



# Effects of crude glycerin levels on nutritional characteristics and performance of sheep fed mixed forage diets

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## Editors:

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**ABSTRACT** - Two studies were carried out to investigate the effects of crude glycerin (CG) levels in feedlot sheep diets as a substitute for ground corn. In the first study, four castrated rumen-cannulated sheep were used to assess the effects of CG on nutrient intake, digestibility, rumen characteristics, and blood glucose. In the second study, 40 crossbred female lambs were employed to evaluate the influence of CG levels in the diet on nutrient intake, digestibility, and animal performance. Crude glycerin levels did not affect nutrient intake in either study, except for crude fat (CF) intake, which increased linearly as neutral detergent fiber decreased. However, the digestibility of dry matter, CF, total carbohydrates, and non-fibrous carbohydrates increased linearly, with CF being quadratically affected in both studies. Rumen pH (mean = 6.1) and blood glucose (mean = 61.72 mg/dL) remained unaffected by CG levels, whereas ruminal ammonia nitrogen displayed a quadratic response. Additionally, CG levels did not impact the performance (mean = 204.6 g/d) in female feedlot lambs, suggesting that CG can be included in sheep diets at up to 30% in a mixed forage diet.

**Keywords:** biodiesel, byproduct, digestibility, feedlot, nutritional value, ruminants

## 1. Introduction

Byproducts from the biodiesel industry have become increasingly available in countries like Brazil, where the federal government encourages the use of biodiesel as a substitute for fossil fuels to mitigate greenhouse gas emissions. One significant byproduct is crude glycerin (CG), which results from the conversion of vegetable oils and animal fat into biodiesel. Crude glycerin is composed of 75-80% glycerol, 7-13% fatty acids, 2-3% minerals from catalysts, and 0.5% alcohol and water (Donkin, 2008).

Given its high energy content, the use of CG in animal nutrition can be advantageous, especially when its cost is lower than that of other high-energy ingredients like corn (Mach et al., 2009). Furthermore, incorporating CG into animal diets is convenient since it is not used in human nutrition, and its disposal as organic waste could pose a significant environmental impact (Silva et al., 2014).

Research on the effects of CG in ruminant diets has yielded conflicting results. Some studies found no impact on dry matter intake (DMI) or on animal performance (Mach et al., 2009; Terré et al., 2011; Avila-Stagno et al., 2013; Chanjula et al., 2014), while others reported negative effects (Musselman et al., 2008; Gunn et al., 2010b; Lage et al., 2014; Benedeti et al., 2016; Saleem and Singer, 2018) and positive effects (Andrade et al., 2018). In most studies, the maximum inclusion levels of CG ranged from 10 to 21% of the diet dry matter (DM), with only two studies evaluating 45%.

The decrease in DMI observed in some studies can be attributed to some factors such as the high energy content of CG, an increase in propionate oxidation in the liver (Allen et al., 2009) as well as a decrease in rumen pH caused by its rapid fermentation compared with starch, which could lead to a reduction in cellulolytic activity. The negative impact on DMI may explain the decrease in animal performance, particularly in beef cattle, while the positive effect has been associated with an increase in nutrient digestibility (Rico et al., 2012).

Contradictory findings regarding the use of CG in ruminant diets have also emerged in recent meta-analyses. One meta-analysis, utilizing data from *in vitro* studies, concluded that glycerin did not exert negative effects on rumen fermentation (Syahniar et al., 2016). However, in another meta-analysis incorporating data from *in vivo* studies, a linear decrease in DMI and animal performance was observed in beef cattle fed varying levels of glycerin (0 to 300 g/kg of DM diet). Interestingly, CG levels did not show any effect on milk production in dairy cows (Syahniar et al., 2021). A third meta-analysis suggested that crude glycerin inclusions up to 200 g/kg did not negatively impact productive performance but enhanced feed efficiency in beef cattle (Torres et al., 2021a). For dairy cows, glycerin inclusions of up to 100 g/kg in the diet did not negatively affect milk yield or composition, but levels above 150 g/kg reduced milk fat and its fatty acid profile (Torres et al., 2021b).

The likely explanation for these observations is that CG is prone to decreasing DMI in animals fed high-grain diets, such as feedlot beef cattle, resulting in a direct negative effect on animal performance. However, for dairy cows, despite the negative impact on DMI, the increase in propionate supply to liver gluconeogenesis induced by CG may compensate for the lower DMI (Syahniar et al., 2021). These effects could also be influenced by the forage levels in the diets.

Given that negative effects of CG are more commonly observed in animals fed grain-based diets, even at low inclusion levels of CG (up to 10-12% in the diet), especially when hay is the roughage, it is hypothesized that in mixed forage diets, CG could exhibit minimal negative effects on animal performance. Therefore, this study was carried out to evaluate higher CG levels, commonly evaluated in the literature, on the intake and digestibility of nutrients, as well as on the animal performance of sheep.

## 2. Material and Methods

### 2.1. Location

The study was carried out in Santo Antonio do Leverger, Mato Grosso, Brazil (15°51'17" S, 56°4'13" W, 144 m above sea level). Research on animals was conducted according to the institutional committee on animal use (23108.050556/2020-04).

### 2.2. Study 1: Nutrient intake and digestibility and ruminal parameters

Four castrated rumen-cannulated hair sheep, with an average body weight of 35.02 ± 4.08 kg, were distributed in a 4 × 4 Latin Square design. Each experimental period lasted 14 days, with the initial nine days dedicated to animal adaptation, followed by five days for data collection. The animals were housed in individual pens (4.06 m<sup>2</sup>) with a concrete floor, equipped with a feed bunk and a water fountain.

The treatments comprised four CG levels (0, 100, 200, and 300 g.kg<sup>-1</sup> DM) in a standard diet containing 400 g of roughage (corn silage) and 600 g of concentrate per kilogram of DM. The concentrate was composed of ground corn, soybean meal, urea/ammonium sulfate (9:1), and a mineral mixture (refer to Tables 1 and 2), with ground corn being replaced with CG. Diets were formulated to be isonitrogenous, with 16% crude protein (CP) (NRC, 2007), and were delivered twice daily (08:00 and 16:00 h) as a total mixed ration, allowing 10% for orts, which were measured and sampled daily. Dry matter intake was calculated by subtracting the DM in the orts from the DM amount in the diet delivered daily.

Digestibility coefficients of DM and nutrients were calculated as the amount of DM or nutrient ingested minus its respective fecal excretion. Fecal samples were collected twice daily (at 09:00 and 15:00 h) directly from the rectal ampulla of the animals during the 10th to 12th days of each experimental period. Fecal excretion was estimated using indigestible neutral detergent fiber (iNDF) as an internal marker. The marker was measured in dietary ingredients, orts, and fecal samples by incubation into the rumen of two rumen-cannulated cattle for 144 h (Berchielli et al., 2000), followed by neutral detergent fiber (NDF) determination.

On the 13th day of the experimental period, rumen fluid samples were manually collected before and 2, 4, 6, and 8 h after the morning feeding. The collected samples underwent immediate filtration using four layers of cheesecloths and were subjected to pH measurement using a digital pH meter (Q400BD-Quimis®). Subsequently, 50 mL of rumen fluid were collected into cone tubes containing 1 mL of H<sub>2</sub>SO<sub>4</sub> solution (1:1), frozen, and later used for the determination of ruminal ammonia nitrogen (RAN), which was measured through analysis in a micro-Kjeldahl system without acid digestion, followed by distillation with KOH 2N (Detmann et al., 2012).

On the 14th day, blood samples were obtained from the auricular vein before and 2, 4, 6, and 8 h after morning feeding to determine glucose concentration using a digital glucometer (brand Accu-Chek®) equipped with test strips.

**Table 1 - Chemical composition of ingredients used in experimental diets**

Item (g.kg <sup>-1</sup> DM)	Ingredient			
	CS	GC	SM	CG
Dry matter (g.kg <sup>-1</sup> )	387.2	920.9	933.0	878.9
Ash	38.7	11.6	65.1	73.6
Organic matter	961.2	988.3	934.8	926.3
Crude protein	81.6	90.3	517.0	-
Crude fat	77.1	30.6	12.5	155.0
Total carbohydrates	862.4	867.2	405.3	771.3
aNDFom	540.5	103.5	111.8	-
Non-fibrous carbohydrates	351.9	751.6	293.5	771.3
iNDF	183.9	56.1	28.9	-

CS - corn silage; GC - ground corn; SM - soybean meal; CG - crude glycerin; aNDFom - neutral detergent fiber expressed exclusive of ash and nitrogenous compounds; iNDF - indigestible neutral detergent fiber.

### 2.3. Study 2: Animal performance

Forty female hair sheep, averaging 21.72 kg ( $\pm$  1.39 kg) and four months old, were allocated in a randomized block design, with initial body weight serving as the block criterion (two blocks). The animals (two per pen) were accommodated in 20 concrete floor pens (4.06 m<sup>2</sup>) equipped with a feed bunk and a water fountain.

**Table 2** - Composition of experimental diets

Item (study 1)	CG level (g.kg <sup>-1</sup> DM)				
	0	100	200	300	
<b>Ingredient (g.kg<sup>-1</sup> DM)</b>					
Corn silage	400.0	400.0	400.0	400.0	
Ground corn	412.0	292.0	170.0	48.0	
Soybean meal	160.0	180.0	202.0	224.0	
Crude glycerin (CG) <sup>1</sup>	0.0	100.0	200.0	300.0	
Mineral mixture	18.0	18.0	18.0	18.0	
Urea/ammonium sulfate (9:1)	10.0	10.0	10.0	10.0	
<b>Chemical composition (g.kg<sup>-1</sup> DM)</b>					
Dry matter	693.0	689.0	685.0	681.0	
Ash	36.0	44.0	51.0	58.0	
Organic matter	868.0	857.0	846.0	834.0	
Crude protein	152.0	152.0	152.0	152.0	
Crude fat	21.0	33.0	45.0	57.0	
Total carbohydrates	763.0	744.0	724.0	704.0	
aNDFom	302.0	287.0	271.0	255.0	
Non-fibrous carbohydrates	461.0	457.0	454.0	450.0	
iNDF	125.0	119.0	113.0	106.0	
Item (study 2)	CG level (g.kg <sup>-1</sup> DM)				
	0	70	140	210	280
<b>Ingredient (g.kg<sup>-1</sup> DM)</b>					
Corn silage	400.0	400.0	400.0	400.0	400.0
Ground corn	432.0	352.0	262.0	182.0	92.0
Soybean meal	140.0	150.0	170.0	180.0	200.0
Crude glycerin <sup>1</sup>	0.0	70.0	140.0	210.0	280.0
Mineral mixture <sup>2</sup>	18.0	18.0	18.0	18.0	18.0
Urea/ammonium sulfate (9:1)	10.0	10.0	10.0	10.0	10.0
<b>Chemical composition (g.kg<sup>-1</sup> DM)</b>					
Dry matter	682.0	682.0	682.0	682.0	683.0
Ash	47.0	52.0	57.0	62.0	68.0
Organic matter	942.0	940.0	937.0	934.0	931.0
Crude protein	154.0	152.0	154.0	152.0	154.0
Ether extract	21.0	30.0	38.0	47.0	55.0
Total carbohydrates	776.0	765.0	749.0	738.0	722.0
aNDFom	303.0	292.0	281.0	270.0	259.0
Non-fibrous carbohydrates	509.0	506.0	498.0	495.0	487.0
iNDF	110.0	116.0	104.0	105.0	100.0

aNDFom - neutral detergent fiber expressed exclusive of ash and nitrogenous compounds; iNDF - indigestible neutral detergent fiber.

<sup>1</sup> Crude glycerin composition: dry matter, 915 g.kg<sup>-1</sup>; glycerol, 831 g.kg<sup>-1</sup> DM; ash, 60 g.kg<sup>-1</sup> DM; ether extract, 16 g.kg<sup>-1</sup> DM; methanol, 2 g.kg<sup>-1</sup> DM; sodium, 5 g.kg<sup>-1</sup> DM.

<sup>2</sup> Commercial mineral mixture for sheep – guaranteed levels (kg<sup>-1</sup>): calcium, 177 g; phosphorus, 80 g; sulfur, 20 g; sodium, 108 g; cobalt, 40 mg; copper, 550 mg; iodine, 60 mg; selenium, 15 mg; manganese, 1200 mg; zinc, 3000 mg.

The experimental diets comprised 40% roughage (corn silage) and 60% concentrate, consisting of ground corn, soybean meal, urea/ammonium sulfate (9:1), and a mineral mixture. In these diets, CG replaced ground corn at varying levels (0, 70, 140, 210, and 280 g.kg<sup>-1</sup> DM; Table 2). Diets were formulated to be isonitrogenous, containing 16% crude protein (NRC, 2007), calculated for animals with a potential daily weight gain of 250 g.day<sup>-1</sup>. Feeding occurred twice daily (08:00 and 16:00 h), allowing for 10% orts, which were monitored and sampled daily. Dry matter intake was calculated as the difference between the DM of the supplied diet and the respective orts.

The study lasted 78 days, with the initial 15 days allocated for diet and environment adaptation and the subsequent 63 days for intake and performance data collection. On days 32, 33, and 34, as well as on days 54, 55, and 56 of the study, samples of corn silage, concentrate, and feces were collected, dried at

60 ± 5 °C for 72 h in a forced-air oven, and ground to 1 mm for chemical composition analysis. Fecal samples were collected for fecal excretion and digestibility calculations, following the procedures outlined in Study 1.

Animals were weighed at the beginning and end of the study after a 16-h solid fasting period to determine initial body weight (IBW) and final body weight (FBW), respectively. Total weight gain (TWG) was calculated as the difference between FBW and IBW, while average daily gain (ADG) was determined by dividing TWG by the experimental days (63 days).

### 2.3.1. Chemical composition analysis

All samples obtained from both studies, including silage, Orts, and feces, underwent a drying process in a forced-air oven at 55 °C for 72 h to achieve partial drying. Subsequently, these samples were ground in a Wiley-type mill equipped with a 1-mm sieve. The analyzed parameters included DM (method 930.15), ash (method 942.05), and crude protein (method 984.13), according to AOAC (2000). Additionally, crude fat (CF) was determined according to AOCS (2005; Method Am 5-04). Organic matter (OM) content was calculated as the difference between DM and ash using method 942.05 (AOAC, 2000).

The analysis of neutral detergent fiber (aNDFom) utilized the filter bag system under the pressurization method with an ANKOM<sup>220</sup> fiber analyzer (ANKOM technology). This analysis involved heat-stable  $\alpha$ -amylase without sodium sulfite, adapted from Van Soest et al. (1991), with residue correction for nitrogen compounds following the method described by Licitra et al. (1996). Non-fibrous carbohydrates (NFC) were calculated using the following equation:  $NFC = 100 - [(CP - CP \text{ derived from urea} + \text{urea in the diet}) + \text{aNDFom} + CF + \text{ash}]$  (Hall, 2000). Total carbohydrates (TC) were determined according to Sniffen et al. (1992).

### 2.4. Statistical analysis

In both studies, data were analyzed using the generalized linear mixed models (GLIMMIX) procedure of SAS<sup>®</sup> Studio software (University Edition version). For Exp. 1, the following statistical models were considered for a Latin square design:

$$y_{ijk} = \mu + \tau_i + a_j + p_k + e_{ijk} \quad (1)$$

in which  $y_{ijk}$  denotes the response variable observed in treatment  $i$  ( $\tau$ ), animal  $j$  ( $a$ ), and experimental period  $k$  ( $p$ ). The overall mean ( $\mu$ ) and crude glycerin levels ( $\tau$ ; 0, 10, 20, and 30% of the diet DM) denote fixed effects. The random effects are represented by the effects of animal ( $a_j$ ) and experimental period ( $p_k$ ) and random effects ( $e_{ijk}$ ). The variables pH, ammonia, and glucose in Exp.1 were assessed as repeated measurements over time. Consequently, the experimental model chosen for analysis using these statistical approaches was as follows:

$$y_{ijkl} = \mu + \tau_i + a_j + p_k + t_l + (\tau \times t)_{il} + e_{ijkl} \quad (2)$$

The parameters outlined in this model mirror those in equation 1, with the following exceptions:  $y_{ijkl}$  denotes the result of the measurement taken at time  $l$  from the experimental unit (animal  $\times$  period) that received treatment  $i$ ;  $t_l$  represents the effect of time  $l$ ;  $(\tau \times t)_{il}$  indicates the interaction between treatment  $i$  and time  $l$ ; and  $e_{ijkl}$  is the random error associated with the measurement taken in the experimental unit at time  $l$  that received treatment  $i$ .

For Exp. 2, the following statistical model was considered:

$$y_{ij} = \mu + \tau_i + b_j + e_{ij} \quad (3)$$

in which  $y_{ij}$  refers to the response variable observed in treatment  $i$  ( $i = 1, 2, 3, \text{ and } 4$ ) and experimental block  $j$  ( $j = 1, 2, 3, \text{ and } 4$ ). The fixed effects are represented by the overall mean ( $\mu$ ) and CG levels ( $\tau$ ; 0, 7, 14, 21, and 28% of the diet DM). The random effects are represented by the block ( $b_j$ ) and the experimental error ( $e_{ij}$ ) of each experimental unit, i.e., pens.

In both experiments (Exp. 1 with Eqs. 1 and 2; Exp. 2 with Eq. 3), the null hypotheses regarding treatments and their linear and quadratic effects were rejected when  $P < 0.05$ . Trends in treatment effects were considered when  $0.05 \leq P < 0.10$ . The option of least square means (LSMEANS) was used to estimate individual means for each treatment when there was no regression effect.

### 3. Results

#### 3.1. Study 1: Nutrient intake, digestibility, and ruminal parameters

No statistical difference was detected for the intakes of DM ( $P = 0.236$ ), OM ( $P = 0.172$ ), or CP ( $P = 0.102$ ) in response to CG levels. However, CF intake exhibited a linear increase ( $P < 0.001$ ), whereas aNDFom intake linearly decreased ( $P = 0.005$ ). There was also a tendency toward a decrease in the intakes of TC ( $P = 0.078$ ) and NFC ( $P = 0.064$ ) as the CG levels were raised (Table 3).

Crude glycerin levels led to a linear increase in digestibility coefficients of DM ( $P = 0.035$ ), OM ( $P = 0.019$ ), TC ( $P = 0.017$ ), and NFC ( $P = 0.024$ ). A quadratic effect was observed on CF digestibility ( $P = 0.002$ ) (Table 3). There was a tendency for a linear increase in CP digestibility ( $P = 0.098$ ), and no effect of CG levels on aNDFom digestibility ( $P > 0.05$ ).

The interaction effect between CG levels and sampling time was not significant for rumen pH ( $P = 0.778$ ) or RAN ( $P = 0.970$ ). Moreover, no statistical difference was detected for rumen pH ( $P = 0.239$ ) in response to increasing CG levels (Table 4), averaging 6.10 (Table 4). However, sampling time had a quadratic impact ( $P = 0.006$ ) on rumen pH (Figure 1). Crude glycerin levels had a quadratic effect on RAN ( $P = 0.041$ ), while sampling time caused RAN to decrease linearly ( $P = 0.018$ ) (Figure 2).

No interaction effect between CG levels and sampling time was detected for blood glucose ( $P = 0.157$ ), with an average of 62.25 mg.dL<sup>-1</sup>. Nonetheless, sampling time had a quadratic effect ( $P < 0.001$ ) on blood glucose (Table 4, Figure 3).

**Table 3 - Effect of crude glycerin (CG) levels on nutrient intake and digestibility (Study 1)**

Item	CG level (g.kg <sup>-1</sup> DM)				SE	P-value	
	0	100	200	300		L	Q
	Intake (g.d <sup>-1</sup> )						
DM	1158.6	1260.8	1119.0	1034.5	122.3	0.236	0.330
OM	1011.5	1085.8	959.9	889.3	102.9	0.172	0.359
CP	173.3	181.9	164.9	133.0	22.5	0.102	0.249
CF	26.1	42.7	59.2	75.8	4.8	<0.001 <sup>a</sup>	0.246
TC	895.6	947.9	813.9	749.5	87.9	0.078	0.390
aNDFom	382.7	347.7	312.7	277.7	44.3	0.005 <sup>b</sup>	0.668
NFC	560.2	620.6	507.1	433.5	54.3	0.064	0.211
	Digestibility (g.kg <sup>-1</sup> DM)						
DM	559.2	509.5	657.6	703.7	38.7	0.035 <sup>c</sup>	0.162
OM	596.7	652.8	705.0	752.2	37.7	0.019 <sup>d</sup>	0.292
CP	356.3	488.5	601.2	517.0	63.3	0.098	0.139
CF	591.4	781.5	900.9	906.3	14.0	0.002	0.031 <sup>e</sup>
TC	631.7	677.6	720.2	759.2	19.0	0.017 <sup>f</sup>	0.191
aNDFom	388.2	334.3	434.1	397.1	71.9	0.485	0.841
NFC	762.4	805.9	843.1	874.3	20.0	0.024 <sup>g</sup>	0.120

DM - dry matter; OM - organic matter; CP - crude protein; CF - crude fat; TC - total carbohydrates; aNDFom - neutral detergent fiber expressed exclusive of ash and nitrogenous compounds; NFC - non-fibrous carbohydrates; SE - standard error; L - linear effect; Q - quadratic effect.

$$^a y = 26.06 + 1.66x.$$

$$^b y = 382.74 + 3.50x.$$

$$^c y = 0.238 + 0.02073x.$$

$$^d y = 0.3919 + 0.0239x.$$

$$^e y = 0.5252 + 0.0347x + 0.0007x^2.$$

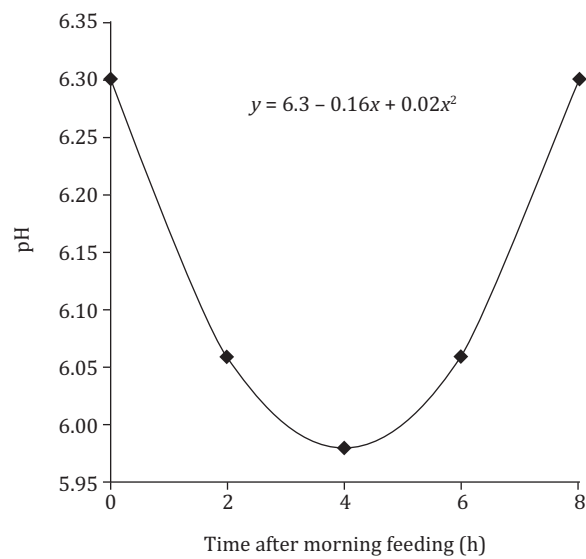
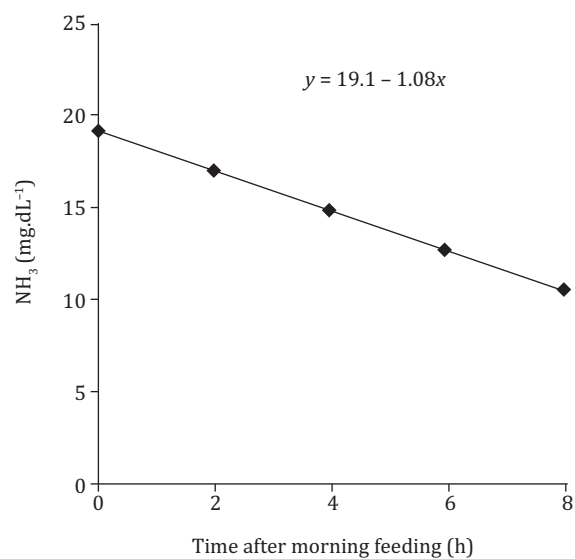
$$^f y = 398.4 + 6.43x.$$

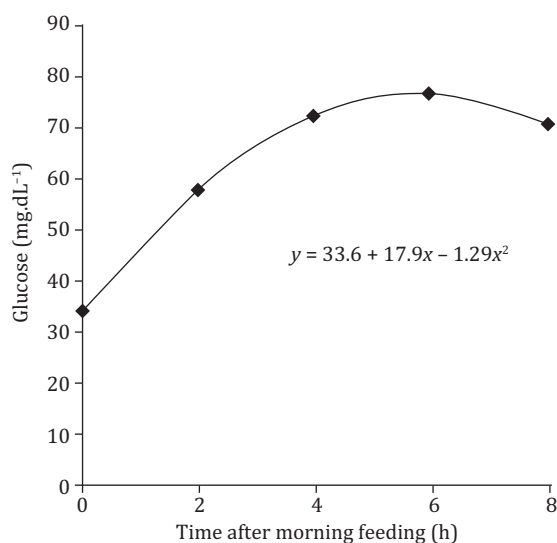
$$^g y = 1.166 + 0.0258x.$$

**Table 4** - Effect of crude glycerin (CG) levels on rumen pH, rumen ammonia nitrogen (RAN), and blood glucose of sheep (Study 1)

Item	CG level (g.kg <sup>-1</sup> DM)				SE	P-value		
	0	100	200	300		L	Q	CG vs. time
pH	6.20	6.09	6.09	6.04	0.11	0.239	0.718	0.778
RAN (mg.dL <sup>-1</sup> )	13.80	18.07	16.95	10.44	2.86	0.523	0.041	0.970
Glucose (mg.dL <sup>-1</sup> )	61.55	62.70	64.35	60.40	4.27	0.924	0.550	0.157

SE - standard error; L - linear effect; Q - quadratic effect.

**Figure 1** - Rumen pH as a function of sampling time, after morning feeding.**Figure 2** - Rumen ammonia nitrogen as a function of sampling time, after morning feeding.



**Figure 3** - Blood glucose concentrations as a function of sampling time, after morning feeding.

### 3.2. Study 2: Nutrient intake, digestibility, and animal performance

Crude glycerin levels did not affect ( $P>0.05$ ) the intake of nutrients, except CF, which linearly ( $P<0.001$ ) increased (Table 5). The digestibility of DM, OM, aNDFom, and TC also increased linearly ( $P<0.05$ ), while the digestibility of CP ( $P = 0.007$ ) and CF ( $P = 0.037$ ) was quadratically affected by CG levels (Table 5). Crude glycerin levels did not affect ( $P>0.05$ ) animal performance, with mean values of 12.1 kg and 200.0 g observed for TWG and ADG, respectively (Table 6).

**Table 5** - Effect of crude glycerin (CG) levels on nutrient intake and digestibility of feedlot lambs (Study 2)

Item	CG level (g.kg <sup>-1</sup> DM)					SE	P-value	
	0	70	140	210	280		L	Q
Intake (g.d <sup>-1</sup> )								
DM	1340.4	1494.9	1499.2	1419.9	1412.0	44.0	0.822	0.267
OM	1277.9	1418.0	1413.2	1333.3	1318.6	41.6	0.991	0.269
CP	205.4	227.9	231.2	213.5	213.8	7.8	0.954	0.238
CF	33.1	45.1	57.0	69.0	81.0	2.9	<0.001 <sup>a</sup>	0.524
TC	1085.3	1191.4	1169.5	1093.0	1066.7	34.6	0.578	0.270
aNDFom	353.5	382.6	386.7	357.7	344.9	13.5	0.580	0.209
NFC	690.2	764.8	740.6	701.0	680.8	26.4	0.600	0.278
Digestibility (g.kg <sup>-1</sup> DM)								
DM	505.5	531.1	557.8	585.8	615.5	14.0	<0.001 <sup>b</sup>	0.121
OM	530.6	554.1	578.5	604.0	630.5	13.8	0.001 <sup>c</sup>	0.084
CP	516.6	358.7	422.8	570.6	599.2	23.6	0.021	0.007 <sup>d</sup>
CF	533.1	650.3	785.4	874.3	950.3	29.5	<0.001	0.037 <sup>e</sup>
TC	572.0	591.0	610.0	630.0	650.0	12.7	0.002 <sup>f</sup>	0.128
aNDFom	164.1	183.6	205.4	229.8	256.9	20.2	0.025 <sup>g</sup>	0.188
NFC	753.8	766.4	779.2	792.2	805.3	10.3	0.008 <sup>h</sup>	0.169

DM - dry matter; OM - organic matter; CP - crude protein; CF - crude fat; TC - total carbohydrates; aNDFom - neutral detergent fiber expressed exclusive of ash and nitrogenous compounds; NFC - non-fibrous carbohydrates; SE - standard error; L - linear effect; Q - quadratic effect.

<sup>a</sup>  $y = 33.11 + 1.71x$ .

<sup>b</sup>  $y = -2.93 + 0.0074x$ .

<sup>c</sup>  $y = -2.88 + 0.0065x$ .

<sup>d</sup>  $y = 52.04 - 0.5732x + 0.0314x^2$ .

<sup>e</sup>  $y = -3.0074 + 0.04297x - 0.0007x^2$ .

<sup>f</sup>  $y = -4.09 + 0.0163x$ .

<sup>g</sup>  $y = -2.50 + 0.0026x$ .

<sup>h</sup>  $y = -2.80 + 0.0048x$ .

**Table 6** - Effect of crude glycerin levels (CG) on the performance of feedlot lambs (Study 2)

Item	CG level (g.kg <sup>-1</sup> DM)					SE	P-value	
	0	70	140	210	280		L	Q
Initial body weight (kg)	21.5	21.9	22.1	21.5	21.7	1.0	0.987	0.731
Final body weight (kg)	32.8	35.8	34.3	32.5	34.6	1.4	0.963	0.689
Total weight gain (kg)	11.3	13.9	12.3	11.0	12.9	1.0	0.934	0.796
Average daily gain (g)	189.0	231.0	205.0	183.0	215.0	16.0	0.934	0.797

SE - standard error; L - linear effect; Q - quadratic effect.

## 4. Discussion

According to Van Soest (1994), the nutritional value of feed can be measured in terms of its impact on intake, digestibility, and nutrient utilization efficiency, with intake considered a major factor influencing animal performance (Mertens, 1987). In this study, CG levels did not affect DMI in either trial, aligning with findings from other studies involving CG-fed finishing lambs (Gunn et al., 2010a), dairy cows (Werner Omazic et al., 2015), or goats (Chanjula et al., 2015). However, some studies reported a reduction in feed intake in response to glycerol supplementation in lambs (Gunn et al., 2010b; Avila-Stagno et al., 2013; Lage et al., 2014; Saleem and Singer, 2018), beef heifers (Parsons et al., 2009), finishing beef steers (Pyatt et al., 2007; Moore et al., 2011; Hales et al., 2015; Chanjula et al., 2016), and dairy cows (Paiva et al., 2016).

Orrico Junior and Orrico (2015) pointed out that the reduced DMI observed in studies with glycerol supplementation could be attributed to the higher energy density of diets containing glycerol, which appears more prevalent in high-grain diets (high-corn diets), whose energy content is too high and DMI is regulated by physiological mechanisms, such as the energy requirements of animals. Benedeti et al. (2016) estimated that glycerol contains 12% more metabolizable energy than corn (3.64 vs. 3.25 Mcal/kg).

The linear decrease in NDF intake and linear increase in CF intake in response to dietary CG levels could be explained by the lower and higher amounts of these nutrients, respectively, in crude glycerol compared with ground corn (Table 1).

In this study, CG levels linearly increased the digestibility of DM, OM, TC, and NFC, but had a quadratic effect on CF digestibility and tended to increase CP digestibility. In contrast, the diet containing 30% CG presented a DM digestibility 25.76% higher than that observed for the control diet. Similar positive effects of CG on nutrient digestibility have been noted by several authors (Wang et al., 2009; Barros et al., 2015; Hales et al., 2015; Benedeti et al., 2016; Paiva et al., 2016; Saleem and Singer, 2018), while others reported no significant effect (Lage et al., 2010; Beck, 2011; Chanjula et al., 2014).

The increase in DM and OM digestibility with increasing CG levels might be due to the substantial disappearance of glycerol from CG in the digestive tract. This includes its high absorption by the rumen epithelium and rapid fermentation by rumen microorganisms, resulting in 50 to 80% of glycerol disappearing within approximately 4 h (Rémond et al., 1993; Donkin, 2008; Werner Omazic et al., 2015), explaining its higher metabolizable energy content compared with that of corn. Additionally, in some studies, the enhanced diet digestibility has been attributed to a reduction in DM intake, which would lead to a longer residence time of digesta in the digestive tract, thereby enhancing digestive efficiency (Van Soest, 1994).

Some authors have reported negative effects of CG on NDF digestibility (Shin et al., 2012; van Cleef et al., 2015), attributing it to the inhibition of fibrolytic organisms in the rumen (Roger et al., 1992). However, in this study, no effect of CG levels on NDF digestibility was observed in study 1, aligning with results found by Hess et al. (2009), whereas others reported an increase in NDF digestibility

(Wang et al., 2009; Benedeti et al., 2016), supporting the data observed in study 2. This divergence in the literature regarding the effect of CG on NDF digestibility may be explained by its interaction with diet composition (high or low NDF) and appears to be more significant for animals fed forage-based diets (high NDF content).

Furthermore, in this study, CG levels did not influence rumen pH, despite the potential fermentative differences between glycerol and starch. The substitution of starch with glycerol is thought to benefit rumen pH, as glycerol fermentation does not produce lactate, contrary to starch fermentation (Krause and Oetzel, 2006; Nagaraja and Titgemeyer, 2007). Nevertheless, in some studies, CG levels were associated with a decrease in rumen pH, attributed to the quicker fermentation of glycerol compared with starch (Mach et al., 2009; Burakowska et al., 2020).

There was no effect of CG on RAN, whose concentrations in this study remained above 5 mg.dL<sup>-1</sup>, considered the minimum for the adequate activity of the rumen microbial population (Satter and Slyter, 1974). The concentration of RAN can vary based on the amount of rumen-degradable protein in the diet, its digestion rate, and energy availability, primarily from carbohydrate fermentation (Russell et al., 1992). Thus, the substitution of starch by glycerol as an energy source does not appear to alter the energy available for rumen microorganisms.

Crude glycerin levels did not affect blood glucose concentrations, but sampling time exhibited a quadratic behavior. This response could be explained by the increased availability of glucose precursors for hepatic gluconeogenesis, such as rumen propionate and glycerol found in CG. Similar to our results, some authors (Mach et al., 2009; Terré et al., 2011; Bajramaj et al., 2017) did not find effects of glycerol on plasma glucose concentrations in lambs, Holstein bulls, or dairy cows, but Gunn et al. (2010b) observed a decrease in plasma glucose concentrations when glycerol was offered up to 45% DM of diets.

Despite the increased nutrient digestibility, CG levels did not impact animal performance, expressed as TWG or ADG. This could be explained by the fact that DMI was not affected, which is the most crucial variable influencing animal performance. Dry matter intake corresponds to 60 to 90% of the variation observed in digestible energy intake, whereas digestibility explains only 10-40% of animal performance (Crampton et al., 1960; Reid, 1961; Mertens, 1987).

These results agree with those found by Avila-Stagno et al. (2013), who fed sheep glycerol levels of 70, 140, and 210 g.kg<sup>-1</sup> DM, and Chanjula et al. (2015), who evaluated glycerol doses of 50, 100, and 200 g.kg<sup>-1</sup> DM. However, there are reports of a quadratic effect of CG levels on DMI (Saleem and Singer, 2018; Andrade et al., 2018), as well as a linear increase in intake when glycerol doses of 60-200 g.kg<sup>-1</sup> DM were offered (Gunn et al., 2010a; Souza et al., 2015). The inconsistency of the results can be attributed to the method of administration, composition of the diets, purity, and level of inclusion in the diets (Saleem and Singer, 2018).

Contradictory observations regarding CG have also been reported in recent meta-analyses. One study (Syahniar et al., 2021) found negative effects of CG levels (ranging from 0 to 300 g/kg of diet DM) on DMI and animal performance in beef cattle but no effect on milk yield in dairy cows. Another meta-analysis concluded that CG up to 200 g/kg did not have a negative effect on productive performance but increased feed efficiency in beef cattle (Torres et al., 2021a). For dairy cows, glycerin inclusions of up to 100 g/kg in the diet did not negatively affect milk yield or composition, but when fed above 150 g/kg, reduced milk fat and its fatty acid profile (Torres et al., 2021b).

The negative effect of CG on DMI is often associated with animals fed high-grain diets, in which CG could reduce DMI due to its higher energy density compared with corn. Additionally, the potential increase in propionate production in the rumen when high levels of CG are fed might decrease DMI due to propionate oxidation in the liver (Allen et al., 2009). Moreover, CG might have negative effects on the rumen environment, such as a decrease in rumen pH (Mach et al., 2009; Burakowska et al., 2020), leading to a reduction in cellulolytic activity in the rumen.

## 5. Conclusions

Crude glycerin can be given to sheep at up to 280-300 g/kg of dry matter, replacing ground corn in a mixed forage diet (40% corn silage), without causing negative effects on nutritional characteristics, rumen fermentation, and animal performance.

## Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Author contributions

**Conceptualization:** Silva Neto, I. M.; Galati, R. L. and Cabral, L. S. **Data curation:** Fonseca, A. S. R.; Silva Neto, I. M.; Abreu, M. L. C.; Costa, A. C.; Vieira, K. P. N. and Carvalho, L. B. **Formal analysis:** Fonseca, A. S. R.; Silva Neto, I. M.; Abreu, M. L. C.; Galati, R. L. and Cabral, L. S. **Funding acquisition:** Galati, R. L. and Cabral, L. S. **Investigation:** Fonseca, A. S. R.; Silva Neto, I. M.; Costa, A. C.; Vieira, K. P. N.; Carvalho, L. B.; Galati, R. L. and Petter, F. A. **Methodology:** Fonseca, A. S. R.; Silva Neto, I. M.; Costa, A. C.; Vieira, K. P. N.; Carvalho, L. B.; Galati, R. L.; Petter, F. A. and Cabral, L. S. **Project administration:** Silva Neto, I. M. and Cabral, L. S. **Resources:** Cabral, L. S. **Software:** Abreu, M. L. C.; Costa, A. C.; Carvalho, L. B.; Petter, F. A. and Cabral, L. S. **Supervision:** Costa, A. C.; Galati, R. L. and Cabral, L. S. **Validation:** Cabral, L. S. **Visualization:** Fonseca, A. S. R. and Cabral, L. S. **Writing – original draft:** Fonseca, A. S. R.; Silva Neto, I. M.; Abreu, M. L. C.; Costa, A. C.; Vieira, K. P. N.; Carvalho, L. B.; Galati, R. L. Petter, F. A. and Cabral, L. S. **Writing – review & editing:** Costa, A. C.; Vieira, K. P. N.; Carvalho, L. B.; Galati, R. L. and Petter, F. A.

## Conflict of interest

The authors declare no conflict of interest.

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