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Nutritional and metabolic parameters in lambs fed diets containing crude glycerine

Parâmetros nutricionais e metabólicos em cordeiros alimentados com dietas contendo glicerina bruta

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Highlights

Crude glycerin at 40.0 g kg DM^{-1} provides highest intake of dry matter.
Crude glycerin does not influence the nutrients apparent digestibility.
Crude glycerin does not influence ruminal pH, ammonia and blood urea.

Abstract

The objective of this work was to evaluate the intake, apparent digestibility, nitrogen balance, ruminal and blood parameters in lambs fed diets containing of crude glycerin. Four castrated lambs of Santa Ines breed with an average weight of 25.0 kg were used. The evaluated diets contained four levels of crude glycerin (0.0, 50.0, 100.0 and 150.0 g kg DM^{-1}). All diets were formulated to meet the nutrient requirements of growing and finishing lambs. The ingredients were: *Brachiaria dyctioneura* hay as roughage, ground corn grain, soybean meal, urea, mineral salt and crude glycerin. The complete diet provided to the animals was composed of 500 g kg^{-1} roughage and 500 g kg^{-1} concentrate on DM basis. A 4 x 4 Latin square experimental design was used. The highest intake ($P<0.05$) of dry matter (129.08 g kg $BW^{-0.75}$), neutral detergent fiber (62.91 g kg $BW^{-0.75}$), acid detergent fiber (41.10 g kg $BW^{-0.75}$), total carbohydrate (99.92 g kg $BW^{-0.75}$) and organic matter (116.89 g kg $BW^{-0.75}$) was achieved with diets varying from 39.2 to 44.7 g kg DM^{-1} crude glycerin. Crude protein (17.84 g kg $BW^{-0.75}$) and ether extract (2,70 g kg $BW^{-0.75}$) intake was higher ($P<0.05$) with diets containing 39.0 and 77.1 g kg DM^{-1} of crude glycerin, respectively. Consumed

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and absorbed N displayed a quadratic effect, whereas retained N displayed a linear decreasing effect in function of crude glycerin levels.
Ruminal concentrations of acetate, propionate, and butyrate, and blood glucose was influenced (P<0.05) by the postprandial hour vs. experimental diet interaction. Glycerin levels in the diet did not influence (P>0.05) the apparent digestibility and ruminal N-NH3, pH, ciliate protozoa and blood urea. Therefore, diets containing crude glycerin influenced dry matter intake, with the highest value being around 40 g kg DM^{-1}. Likewise, diets containing crude glycerin influenced the N consumed and absorbed with maximum crude glycerin points of 36.9 and 28.4 g kg DM^{-1}.
Key words: Agroindustry. Animal production. Diet. Glycerol. Ruminant nutrition.

Resumo

O objetivo deste estudo foi avaliar o consumo, digestibilidade aparente, balanço de nitrogênio, parâmetros ruminais e sanguíneos em cordeiros alimentados com dietas contendo glicerina bruta. Foram utilizados quatro cordeiros castrados da raça Santa Inês com peso médio de 25,0 kg. As dietas avaliadas continham quatro níveis de glicerina bruta (0,0, 50,0, 100,0 e 150,0 g kg MS^{-1}). Todas as dietas foram formuladas para atenderem às exigências nutricionais de cordeiros em crescimento e terminação. Os ingredientes foram: feno de *Brachiaria dyctioneura* como volumoso, grão de milho moído, farelo de soja, ureia, sal mineral e glicerina bruta. A dieta completa fornecida aos animais foi composta por 500 g kg^{-1} de volumoso e 500 g kg^{-1} de concentrado com base na MS. Foi utilizado delineamento experimental em quadrado latino 4 x 4. O maior consumo (P<0,05) de matéria seca (129,08 g $PC^{-0,75}$), fibra em detergente neutro (62,91 g $PC^{-0,75}$), fibra em detergente ácido (41,10 g $PC^{-0,75}$), carboidrato total (99,92 g $PC^{-0,75}$) e matéria orgânica (116,89 g $PC^{-0,75}$) foi alcançada com dietas variando de 39,2 a 44,7 g kg DM^{-1} de glicerina bruta. O consumo de proteína bruta (17,84 g $PC^{-0,75}$) e extrato etéreo (2,70 g $PC^{-0,75}$) foi maior (P<0,05) com dietas contendo 39,0 e 77,1 g kg MS^{-1} de glicerina bruta, respectivamente. O N consumido e absorvido apresentou efeito quadrático, enquanto o N retido apresentou efeito linear decrescente em função dos níveis de glicerina bruta. As concentrações ruminais de acetato, propionato e butirato, e a glicose plasmática foram influenciados (P<0,05) pela interação entre dieta experimental e hora pós-prandial. Os níveis de glicerina na dieta não influenciaram (P>0,05) a digestibilidade aparente e as concentrações ruminais de N-NH3, pH, protozoários ciliados e ureia sanguínea. Deste modo, as dietas contendo glicerina bruta influenciaram o consumo de matéria seca, que alcançou o maior valor com a inclusão de 40 g kg MS^{-1} de glicerina bruta. Da mesma forma, as dietas contendo glicerina bruta influenciaram o N consumido e absorvido com pontos máximos de glicerina bruta de 36,9 e 28,4 g kg MS^{-1}.
Palavras-chave: Agroindústria. Dieta. Glicerol. Nutrição de ruminantes. Produção animal.

Introduction

The search for food resources that allow animals to reach their maximum production potential at low cost has been constant, and even a challenge for researchers in the area of animal nutrition. The use of by-products generated from industrial processes presents one alternative to the achievement of these goals, and at the same

time alleviate the environmental problems caused by technological growth. One of these by-products is the crude glycerin that comes from the biodiesel production, and is composed of water, ash, lipids, sodium, phosphorus, calcium and glycerol (J. S. Oliveira et al., 2013).

Glycerol has high importance for gluconeogenesis, which is the main mechanism of glucose production from non-carbohydrate compounds. Metabolically, glycerol can be derived from lipolysis in adipose tissue, from the hydrolysis of triglycerides of blood lipoproteins and dietary fat (Rotondo et al., 2017).

Given this energy component and the high cost of producing sheep feed, crude glycerin is an interesting alternative for the substitution of energetic foods, such as corn. Crude glycerin is presumed to enhance the nutritional potential of diets through a suitable balance and combination of ingredients that maximizing microbial growth and improve food intake and digestion without impacting animal health, allowing the animal to attain its production potential at a lower cost (Zacaroni & Souto, 2019).

Nevertheless, when replacing corn with crude glycerin, the non-fibrous carbohydrate profile of the diet can be altered, reducing the participation of starch as a substrate in microbial fermentation. Therefore, a reduction in nutrient intake may occur, which is mainly affected by the characteristics of the diet (B. C. Oliveira et al., 2017). However, to prove the efficiency of crude glycerin it is necessary to measure and evaluate some nutritional parameters. Daily food intake, nutrient digestibility, nitrogen balance, ruminal and blood parameters can act on satiety receptors as a response to the result of the interaction between the metabolism of the animal and the physical and chemical properties of the diet (Silva, 2006). Thus, the objective of this work was to evaluate the nutrient intake, nutrient apparent digestibility, nitrogen balance, ruminal and blood parameters in lambs fed levels of crude glycerin in the diet.

Material and Methods

The experiment was carried out at the experimental Farm of the State University of Londrina (UEL), Paraná, Brazil. All procedures involving animals were approved by the ethics committee of the UEL, under protocol number CEEA 60/2010.

Four castrated lambs of Santa Ines breed with an average weight of 25.0 kg newly weaned were used in the study. The evaluated diets contained 0, 50, 100 and 150 g kg^{-1} of crude glycerin, based on dry matter (DM; Table 1). All diets were formulated to meet the nutrient requirements of early maturity lambs with daily gain of 200 g kg^{-1} (National Research Council [NRC], 2007). The ingredients were: *Brachiaria dyctioneura* hay as roughage, ground corn grain, soybean meal, urea, mineral salt and crude glycerin. The complete diet provided to the animals was composed of 500 g kg^{-1} roughage and 500 g kg^{-1} concentrate on DM basis (Table 1).

Table 1
Ingredient and nutrient composition of diets containing crude glycerin levels for lambs

	Crude glycerin (g kg DM^{-1})			
Composition (g kg DM^{-1})	0	50	100	150
Ingredient composition				
Brachiaria dyctioneura hay	500.0	500.0	500.0	500.0
ground corn grain	260.0	200.0	130.0	0.0
Soybean meal	220.0	230.0	250.0	330.0
Urea	10.0	10.0	10.0	0.0
Mineral Supplement[1]	10.0	10.0	10.0	20.0
Crude glycerin	0.0	50.0	100.0	150.0
Chemical composition				
Dry matter	884.7	883.2	881.7	880.5
Crude protein	160.3	160.0	163.0	159.8
Neutral detergent fibre	434.2	426.6	418.7	408.8
Acid detergent fibre	218.0	216.9	216.0	216.0
Ether extract	25.0	23.6	22.3	21.7
Total digestible nutrients	682.4	685.5	688.5	691.7
Organic matter	943.1	944.4	945.4	944.5
Total carbohydrates	757.8	761.1	760.3	763.0

[1]Commercial mineral supplement contained the following guarantee levels kg^{-1}: calcium 135 g, phosphorus 65 g, sodium 107 g, sulphur 12 g, magnesium 6,000 mg, cobalt 175 mg, copper 100 mg, iodine 175 mg, manganese 1,440 mg, selenium 27 mg, zinc 6,000 mg, iron 1,000 mg, fluorine 650 mg, crude protein (CP) 30 g, total digestible nutrients (TDN) 100 g. DM= Dry matter; TDN = [88.9 - (0.779 x ADF%)].

To formulate the diets, we used chemical compositions of ingredients determined at the Laboratory of Animal Nutrition, according to the methodologies described by Association of Official Analytical of Chemists [AOAC] (2000, 2006). The feed was subjected to chemical analyses in order to determine dry matter (DM, method 930.15), ash (ash method 923.03), crude protein (CP, method 990.03), ether extract (EE, method 920.39), hemicellulose (HEM) according to AOAC (2000); organic matter (OM, method 942.05) following AOAC (2006); neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (LIG), as reported by Detmann et al. (2012). Total carbohydrate (TC) contents were calculated according to recommendations of Sniffen et al. (1992): TC = 100 - (%CP + %EE + %Ash). Total digestible nutrients (TDN) of hay, ground corn grain and soybean meal used to balance the diets were estimated by the equation proposed by T. Patterson et al. (2000): TDN = [88.9 - (0.779 x ADF%)]. The value assigned to the TDN of crude glycerin was 934.0 g kg DM-1 as reported by Monnerat (2012).

The lambs were housed in individual stalls with slated floor, equipped with feeders, mineral supplementation troughs and

drinkers, and were distributed in a 4x4 Latin square with four diets and periods of 13 days each. Animals were adapted to the cages before the beginning of the experiment. There was an initial period of 10 days for the lambs to adapt to their diets and management routines. Then, there was the experimental period lasting 52 days, in which the first 7 days were for the animals to adapt to the experimental conditions and installation of the collection bags, followed by 6 days of collection of food (offered and leftovers) and feces for determination of nutrient intake and apparent digestibility.

The diets were provided twice a day at 07h30 and 16h30. Water was provided *ad libitum.* The feed supply was adjusted every morning, before the first meal, using the weights of food and leftovers. During the collection period, daily weighing of the food provided, the leftovers and total feces was performed, and an aliquot of each was taken to provide representative composite samples for each animal. Later, all samples were analyzed in the Laboratory of Animal Nutrition of the State University of Londrina - UEL.

Faeces were collected twice a day, at 07:30 h and 16:30 h, through the collection bags. The faeces from each animal were weighed daily and after homogenization of the material an aliquot of approximately 20% of the total was removed per day to prepare a composite sample per animal. Faecal samples were placed in plastic bags, labelled and stored in a freezer for further analysis. These samples and samples of the feed supplied and the leftovers, were subjected to pre-drying at 55°C for 72 hours. After pre-drying, samples were processed in a Wiley mill, with 1 mm sieve, and subsequently analysed.

The determination of feed intake and nutrients digestibility coefficient was performed according to Silva and Leão (1979). The metabolic cages had a slatted floor and a tapered bottom. Buckets equipped with screens were placed for urine collection (to avoid dirt contamination). In order to determine nitrogen balance, 20 mL of HCL 1:1 was placed daily in the urine collectors. The total production of urine was measured using a measuring cylinder, and a portion corresponding to 10% of total urine excreted by the animal was collected and stored under refrigeration for further analysis. The balance of nitrogen compounds was obtained by the difference between the total nitrogen intake and total nitrogen excreted in faeces and urine (Silva & Leão, 1979).

Blood samples from all animals were collected in anticoagulant-containing tubes, through puncture of the jugular vein on the tenth day of each period at 0, 3 and 6 h postprandial time. The tubes were immediately centrifuged, the supernatant was transferred to 1.5 mL microtubes, identified and frozen for further analysis of blood concentration of glucose and urea using a commercially available Laborclin brand kit. The analysis was performed using a Bell photonics® SF325NM spectrophotometer.

Ruminal fermentation parameters (pH, ammonia [N-NH3] concentration and short chain fatty acids [SCFAs]) were evaluated at the 13th day and liquid ruminal samples were collected to quantify and identify ciliate protozoa. Ruminal fluid sampling was performed after 12-hour fasting (collection period zero) and then at 3, 6 and 9 hours after feeding (Zeoula et al., 2003). Each animal was sampled around 100 mL of ruminal fluid

through an esophageal tube (a flexible 1.5 m-length and 0.3 cm-thick probe with 1.27 cm internal diameter, rounded at the tip, and adapted to a vacuum pump). Immediately after the ruminal fluid was collected, pH was measured with a digital potentiometer calibrated with buffer solutions of pH 4,0 and 7,0. Then, the liquid was filtered with double gauze, placed into hermetically sealed containers within eight drops of sulfuric acid (50% $v.v^{-1}$) and frozen at -18ºC for later determination of $N\text{-}NH_3$ and SCFAs.

The $N\text{-}NH_3$ content was determined by distilling 5mL of ruminal fluid added to 10 mL 155 KCl solution and 250 mg of magnesium oxide (P.A.) in a micro Kjeldahl distiller and by titration with H_2SO_4 at 0.01N Vieira (1980). The following formulas were used for calculation: $N\text{-}NH_3$ 100 mL^{-1} = ((V2 - V3) x N x 0.014007 x 1000 x 100) $V1^{-1}$), where $N\text{-}NH_3$ 100 mL^{-1} = ammoniacal nitrogen concentration in 100 mL of ruminal fluid sampled; V1 = volume in mL of ruminal fluid used in the analysis; V2 = sulfuric acid volume used in titration, in mL; V3 = sulfuric acid volume used in reference titration, in mL; N = sulfuric acid normality.

The analysis of SCFAs was performed using an Agilent model 6890N gas chromatograph, with a 30 m long x 0.25 mm internal diameter polyethylene glycol capillary column. A specific chromatographic column that supports low pH values was acquired and the methodology described by Bock et al (1991) was applied. In the chromatographic separation process, temperatures of 250 ºC were used for the split injector, 100-185 ºC of heating ramp with increment of 15 ºC min^{-1} for the column, 300 ºC in the flame ionization detector, gas flow rate of 1.5 mL min^{-1} (drag gas H_2), and detection system of 350 mL min^{-1} (synthetic air) and 30 mL min^{-1} (H_2), and for N_2, 25 mL min^{-1} (gas saver). The sample volume injected was 0.1 mL.

In order to quantify and identify protozoal population, ruminal fluid was filtered with gauze, then 20 mL was fixed at the same volume of 18.5%-formalin solution, according to Dehority (1984), adapted by D'Agosto and Carneiro (1999). The ciliate protozoa genera were identified and evaluated quantitatively in a Sedgewick-Rafter counting chamber with a capacity of 1 mL and a common optical microscope provided with a grid with an area of 0.4362 mm^2, according to the recommendations of Dehority (1984), with modifications proposed by D'Agosto and Carneiro (1999). The results were expressed as number of ciliates per mL of ruminal content. The identification of ciliate protozoa was performed according to the identification key described by Ogimoto and Imai (1981).

All data were subjected to statistical analyses as a 4 x 4 latin square experimental design. The data were tested for normality and homogeneity of variance. Then, the data of nutrient intake, nutrient digestibility and nitrogen balance were subjected to regression analysis, where crude glycerin level was the independent variable. The regression was tested, and when were observed quadratic effect of the replacement levels of crude glycerin in studied characteristics, we derived the equations to obtain the maximum (maximum point = max.p) or minimum level (minimum point = min.p) that affected these characteristics. The data of ruminal and blood parameters were subjected to analysis of variance (ANOVA) regarding the independent effects of crude glycerin level and postprandial hour, and their interactions. When the effect of crude glycerin was

significant, the regression analysis was performed as previously described; and the effect of postprandial hour was significant, the means were compared by Tukey test. The data of ruminal protozoa count was subjected to ANOVA in relation to the crude glycerine level. The analyses were performed in the statistical package R (R Core Team [R], 2014) at 0.05 of probability level.

Results and Discussion

Intake, digestibility and nitrogen balance

The addition of crude glycerin influenced the intake (P<0.05) of nutrients expressed as kg lamb^{-1} day^{-1} and g kg BW$^{-0.75}$, displaying a quadratic effect for all evaluated parameters (Table 2).

Table 2
Nutrients intake in lambs fed diets containing crude glycerin levels

Item	Equation	R^2	Inflection Point		P-Value	
			Crude glicerin	Value	Linear	Quadratic
Daily intake (kg lamb^{-1} day^{-1})						
DM	DM=0.00004 CG²+ 0.0028 CG + 1.3865	0.96	35.0	1.530	<0.001	<0.05
CP	CP = 0.000005 CG² +0.0004 CG + 0,1905	0.99	40.0	0.214	<0.001	<0.05
EE	EE = -0.000001 CG²+ 0,00009 CG + 0.0295	0.75	45.0	0.031	<0.001	<0.05
NDF	NDF = -0.00002 CG²+ 0.0015 CG + 0.6665	0.85	37.5	0.694	<0.001	<0.05
ADF	ADF = -0.00001 CG²+ 0.0011 CG + 0.434	0.93	55.0	0.464	<0.001	<0.05
CT	CT = -0.00003 CG² + 0.0019 CG + 1.082	0.95	31.6	1.112	<0.001	<0.05
OM	OM = -0.00003 CG² + 0.0025CG + 1.2585	0.97	41.6	1.310	<0.001	<0.05
Intake relative to metabolic weight (g kg BW$^{-0.75}$)						
DM	DM = -0.003 CG² + 0.2387 CG + 124.33	0.94	39.8	129.08	<0.001	<0.05
CP	CP = -0.0004 CG² + 0.0312 CG + 17.231	0.98	39.0	17.84	<0.001	<0.05
EE	EE = -0.00007 CG² + 0.0108 CG + 2.288	0.11	77.1	2.70	<0.001	<0.05
NDF	NDF = -0.0015 CG² + 0.1341 CG + 59.911	0.80	44.7	62.91	<0.001	<0.05
ADF	ADF = -0.0009 CG² + 0.0798 CG + 39.311	0.90	44.3	41.10	<0.001	<0.05
CT	CT = -0.0022 CG² + 0.1725CG + 96.541	0.96	39.2	99.92	<0.001	<0.05
OM	OM = -0.0027CG² + 0.2185CG + 112.47	0.94	40.4	116.89	<0.001	<0.05

DM= Dry matter; CP= Crude protein; EE= Ether extract; NDF= Neutral detergent fibre; ADF= Acid detergent fibre; TC= Total carbohydrate; OM= Organic matter; CG= Crude Glycerin g kg DM^{-1}; BW=body weight; R^2= coefficient of determination.

In general, the highest nutrients intake in kg lamb^{-1} day^{-1} was obtained with diets containing from 37 to 50 g kg DM^{-1} of crude glycerin. Only EE in g kg BW$^{-0.75}$ showed higher intake for diets containing 79.3 g kg DM^{-1} of crude glycerin (Table 2).

Dry matter intake (DMI, kg lamb^{-1} day^{-1}) in animals fed up to 100 g kg DM^{-1} crude glycerin was above those recommended by the NRC (2007) for early finishing lambs (1.20 kg lambs^{-1} day^{-1}) with daily gain of 200 g kg^{-1}. However, the decrease in DMI and NDFI was observed with 150 g kg DM^{-1} crude glycerin where possibly the presence of glycerin may have compromised the degradation of NDF in the rumen (Ribeiro et al., 2018). Because glycerol is also a SCFA, the molecule can coat the vegetable fiber; this coating can harm cellulolytic bacteria, since for cellulase to be released, an enzymatic complex must first be formed, which only may exist by attaching the fiber with the bacteria (Nagaraja, 1997).

The crude protein intake (CPI) by lambs fed diets with up to 100 g kg DM^{-1} of crude glycerin ranged from 180 to 200 g lamb^{-1} day^{-1}, in accordance with the NRC (2007), which recommends a CPI above 167 g lamb^{-1} day^{-1}. The lower intake observed with the 150 g kg DM^{-1} inclusion level may be due to the lower DMI observed for this glycerin level ($P<0.05$, Table 2).

The reduction in feed intake can also occur due to psychogenic mechanism, related to the texture of the food, which can modify the intensity of intake (B. C. Oliveira et al., 2017). Van Cleef et al. (2018) stated that up to a certain level of inclusion of glycerin in the diet, it can help in the aggregation of particles and facilitate the intake, due to the moisture contained in this molecule. However, when increasing levels, it is possible that the texture of the diet becomes more wet, making it difficult for the animal to consume it. This was observed in our study with the diet containing 150 g kg DM^{-1} crude glycerin.

When evaluating four crude glycerin levels (0, 70, 170 and 210 g kg^{-1}) in the diet, Ribeiro et al. (2018) observed the lowest DMI (1.11, 1.19, 0.999, 0.863 kg d^{-1}, respectively) and CPI (188, 204, 173, 140 g d^{-1}, respectively) for the highest inclusion level. Likewise, Almeida et al. (2018) worked with the inclusion of crude glycerin at levels of 0, 100, 200 and 300 g kg DM^{-1} in the diet of lambs and observed a decrease in DMI. However, Chanjula et al. (2015) did not observe differences in the DMI, CPI and NDFI when including 0, 50, 100 and 200 g kg^{-1} of crude glycerin in the diet of goats, as well as, Terré et al. (2011) that also worked with added crude glycerin in the diet of lambs and reported that there was no change in the DMI.

The differences found between different studies may be related to the physical and chemical composition of crude glycerin that varies according to the production method, which may cause a change in rumen metabolism, causing the animal to reject or not reject the diets containing a higher proportion of this ingredient. According to Chung et al. (2007) mineral salts and methanol, which are the products used in the transesterification process, may influence the palatability of crude glycerin.

The addition of glycerin to the lamb diet did not influence ($P>0.05$) the evaluated digestibility coefficients (Table 3). When they evaluated the levels 0, 60, 120 and 180 g kg^{-1} of crude glycerin to replace corn, G. P. Andrade et al. (2018) observed a quadratic

effect for the digestibility coefficients of DM, CP and NDFap. However, Barros et al. (2015) observed a decreasing linear effect on the digestibility coefficients of DM and non-fibrous carbohydrates with increasing inclusion of crude glycerin (0, 26.5, 53.3, 80.6 and 108.4 g kg^{-1}). Regardless of the various factors that may influence the nutrients digestibility, such as composition of food and nutritional level of the diet, the results show that crude glycerin up to 150 g kg DM^{-1} and does not interfere with the nutrients digestibility.

The addition of crude glycerin promoted a quadratic effect ($P<0.05$) on the consumed and absorbed nitrogen (Table 4). The maximum intake and absorption of nitrogen occurred at levels of 36.9 and 28.4 g kg DM^{-1} of crude glycerin in the diet, respectively. Retained nitrogen (g day^{-1}) showed a decreasing linear effect with the addition of crude glycerin to the diet. There was no effect of diets on the faecal nitrogen and urinary nitrogen (g day^{-1}) (Table 4).

The relationship between retained and absorbed nitrogen reflects the use of nitrogen for protein synthesis, to form new tissues, new enzyme systems or to replace damaged tissues or epithelia. The result of this work depends particularly on the composition of the nitrogenous compounds that reach the tissues from the intestinal absorption, and the results indicate that the proteins or other nitrogenous compounds from the diets were well used by lambs, because the nitrogen balance is highly influenced by the content and the bromatological characteristic of the concentrate (Van Soest, 1994). Given that the nitrogen balance is an important tool to determine the efficiency of nitrogen utilization by ruminants and their losses to the environment (Hristov et al., 2019), it is important to note that there was no negative nitrogen balance in the animals.

Table 3
Nutrients apparent digestibility of diets containing crude glycerin levels

Nutrient digestibility (g kg DM^{-1})	Crude glycerin (g kg DM^{-1})				CV (%)	P-value
	0	50	100	150		
Dry matter	688.1	697.7	606.0	611.9		
Organic matter	681.8	692.1	599.2	600.8	8.33	0.095
Crude protein	816.0	814.4	780.0	779.3	3.50	0.194
Ether extract	705.9	670.1	657.8	712.5	5.74	0.240
Neutral detergent fibre	684.0	688.2	628.5	662.7	7.96	0.433
Acid detergent fibre	564.9	593.1	475.3	535.9	14.13	0.262
Total carbohydrates	674.8	689.7	581.7	590.6	9.23	0.081

CV = coefficient of variation.

Table 4
Nitrogen balance in lambs fed diets containing crude glycerin levels

Variables	Crude glycerin (g kg DM^{-1})				Equation	Maximun Point	R^2	P-value
	0	50	100	150				
consumed N, g day^{-1}	30.94	31.36	29.42	22.13	30.791+0.059CG -0.0008 CG²	36,9	0,99	0.001
faeces N, g day^{-1}	2.82	3.39	3.85	3.09	Ŷ= 3.29	--	--	0.121
urine N, g day^{-1}	4.88	5.73	5.91	5.66	Ŷ= 5.55	--	--	0.164
retained N, g day^{-1}	23.25	22.24	19.65	13.37	24.462 - 0.0645CG	--	0,88	0.003
absorbed N, g day^{-1}	28.13	27.97	25.57	19.04	28.036+0.0362 CG-0.0006CG²	28,4	0,99	0.005

CG = Crude Glycerin; N=nitrogen; R2= coefficient of determination.

Regarding to the faecal and urinary nitrogen, no influences of the crude glycerin in the diets was observed. It is important to note that the main factor that affects the loss of nitrogen through faeces is the roughage:concentrate ratio, because the higher the level of concentrate in the diet, the higher the passage rate, followed by higher release of nitrogen from microbial activity (Van Soest, 1994). In the diets used in this experiment, the roughage:concentrate ratio was similar for all treatments (50:50; Table 1), and this ratio may have contributed to the results. However, the amount of urinary nitrogen is related to the amount of crude protein in the diet and nitrogen consumed, so the higher the intake, the higher the amount of ammonia produced. If this amount exceeds the use by ruminal microorganisms, there is a higher synthesis of urea in the liver, resulting in an increase in the excretion of nitrogen through the urine (Reece, 2006). According to Van Soest (1994), meeting nitrogen requirements avoids the mobilization of reserve nitrogen from the animal, consequently limiting the excretion of urinary nitrogen.

Ruminal and blood parameters

The levels of crude glycerin did not influenced the values of N-NH3 in the lamb's diet (P>0.05, Table 5). Therefore, the levels of N-NH_3 in the ruminal fluid was affected by time (P<0.05), with the highest levels observed three hours after feeding. Ruminal pH was not influenced (P>0.05) by the experimental diets (Table 5). The pH and N-NH_3 concentrations in the rumen fluid of lambs are within the values considered adequate for the development of ruminal microorganisms. According to Van Soest (1994), the pH of the rumen should remain between 6.0 and 7.0, and according to Sampaio et al. (2010), 10 mg g^{-1} are the minimum necessary concentrations of N-NH_3 in ruminal fluid. Based on J. A. Patterson and Ricke (2015), the results obtained in the present study may be due to two mechanisms, the first is due to the chemical structure of glycerol as it contains three hydroxyl groups and the absence of a hydrophobic chain, making the medium where the glycerol is dissolved is less suitable for the activity of proteolytic enzymes. Another possible

explanation is due to the presence of alcohol in crude glycerin, which binds with the protein and increasing resistance to proteolytic activity in the rumen.

Table 5
Ruminal parameters in lambs fed diets containing crude glycerin levels

	Crude glycerin (g kg DM^{-1})				Mean
	0	50	100	150	
			NH_3 (mg dL^{-1})		
Before feeding	15.37	11.46	13.74	13.24	13.45b
3 hours after feeding	17.51	16.08	15.18	16.25	16.26a
6 hours after feeding	13.91	12.70	11.99	14.51	13.28b
Mean	15.60	13.41	13.63	14.66	Ŷ= 14.33
			pH		
Before feeding	6.49	6.66	6.59	6.34	6.52
3 hours after feeding	6.60	6.42	6.62	6.52	6.54
6 hours after feeding	6.60	6.57	6.75	6.60	6.63
Mean	6.56	6.55	6.65	6.49	Ŷ= 6.56
			Acetate (mM)		
Before feeding	56.70b	56.91	56.18	56.52b	56.58
3 hours after feeding	58.64a	57.85	56.97	57.21ab	57.67
6 hours after feeding	58.18ab	57.22	57.50	58.66a	57.89
Mean	57.84	57.33	56.89	57.46	Ŷ= 57.38
			Propionate (mM)		
Before feeding	18.06b	17.36b	17.74b	18.71	17.97
3 hours after feeding	19.47a	18.48a	18.59ab	19.29	18.96
6 hours after feeding	18.87ab	19.13a	18.92a	19.05	18.99
Mean	18.80	18.60	18.67	18.75	Ŷ= 18.64
			Butyrate (mM)		
Before feeding	14.24b	14.72	14.58	14.47b	14.50
3 hours after feeding	15.30ab	15.60	15.61	15.55ab	15.52
6 hours after feeding	16.52a	16.07	15.89	16.56a	16.29
Mean	15.36	15.46	15.36	15.56	Ŷ= 15.43

Lowercase letters in the column differ by the Tukey test at 5%.

Table 6
Blood parameters in lambs fed diets containing crude glycerin levels

	Crude glycerin (g kg DM^{-1})				Mean
	0	50	100	150	
	Glucose (mg dL^{-1})				
Before feeding	32.54b	34.29b	30.37b	33.50b	32.68
3 hours after feeding	37.51a	36.49ab	34.60a	37.97a	36.64
6 hours after feeding	39.15a	39.79a	36.93a	42.14a	39.50
Mean	36.40	36.86	33.97	37.87	Ŷ= 36.27
	Urea (mg dL^{-1})				
Before feeding	22.64	21.86	23.38	22.25	22.53
3 hours after feeding	24.44	22.86	26.00	23.87	24.29
6 hours after feeding	23.42	23.91	23.75	24.65	23.93
Mean	23.50	22.88	24.39	23.59	Ŷ= 23.59

Lowercase letters in the column differ by the Tukey test at 5%.

Regarding the results for blood glucose, it was found that there were small variations to the values obtained due to highly efficient homeostatic mechanisms that involve endocrine controls by insulin and glucagon on glycogen and glucocorticoids on gluconeogenesis (Kuo et al., 2015). These homeostatic mechanisms that control glycaemia make it difficult to establish a clear relationship between nutritional status and glucose levels because tissues use free fatty acids and ketone bodies as an energy source, and the liver of these animals has a high gluconeogenic activity. Thus, much of the glucose available to ruminants originates from gluconeogenesis through propionate that enters the portal vein, or from glycerol that is absorbed without fermentation, and also through the mobilization of proteins present in muscle tissue, in order to obtain gluconeogenic amino acids (Cunningham, 2004; Aschenbach et al., 2010).

The diet containing crude glycerin did not influence (P>0.05) the plasma urea, which presented a mean value of 23.59 mg dL^{-1} (Table 6). One of the main indicators of protein metabolism is the concentration of urea, which is synthetized in the liver in amounts proportional to the concentration of ammonia produced in the rumen. Urea concentration is directly related to the protein levels of the diet and the energy:protein ratio of the diet, and is an indirect indicator of the use of nitrogenous nutrients by ruminal microorganisms (Wittwer, 2000; Getahun et al., 2019). According to Kozloski (2011), when the concentration of urea in the plasma is normal, this indicates that there is less conversion of ammonia to urea in the liver and greater use of nitrogen by the ruminal microorganisms for microbial synthesis.

The number of ciliate protozoa was not influenced (P>0.05) by the experimental diets (Table 7). Ruminal microorganisms are

important for the health and productivity of ruminants (Welkie et al., 2010). According to Kholif (2019), ruminal microorganisms have capacity to adapt rapidly to glycerin intake, indicating that the adaptation of the microorganisms to this substrate is almost immediate.

Table 7
Mean number of protozoa ciliates x 104 mL-1 of rumen fluid in lambs fed diets containing crude glycerin levels

	Crude glycerin (g kg DM^{-1})				Mean	CV (%)	P value
	0	50	100	150			
Isotrichia spp	1.15	1.14	1.15	1.14	1.15	24.3	0.98
Dasytricha spp	2.10	2.08	2.04	2.07	2.07	6.8	0.95
Diplodinium	2.20	2.14	2.14	2.17	2.16	4.5	0.77
Entodinium	2.16	2.11	2.13	2.13	2.13	18.5	0.99
Eupidinium	1.56	1.57	1.54	1.56	1.56	16.5	0.99
Total	9.55	9.42	9.38	9.47	9.43	7.2	0.98

CV = coefficient of variation.

Conclusion

Crude glycerin does not influence the nutrients apparent digestibility, ruminal pH, $N\text{-}NH_3$, blood urea concentrations and the number of rumen protozoa, but affects acetate, propionate, and butyrate concentrations in the rumen, and blood glucose concentration, with higher values at 6 hours after feeding. Diets containing crude glycerin influenced dry matter intake, with the highest value being around 40 g kg DM^{-1}. Likewise, diets containing crude glycerin influenced the N consumed and absorbed with maximum crude glycerin points of 36.9 and 28.4 g kg DM^{-1}.

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