	1.615	0.825	0.525
Feeding	13.2	14.9	16.0	13.5	1.318	0.735	0.120
Idling	22.6	18.8	20.6	27.3	2.923	0.243	0.086
Efficiency (g/h)							
Feeding DM	548	366	253	246	61.4	0.005	0.261
Feeding NDF	225	146	96.3	98.2	25.4	0.004	0.215
Rumination DM	181	145.4	131	98.9	13.2	<0.001	0.908
Rumination NDF	73.4	57.5	50.1	38.9	5.20	<0.001	0.688
Chewing							
g DM/bolus	2.49	2.20	1.72	1.41	0.18	<0.001	0.965
Chew/bolus	579.73	586.12	611.43	507.86	37.25	0.269	0.158
Chew/d	8.520	9.133	7.773	7.779	7.547	0.296	0.693

*Significant at $P \leq 0.05$.

Table 6. Blood serum metabolites of the crossbred Santa Inês lambs fed diets with crude glycerin

	Crude glycerin (%DM)					*P* value*	
Item	0	7	14	21	SEM	Linear	Quadratic
Total protein (g/dL)	8.05	8.11	8.15	7.56	0.51	0.564	0.571
Albumin (g/dL)	3.12	3.05	3.38	3.02	0.11	0.969	0.278
Globulin (g/dL)	4.93	5.06	4.99	4.54	0.47	0.588	0.580
A:G ratio	0.67	0.65	0.73	0.72	0.06	0.409	0.943
Urea-N (g/dL)	14.31	14.7	13.2	11.8	0.71	0.004	0.438
Glucose (g/dL)	84.4	77.1	65.4	61.6	4.58	<0.001	0.226
Cholesterol (mg/dL)	76.6	85.7	77.5	102	6.67	0.043	0.305
Triglycerides (mg/dL)	78.8	84.6	80.6	95.0	11.11	0.413	0.726
ALT (IU/L)	23.0	18.5	21.4	17.8	2.06	0.207	0.832
AST (IU/L)	133	131	147	129	11.19	0.931	0.526
GGT (IU/L)	5.77	6.26	6.76	10.8	1.57	0.214	0.530

IU = International units.

*Significant at $P \leq 0.05$.

reduction in DMI by the lambs. Is possible infer that those factors only were able to affect the physiological satiety mechanism as from 4.52% of glycerin addition.

In addition, the inclusion of crude glycerin in the diets of the lambs caused significant changes in the chemical composition of the diets (Table 3), and it is likely these variations influenced animal performance. The glycerin used in this study had 30% of total fatty acids (Table 1), and its addition promoted an increase in this fraction in the diets and a reduction in NDF.

The inclusion of byproducts from biodiesel production increases EE content such that values greater than 60 g/kg of EE reduce the DM intake and change the fiber degradation (Oliveira et al., 2015; Costa et al., 2016a; Silva et al., 2016a). Thus, the reduction of NDF intake and digestibility was due to the commitment of this fraction degradation in the rumen, which possibly arose from the deleterious effect that excess lipids (EE) have on the degradation of fiber that persists longer in the rumen and reduces intake.

A DMI reduction was observed in studies that evaluated the glycerin in diets of ruminants (Lage et al., 2010; Barros et al., 2015; Dias et al., 2015; van Cleef et al., 2016; Cunha et al., 2016) with crude glycerin inclusion greater than 50 g/kg and with more limpidity for 80% glycerol.

Intake and digestibility of EE was positively affected by the inclusion of glycerin in the diets of the lambs, which may be associated with the fact that the fatty acid content of glycerol provides greater lipid availability in the small intestine for formation of micelles and absorption (Barros et al., 2015). According to Palmquist (1991), the EE digestibility increase occurs because of the dilution effect of endogenous losses of EE in feces, and this increase accounts for the relationship between the total EE ingested and EE quantified in the feces, which is reflected in greater digestibility (Silva et al., 2016b).

The addition of glycerin caused a reduction in NFC in the diets and consequently promoted the NFC intake reduction. It is common to associate glycerin with a great concentration of NFC. However, the ingredient used in this study was not clean, and it replaced mostly corn bran in the diets, which was the ingredient with the greatest proportion of NFC (Silva et al., 2016b).

The CP intake ranged from 188 to 140 g/d, with a lower intake observed in animals that received diets with the greatest level of crude glycerin inclusion (21%). According to the NRC (2007), lambs with moderate growth (200 g/d) and BW above 20 kg require an intake of 167 g/d of CP. Animals that received levels of 21% crude glycerin in the DM of the diets had a CP intake below the recommended intake, which can be explained by the lower DM intake observed in these animals once the diets were isonitrogenous. NDT intake was also lower than the intake recommended in treatments with a 21% inclusion of crude glycerin (164 g/d of NDT) because NRC (2007) recommends an intake of 800 g/d of NDT for lambs.

Thus, the CP and NDT intakes were lower than the recommended 7% inclusion of crude glycerin associated with the negative effects of crude glycerin in diets based on the digestibility of DM, CP, NDF, and NFC; this digestibility is related to the impairment of microbial activity caused by excess lipids, confirming the coherence of the physiological behavior between consumption and performance growth through the point of inflection of

the curves of quadratic functions for the CP intake (5.51%), TDN intake (4.52%), ADG (3.83%), and FE (5.09%). These results are below the percentage inclusion suggested by Gunn et al. (2010a), who concluded that the addition of 10 to 15% crude glycerin in the diets of lambs would be ideal if reduction in DM intake did not occur. Importantly, these authors worked with glycerin that had purity greater than 80%.

The feeding and REs of DM and NDF were reduced linearly with the inclusion of glycerin and between the treatments with the greatest level of glycerin and the control; there were decreases of 44.9%, 54.7%, 43.7%, and 53.1% in the intake and REs of DM and NDF. The reduction of the intake, digestibility and REs also reflects the deleterious effects of adding glycerin to the fibrous fraction degradation; and according to the NRC (1987), the digestion of fiber directly affects the digestion of other nutrients because this fraction limits the disappearance rates in the digestive tract, which may cause increased retention time for feeding and ultimately reduce intake.

Another factor that may have negatively influenced the digestibility and rumination of NDF is the increase in the concentration of glycerol in the diets. Paggi et al. (2004) reported that the cellulolytic activity decreased as a function of the rumen glycerol concentrations based on in vitro studies. This result occurred because, among the affected microorganisms, the fibrolytic ones are the most sensitive. Therefore, the NDF digestibility had the most significant reduction, with digestibility lower than 50% in the 21% crude glycerin treatment compared to the control treatment. By committing the degradation of the plant cell wall, there is an indirect compromise in the availability of other compounds, such as proteins and NFC, which remain physically protected against microbial attack by the plant matrix.

Glucose and urea-N serum concentrations were also reduced by the inclusion of glycerin, which can be explained by the lower CP intake and reduced digestibility of this fraction. This result is similar to the result observed in some studies with the addition of glycerol in diets (Krebs and Lund, 1966; Gunn et al., 2010b) and can be explained in this study by reducing the TDN fraction of the diets of the lambs.

The measurement of cholesterol and triglycerides can be used to access the energy status of the animal. In the present study, the cholesterol levels were affected by the inclusion of glycerin in the diets, and this outcome can be explained by the greater lipid content in the diets with added crude glycerin (Krebs and Lund, 1966). Therefore, it was assumed that greater concentrations of dietary lipids resulted in increased availability of absorbed fatty acids that may have been converted to acetate and then to cholesterol synthesis (Costa et al., 2016b).

The serum concentration of total proteins, albumin, and globulins demonstrated that animals that received the greatest amount of glycerin were not in a nutritionally deficient state since it is known that albumin can be used to predict if protein requirements are being met (Gunn et al., 2010b). The activities of the liver enzymes ALT, AST, and GGT were not affected by the inclusion of crude glycerin in the diet, which was also indicative of the absence of acute or chronic liver disease (Costa et al., 2016b).

CONCLUSIONS

The inclusion of up to 4.7% crude glycerin in the diets of the lambs improved nutrient intake and performance growth despite reductions in nutrient digestibility, glucose and urea-N concentrations, as well as feeding and RE of DM and NDF. Crude glycerin did not compromise the healthy status of the animals during the feedlot period after monitoring the serum metabolites.

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