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Rumen fermentation and degradability in sheep fed crude glycerin from biodiesel production from frying oils

Fermentação e degradabilidade ruminal em ovinos alimentados com glicerina bruta oriunda da produção de biodiesel a partir de óleos de fritura

Elizângela Soares Menezes[1]*; Marcos Jácome de Araújo[2]; Jacira Neves da Costa Torreão[2]; Carlo Aldrovandi Torreão Marques[3]; Leilson Rocha Bezerra[4]; Ricardo Loiola Edvan[2]; Silvokleio da Costa Silva[5]; Tiago Gutemberg de Jesus Gomes[6]; Francisca Jakeline Carvalho Viana[7]

Highlights

Crude glycerin inclusion did not influence total diet dry matter intake.
Crude glycerin inclusion increased concentrate crude protein degradability.
Low-quality crude glycerin can be included at up to 75 g kg^{-1} of the total diet DM.

Abstract

The objective of this study was to evaluate the dietary inclusion of crude glycerin (CG) derived from biodiesel production from residual frying oils, replacing corn, on intake, fermentation parameters, and rumen degradability of dry matter (DM) and crude protein (CP). Four rumen-cannulated sheep were fed diets containing 0, 25, 50, and 75 g CG kg^{-1} of the total diet DM. A 4×4 Latin square design was adopted. To determine degradability, concentrate samples were incubated in the rumen at 0, 3, 6, 12, 18, 24, 48, and 72 h. Orthogonal contrasts were tested for linear and quadratic effects, which were considered significant when $P < 0.05$ and a trend when $P < 0.10$. The inclusion of CG did not influence the intakes of DM (average = 991.5 g day^{-1}) or CP (average = 114.65 g day^{-1}); however, ether extract (EE) intake increased linearly

[1] Master's Student in the Graduate Program in Animal Science, PPGZ, Universidade Federal do Piauí, UFPI, Bom Jesus, PI, Brazil. E-mail: eliz-menezes@hotmail.com
[2] Profs., Graduate Program in Animal Science, PPGZ, UFPI, Bom Jesus, PI, Brazil. E-mail: jacome@ufpi.edu.br; jacira.torreao@ufpi.edu.br; edvan@ufpi.edu.br
[3] Prof., Universidade Federal do Rio Grande do Norte, UFRN, Macaíba, RN, Brazil. E-mail: cjtorreao@yahoo.com.br
[4] Prof., Graduate Program in Animal Science and Graduate Program in Science and Health, Universidade Federal de Campina Grande, UFCG, Patos, PB, Brazil. E-mail: leilson@ufpi.edu.br
[5] Prof., UFPI, Bom Jesus, PI, Brazil. E-mail: silvokleio@ufpi.edu.br
[6] Doctoral Student in the Graduate Program in Animal Science, Universidade Estadual Paulista, UNESP, Botucatu, SP, Brazil. E-mail: tiagogutemberg@hotmail.com
[7] Graduate in Animal Science, UFPI, Bom Jesus, PI, Brazil. E-mail: jakeline.zootecnia2014@gmail.com
* Author for correspondence

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(P < 0.05), ranging from 20.8 to 36.7 g day^{-1}, respectively, for the CG levels of 0 and 75 g kg^{-1} diet DM. There was no interaction effect (P > 0.05) between collection time and CG level on rumen pH or N-NH_3. The lowest pH values were observed at 2 h (5.83) and 4 h (5.87) after feeding. Ammoniacal nitrogen (N-NH_3) showed a quadratic decrease (P = 0.02), with a minimum value of 8 mg dL^{-1} at the CG level of 41.5 g kg^{-1} DM. The "A" fraction of the concentrate DM decreased quadratically (P = 0.05), with the lowest value (19.7%) occurring at the CG level of 74.1 g kg^{-1} DM; the "B" fraction and effective degradability (ED; 8% h^{-1}) decreased linearly (P < 0.05); and potential degradability (PD) tended to decrease (P < 0.10) with increasing CG levels. Crude glycerin did not affect the "c" fraction, lag time, or the ED (2% h^{-1}) of concentrate DM. As regards CP, there was also no effect on fractions "A" (average = 41.5%), "B" (average = 51.0%), or PD (average = 92.4%); on the other hand, fraction "c" and ED increased linearly (P < 0.05), whereas lag time decreased linearly (P < 0.05) with increasing CG. In conclusion, the CG used in this study can be added up to the level of 75 g kg^{-1} of the total DM of the sheep diet.
Key words: Alternative feed. Ammoniacal nitrogen. Byproduct. Glycerol. Rumen disappearance.

Resumo

Objetivou-se avaliar a inclusão da glicerina bruta (GB) oriunda da produção de biodiesel, a partir de óleos residuais de frituras, em substituição ao milho, sobre o consumo, parâmetros de fermentação e da degradabilidade ruminal da matéria seca (MS) e da proteína bruta (PB). Utilizou-se quatro ovinos canulados no rúmen, recebendo dietas contendo 0, 25, 50 e 75 g GB kg^{-1} da MS total da dieta. O delineamento utilizado foi o quadrado latino 4×4. Para determinação da degradabilidade, amostras do concentrado foram incubadas no rúmen nos tempos 0h, 3h00, 6h00, 12h00, 18h00, 24h00, 48h00 e 72h00. Contrastes ortogonais foram testados para efeitos linear e quadrático, considerando estes significativos quando o P < 0,05 e tendência quando P < 0,10. A inclusão da GB não influenciou os consumos de MS (média = 991,5 g dia^{-1}) e de PB (média = 114,65 g dia^{-1}), no entanto, o consumo de extrato etéreo (EE) aumentou linearmente (P < 0,05), o qual variou de 20,8 a 36,7 g dia^{-1}, respectivamente, para os níveis de 0 e 75 g GB kg^{-1} MS da dieta. Não houve efeito da interação (P > 0,05) entre o tempo de coleta e o nível de GB para o pH e o N-NH_3 ruminal. Os menores valores de pH foram observados 2 h (5,83) e 4 h (5,87) após a alimentação. O N-NH_3 sofreu redução quadrática (P = 0,02), com valor mínimo de 8 mg dL^{-1} ao nível de 41,5 g GB kg^{-1} MS. A fração "A" da MS do concentrado reduziu de forma quadrática (P = 0,05), com menor valor (19,7%) ao nível de 74,1 g GB kg^{-1} MS, a fração "B" e a degradabilidade efetiva (DE; 8%h^{-1}) reduziram linearmente (P < 0,05) e a degradabilidade potencial (DP) tendeu a reduzir (P < 0,10) com o aumento do nível de GB. Não foi observado efeito da inclusão da GB sobre a fração "c", o *lag time* e a DE (2% h^{-1}) da MS do concentrado. Para a PB, não foi observado efeito paras as frações "A" (média = 41,5%), "B" (média = 51,0%) e "DP" (média =92,4%), por outro lado, a fração "c" e a DE aumentaram linearmente (P < 0,05), ao passo que o *lag time* diminuiu linearmente (P < 0,05) com o aumento do nível de GB. Conclui-se que a GB usada nesta pesquisa pode ser adicionada até 75 g kg^{-1} da MS total da dieta de ovinos.
Palavras-chave: Alimento alternativo. Coproduto. Glicerol. Desaparecimento ruminal. Nitrogênio amoniacal.

Introduction

The use of residual vegetable frying oils by the food industry for the production of biodiesel is a sustainable alternative that aims to minimize the environmental impacts generated by their inadequate disposal in nature. Linked to the biodiesel production process is the generation of crude glycerin, considered the main by-product of biofuel manufacturing (Cooper & Weber, 2012).

The glycerol present in crude glycerin is converted in the rumen to propionate, which acts as a precursor for the hepatic synthesis of glucose, proving its potential application as a gluconeogenic substrate for ruminants (Dias et al., 2016). In addition, the advantages of constituting a promising energy source that is able to partially replace energy concentrates in the diet and that lipids contain 2.25 times more energy than carbohydrates make this by-product an excellent alternative feed supplement (Fávaro et al., 2015).

Several studies have investigated the effectiveness of crude glycerin in ruminant diets as an energy source to replace corn; however, little is known about the effects of low-quality crude glycerin, which has a lower concentration of glycerol and high levels of fatty acids. Thus, it is essential to evaluate the nutritional value, degradability, and fermentation of nutrients in the rumen to determine their optimum levels in the diet of these animals, providing adequate balance (Martins et al., 2017; Oliveira et al., 2020).

The crude glycerin used in most studies available in the literature originates from vegetable oils and contains high levels of glycerol and a low concentration of lipids. Therefore, the number of studies examining the inclusion of crude glycerin derived from biodiesel production from frying oils (low glycerol and high fatty acid content) in animal feed is still limited. Accordingly, our hypothesis is that crude glycerin from biodiesel production from frying oils can be included up to the level of 75 g kg^{-1} of the total diet DM without compromising intake or rumen fermentation and degradation parameters in sheep.

Given this context, the present study was carried out to evaluate the effect of including increasing levels of crude glycerin from biodiesel production from frying oils (low glycerol and high fatty acid content) in the diet, to replace corn, on nutrient intake, rumen pH and ammonia, as well as the *in situ* rumen degradability of concentrate dry matter and crude protein in sheep.

Material and Methods

The experiment was conducted in the Teaching-Production Module of the Goat-Sheep Farming Section at Colégio Técnico de Bom Jesus, on the Professora Cinobelina Elvas campus of the Federal University of Piauí (CPCE/UFPI), located in the municipality of Bom Jesus - PI, Brazil This study was carried out after approval by the Ethics Committee on Animal Experimentation (CEUA) at UFPI, under approval no. 037/14.

Four Santa Inês animals (approximately 24 months old and 54.5±3.5 kg average body weight) equipped with permanent rumen cannulae were kept in a feedlot where they received a diet composed of 60% elephant grass (*Pennisetum purpureum* Schum) and 40% concentrate. The diets contained

different levels of inclusion of crude glycerin (CG) to replace corn (0, 25, 50, and 75 g kg^{-1} of the total diet DM) and were formulated (Table 1) to be isoproteic as well as to meet the nutritional requirements of sheep for maintenance (National Research Council [NRC], 2007).

Table 1
Chemical composition of the experimental diets (dry-matter basis)

Item	Inclusion of crude glycerin (g kg^{-1} DM of the diet)			
	0	25	50	75
Ingredient (g kg^{-1} DM)				
Pennisetum purpureum Schum	600	600	600	600
Ground corn grain	323	298	273	248
Soybean meal	65.0	64.0	63.0	62.0
Urea	0.00	1.00	2.00	3.00
Crude glycerin	0.00	25.0	50.0	75.0
Mineral premix1	12.0	12.0	12.0	12.0
Nutrient (g kg^{-1} DM)				
Dry matter (g kg^{-1} as-fed-basis)	381.7	378.5	376.2	374
Crude protein	114.8	114.6	114.4	114.2
Neutral detergente fiber	551.7	545.4	539.1	532.8
Acid detergente fiber	303.1	301.6	300.0	298.4
Ether extract	29.9	35.3	40.7	46.1
Mineral matter	45.2	45.0	44.8	44.6
Total carbohydrates	810.1	805.1	800.1	795.1
Nonfibrous carbohydrates	258.4	262.5	263.8	265.1

DM = dry matter.
[1] Guaranteed content per kilogram of product is as follows: Ca, 267 g; P, 61 g; Mg, 20 g; S, 35 g; Zn, 6000 mg; Cu, 350 mg; Fe, 3000 mg; Mn, 2000 mg; Co, 20 mg; I, 80 mg; Se, 23 mg; Cr, 60 mg; F (max.) 6000 mg.

The CG used in the experiment originated from catalytic transesterification reactions of triacylglycerols during the production of biodiesel from oils from restaurants, provided by the Water and Sewage Treatment Agency of the State of Piauí S/A (AGESPISA). The glycerin was incorporated manually and homogenized into the concentrate according to the tested levels. Chemical composition (g kg^{-1} DM) analysis revealed the following contents: mineral matter: 7.50, method, 942.05 Association of Official Analytical Chemists [AOAC] (1990), glycerol: 306.2, method, 169 United States Pharmacopeial Convention [USP] (2015), methanol: 1.11, method 467 (USP, 2015), sodium: 1.60, method, 0159 Food and Drug Administration [FDA] (2010),

and fatty acids (g 100^{-1} g fat): 29.43, method, 996.06 (AOAC, 2007).

The experiment lasted 60 days, which were divided into four 15-day periods. These consisted of 10 days of adaptation of the animals to the experimental diets, followed by five days of data collection. A used 4×4 Latin square experimental design was adopted, corresponding to four animals and four levels of CG in the diet, to which the animals were randomly allocated.

Feed was supplied twice daily (07h00 and 16h00), allowing *ad libitum* intake of feed and water. Intake was quantified daily during the collection period, and the amount of orts was established at 10% to ensure unlimited consumption. The intakes of dry matter (DM), crude protein (CP), and ether extract (EE) were determined by recording the amounts of feed supplied and orts.

Ingredients, diets, and orts were analyzed according to AOAC (1990) for the levels of DM (method no. 930.15), CP (method no. 981.10), and EE (method no. 920.39). Total carbohydrates (TC) were determined by the equation proposed by Sniffen et al. (1992): TC = 100 – (%CP + %EE + %MM). Non-fiber carbohydrate (NFC) contents were calculated using the equation recommended by Hall (2000): NFC = 100 – (%MM –%EE – %NDF) – (CP – CPu + U), in which U = % of urea; CPu = CP of urea.

To determine the parameters of rumen fermentation, rumen fluid samples were collected via fistula on the 5th day of collection of each experimental period, at the following predetermined times: before morning feeding (considered time zero) and 2, 4, and 6 h after feed supply. The pH was measured and, subsequently, the ammonia concentration was determined according to the technique described by Detmann et al. (2012).

The rumen-incubated samples referred to the diet concentrate (Table 2), as CG was incorporated directly into the concentrate. The *in situ* rumen degradability parameters of DM and CP were estimated as per Ørskov and McDonald (1979). For this purpose, the composite samples were ground in a Wiley mill equipped with 2-mm sieves. Non-woven fabric ('TNT') bags (100 g cm^{-2}) measuring 4 × 5 cm were made, as recommended by Valente et al. (2011). These mini-bags were sealed at the edges, properly labeled, and placed in a forced-air oven at 55 ºC for 24 h. Afterwards, they were weighed and placed in linen bags, with a small weight anchored with a nylon line attached to the cannula. Incubation was performed in reverse chronological order at 0, 3, 6, 9, 12, 24, 48, and 72 h, when all bags were removed simultaneously. Bags referring to time zero were not inserted into the rumen, but were washed in running water along with the others. After being washed, the bags were dried in a forced-air oven at 55 °C for 72 h, following AOAC (1990) recommendations.

In situ ruminal degradability of DM and CP was determined as the difference in weight between the weighing operation performed before and after ruminal incubation. After obtaining the degradation parameters (A, B, and c), potential degradability (PD) was calculated as per Ørskov and McDonald (1979): $PD\ (\%) = A + B \times (1 - e^{-ct})$, in which PD = fraction degraded in time t; A = soluble fraction (%); B = potentially degradable insoluble fraction (%); c = degradation rate of fraction B (% h^{-1}); and t = time (h^{-1}).

The non-linear parameters A, B, and c were estimated using interactive Gauss-Newton procedures via PROC NLIN of SAS. After determining the model parameters, the effective degradability (ED) of DM and CP was calculated using the model proposed by Ørskov and McDonald (1979), adopting the passage rates (k) of 2, 5, and 8% h^{-1}: ED = A + [(B × c) / (c + k)], in which k = estimated rate of passage of particles in the rumen.

Table 2
Chemical composition of the experimental concentrates (dry-matter basis)

Item	Inclusion of crude glycerin (g kg^{-1} DM of the concentrate)			
	0.00	62.5	125	187
Ingredient (g kg^{-1} DM)				
Ground corn grain	807.5	745.0	682.5	620.0
Soybean meal	162.5	160.0	157.5	155.0
Urea	0.00	2.50	5.00	7.50
Crude glycerin	0.00	62.5	125.0	187.5
Mineral premix[1]	30.0	30.0	30.0	30.0
Nutrient (g kg^{-1} DM)				
Nutrient (g kg^{-1} DM)	899.6	879.5	859.4	839.3
Crude protein	171.3	170.8	170.3	169.8
Neutral detergente fiber	223.2	207.5	191.8	176.0
Ether extract	41.4	54.9	68.4	81.8
Mineral matter	34.5	33.9	33.4	32.9
Nonfibrous carbohydrates	529.6	561.1	564.3	567.6

DM = dry matter.
[1]Guaranteed content per kilogram of product is as follows: Ca, 267 g; P, 61 g; Mg, 20 g; S, 35 g; Zn, 6000 mg; Cu, 350 mg; Fe, 3000 mg; Mn, 2000 mg; Co, 20 mg; I, 80 mg; Se, 23 mg; Cr, 60 mg; F (max.) 6000 mg.

After adjusting the data and using the disappearance value obtained at degradation time zero, the colonization time (CT; lag time) was estimated for DM and CP according to the methodology proposed by Goes et al. (2010): CT = [-ln(A'-A-B) / c], in which parameters A, B, and c were estimated by Gaus Newton's algorithm.

Data were evaluated using the MIXED procedure of SAS software (version 9.0) (Statistical Analyses System Institute [SAS Institute], 2002), including the CG level, period, and CG level × period interaction as fixed effects in the model. Animal nested within treatment was considered a random effect. The data used (observed) to estimate the degradation parameters were analyzed by the interactive method, by applying the NLIN procedure of SAS statistical package (SAS Institute, 2002) for non-linear models.

For the pH and N-NH3 data, the effects of CG inclusion, collection time, and

the interaction between these factors were checked. The influence of treatments on the analyzed variables was assessed using orthogonal contrasts to determine linear and quadratic effects. Contrasts were significant when the P-value was <0.05. A trend was considered when P<0.10. Residuals were plotted against predicted values and were used to check for model assumptions of homoscedasticity, independence, and normality of errors. Data were considered outliers and removed from the database when the studentized residual was outside the ± 2.5 standard deviation range.

The following statistical model was adopted: $Y_{ijkl} = \mu + T_j + P_k + (TP)_{jk} + A_i(T_j) + e_{ijkl}$, in which Y_{ijk} = observed value of the analyzed trait; μ = overall mean; T_j = fixed effect of treatment (%CG); P_k = fixed effect of collection period; $(TP)_{ik}$ = fixed effect of the interaction between CG levels and periods; A_i = random effect of animal nested within treatment level (CG); e_{ijkl} = random error associated with each observation.

Results and Discussion

The inclusion of CG in the diet did not influence (P > 0.05) the intakes of DM or CP. In contrast, EE intake increased linearly (P < 0.05), by around 77%, in the animals fed 75 g CG kg^{-1} DM compared with those which received the control diet (Table 3).

Table 3
Nutrient intake in sheep fed diets containing crude glycerin from biodiesel production from frying oils

	Inclusion of crude glycerin (g kg^{-1} DM of the diet)					*P*-value[1]	
Item	0	25	50	75	SEM	L	Q
Dry matter intake (g day^{-1})					130	0.52	0.98
Ether extract intake (g day^{-1})	807.5	745.0	682.5	620.0	4.24	0.01	0.59
Crude protein intake (g day^{-1})	162.5	160.0	157.5	155.0	13.4	0.70	0.99

[1]Significant at P < 0.05; and considered trends when the P-value was between 0.05 and 0.10; L= linear effect; Q=quadratic effect; SEM=Standard error of the mean.

These results may be related to the degree of purity of the CG used. Regarding EE intake, this finding is directly associated with the increase in its concentration in the diets after the inclusion of CG, which is a byproduct of the industry of biodiesel from oil sources, as well as the presence of glycerol in its composition (Table 1).

The dietary EE content has always been a concern for ruminant nutritionists. An EE content of 7% in the total diet DM has been considered the maximum value of this nutrient so that there is no negative effect on DM intake or rumen fermentation, or a reduction in fiber intake and digestion (Pimentel et al., 2012). In addition, high levels of lipids induce the creation of a physical barrier over feed particles, hindering bacterial cellulolytic activity, in addition to having toxic action on certain species of ruminal microorganisms (Souza & Ribeiro, 2021).

The literature is abundant with results of research with different animal species and different levels of inclusion of CG in the diet. The results are as diverse as possible, varying mainly depending on the quality of the CG used. In diets with a high DM content, the inclusion of this byproduct can have a beneficial effect in reducing dust generation by improving plasticity, as well as by increasing palatability.

Freitas et al. (2020) worked with a CG similar to the one used in this study and concluded that this byproduct can be included at up to 70 g kg-1 of the total diet DM for lactating goats, without prejudice to nutrient intake and digestibility. In an experiment testing the inclusion of CG (up to 20% of the DM) in the diet of goats, Chanjula et al. (2014) did not observe adverse effects on nutrient intake or digestibility, demonstrating that this energy source can be used without changing animal performance.

Oliveira et al. (2020) and Andrade et al. (2018) concluded that the inclusion of up to 18% CG (80.5% glycerol) in lamb diets induced an adequate metabolic response, and that its inclusion at up to 10.9% improved DM intake. Similarly, Hermes et al. (2018) evaluated the weight growth of lambs receiving up to 32% CG (84.54% glycerol) and found no change in animal growth. According to these authors, CG can be used in place of corn without causing losses in the animals' body weight or dimensions.

Barros et al. (2015) evaluated the inclusion of CG (43.90% glycerol) in the diet of crossbred lambs, testing levels up to 10.84% in the diet DM, and found that DM, CP, NDF, and NFC decreased, while EE intake increased. According to the authors, this fact can be attributed to the high fatty acid content (33.60%) of CG. In addition, they also linked this fact to the quality of the glycerin used, which had a low amount of glycerol and a high concentration of methanol.

There was no interaction effect ($P > 0.05$) between collection time and CG inclusion level on pH values or N-NH3 concentration. However, the pH showed an upward trend ($P < 0.10$) with increasing CG levels ($P < 0.10$), with the highest values recorded at 0 h (6.38) and 6 h (6.08) after feeding and the lowest at 2 h (5.83) and 4 h (5.87) after feeding (Table 4).

Since the rumen pH is a chemical factor that is directly influenced by the diet and the roughage: concentrate ratio, in addition to being related to the final products of fermentation and directly influencing the growth of rumen microorganisms, the positive effect of the use of CG on this parameter is evidenced, since higher pH values were observed after its inclusion (Fávaro et al., 2014).

Table 4
Ruminal values of pH and ammonia nitrogen ($N\text{-}NH_3$) in sheep fed diets containing crude glycerin from biodiesel production from frying oils

Hour (H)	Inclusion of crude glycerin (g kg^{-1} DM of the diet)				Mean	SEM	P-value[2]		
	0	25	50	75			CG	H	CG×H
			pH						
0	6.18	6.41	6.26	6.67	6.38A	0.12			
2	5.53	5.91	5.71	6.15	5.83B	0.10	0.09	0.002	0.87
4	5.57	6.01	5.94	5.98	5.87B	0.12			
6	5.61	6.52	6.07	6.13	6.08A	0.12			
Mean	5.72	6.21	6.00	6.23					
			$N\text{-}NH_3$ (mg dL^{-1})						
0	10.01	7.77	7.23	9.09	8.54	0.72			
2	9.77	7.90	7.81	10.21	8.92	0.65			
4	11.45	8.04	9.09	9.53	9.53	0.53	0.02	0.36	0.84
6	10.87	8.08	9.97	9.09	9.50	0.59			
Mean	10.53	7.94	8.54	9.48					

[A,B] Means followed by different capital letters in the same column differ from each other by the Tukey's test ($P < 0.05$); SEM=Standard error of the mean; CG = crude glycerin.
[2]Significant at $P < 0.05$; and considered trends when the P-value was between 0.05 and 0.10.

The pH results found in the present study are within the ideal range of 6.2 to 7.2 for adequate fiber fermentation, degradation rate, and microbial production (Santana et al., 2012; D'Aurea et al., 2017). When the pH is below 6.0, the ruminal degradability of the feed is compromised due to the low development of cellulolytic microorganisms. In addition, there is an increase in the development of propionic and lactic acid-producing microorganisms after the consumption of rapidly fermentable carbohydrates, increasing lag time and rumen degradation time and reducing digestion rate (Van Soest, 1994; Santana et al., 2012; D'Aurea et al., 2017).

The minimum pH values found between 2 and 4 h post-feeding are related to the rate of glycerol disappearance, which is around 50 to 70% within 4 h after glycerin ingestion. This increased the production of propionate as well as short-chain fatty acids (SCFA) and lactate resulting from the fermentation process. The highest values were found before the feed was supplied, as the pH remains close to neutrality during fasting (Santana et al., 2012).

Another factor that may have contributed to the increase in rumen pH was the likely selection of roughage by the animals as the levels of CG in the diet were

increased, although DM intake was not affected. Therefore, our results suggest that the inclusion of CG has beneficial effects on the digestion of NFC and fiber from the diet.

The rumen N-NH_3 concentration decreased quadratically (P = 0.02) as the inclusion of CG in the diet was increased, with a minimum value of 8 mg dL^{-1} detected at the CG level of 41.5 g kg^{-1} DM. In this study, all CG inclusion levels resulted in an N-NH_3 concentration greater than 5 mg dL^{-1}, considered the minimum value for adequate rumen fermentation and sufficient for bacterial growth. These findings also corroborate Van Soest (1994), who considered the value of 10 mg dL^{-1} as the optimum level to increase the ruminal digestion of DM.

It is important to stress that, for effective optimization of rumen fermentation, the N-NH3 concentration must reach values between 19 and 23 mg dL^{-1} (Van Soest, 1994; D'Aurea et al., 2017). However, according to these authors, this value cannot be considered a fixed number, because the ability to produce protein and capture ammonia by bacteria depends on the carbohydrate fermentation rate.

Ammonia is supplied in the rumen by the following routes: consumption of diets containing non-protein nitrogen, recycling of urea through saliva, consumption and ruminal degradation of true protein from the feed, and degradation of dead rumen microorganisms. The removal of this ammonia is achieved through ruminal absorption, passage to the posterior tract, and, also, by incorporation with the true protein (Van Soest, 1994; Fávaro et al., 2014; Costa et al., 2015).

Therefore, the fact that the N-NH_3 concentration raised again at the CG level of 75 g kg^{-1} is attributed to the recycling of urea via saliva. Its increased production by the animals is likely explained by the greater selection of roughage, given that the inclusion of CG did not influence the ingestion or degradation of CP.

When energy is degraded more rapidly than protein, both microbial growth and digestive efficiency decrease. This is characterized by incomplete fermentation, a situation in which the N-deficient microorganisms divert ATP to carbohydrate accumulation over microbial protein synthesis (Van Soest, 1994).

There is a synergism between pH and N-NH3 levels in the rumen that allows an increase in the growth of microorganisms and degradation of the dietary fiber (Ferreira et al., 2015). In this case, despite the divergences found in pH and N-NH_3 values, it can be stated that this interaction occurred, with the pH and N-NH_3 values being within the expected range and DM intake being similar in all diets.

Crude glycerin inclusion had no effect on the fraction "c", lag time, or ED (2% h^{-1}) of DM. Fraction "A" of DM exhibited a quadratic reduction (P < 0.05) as a function of the increase in CG level, reaching a minimum value of 19.7% at the level of 74.1 g kg^{-1}. Fraction "B" decreased linearly (P < 0.05), whereas the PD of DM showed a downward trend (P < 0.10). There was also a trend towards an increase in the undegradable fraction (UF). Digestible energy at 8% h^{-1} increased linearly (P < 0.05), while ED at 5% h^{-1} tended to increase (P < 0.10) with the content inclusion of CG in the diet (Table 5).

Table 5
***In situ* ruminal degradation parameters of the dry matter and crude protein from concentrate containing crude glycerin from biodiesel production from frying oils**

Item	Inclusion of crude glycerin (g kg^{-1} DM of the concentrate)				SEM	P-value[2]	
	0.00	62.5	125	187		L	Q
Dry matter							
A (%)	22.7	18.5	22.2	25.5	1.34	0.16	0.05
B (%)	69.5	68.8	57.1	56.9	3.46	0.01	0.94
c (% h^{-1})	0.03	0.03	0.04	0.04	0.01	0.47	1.00
PD (%)	92.8	87.3	79.4	82.4	3.98	0.08	0.35
UD (%)	7.20	12.7	20.6	17.6	3.98	0.08	0.35
CT (h)	7.93	7.86	7.35	7.53	0.24	0.18	0.63
R^2	89.1	95.8	91.7	92.1	-	-	-
ED (2% h^{-1})	58.4	56.8	59.4	59.6	1.48	0.45	0.61
ED (5% h^{-1})	43.3	42.9	46.7	47.5	1.65	0.07	0.73
ED (8% h^{-1})	36.7	35.5	40.5	41.8	1.55	0.02	0.47
Crude protein							
A (%)	44.5	37.5	43.6	40.2	18.1	0.43	0.34
B (%)	48.7	54.7	48.8	51.7	1.44	0.67	0.32
c (% h^{-1})	0.05	0.07	0.08	0.09	0.01	0.002	0.73
PD (%)	93.2	92.2	92.4	91.8	0.79	0.35	0.80
UD (%)	6.80	7.81	7.62	8.16	0.79	0.35	0.80
CT (h)	6.80	6.70	6.50	6.40	0.08	0.001	0.57
R^2	84.5	95.1	90.2	91.6	-	-	-
ED (2% h^{-1})	79.9	79.6	82.4	82.2	0.61	0.02	0.92
ED (5% h^{-1})	69.7	68.8	73.3	73.0	0.74	0.002	0.71
ED (8% h^{-1})	64.1	62.4	67.4	67.2	0.82	0.004	0.42

DM = dry matter; L= linear effect; Q=quadratic effect; SEM=Standard error of the mean; PD = potential degradability; A = soluble fraction (%); B = potentially degradable insoluble fraction (%); c = degradation rate of fraction B (% h^{-1}); UD = undegradable fraction; CT = colonization time (lag time); ED = effective degradability; 2Significant at $P < 0.05$; and considered trends when the P-value was between 0.05 and 0.10.

The degradability of DM at time zero was around 20% for all treatments. Over time, it increased more rapidly and linearly from 12 h - at the inflection point of the curve -, tending to stabilize after 72 h of incubation (Figure 1). The concentrate with 187 g kg^{-1} CG was the one which showed the highest initial disappearance rate; however, in the end, the concentrates with 0 and 62.5 g kg^{-1} CG stood out among the treatments, obtaining a higher rate of disappearance of DM. This was mainly due to the higher PD values in these two concentrates (Table 5).

None of the concentrates affected fractions "A" or "B", PD, or UF of CP. On the other hand, fraction "c" and ED (2.5 and 8% h^{-1}) increased linearly with increasing CG levels in the concentrate ($P < 0.05$; Table 5). As for CP degradability, no differences were detected until 3 h of incubation in any treatment. However, from that moment onward, the treatments involving 125 and 187 g kg^{-1} CG slightly stood out relative to the others until 48 h, although the treatments with 0 and 125 g kg^{-1} produced a higher percentage of fraction "A". Nonetheless, after this period, all treatments showed similar maximum CP degradation values (Figure 2).

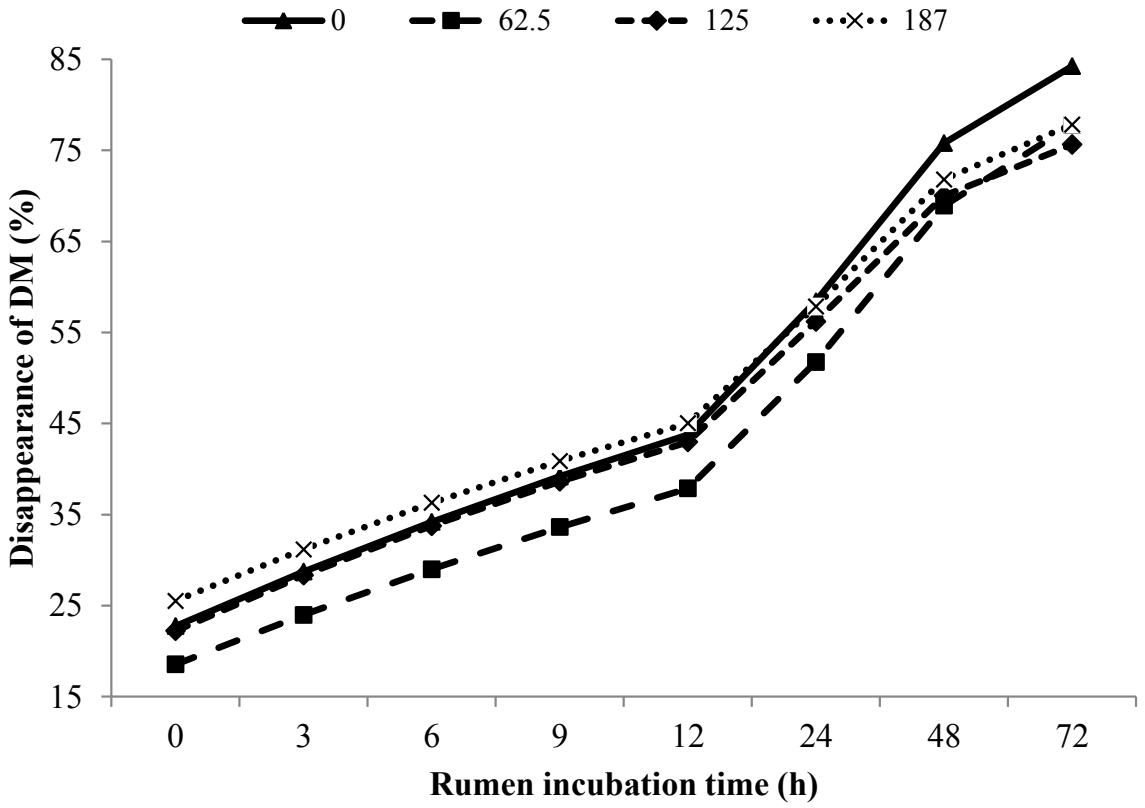

Figure 1. *In situ* dry matter (DM) disappearance rate of the concentrate (g kg^{-1} DM) containing crude glycerin from biodiesel production from frying oils according to rumen incubation time (h).

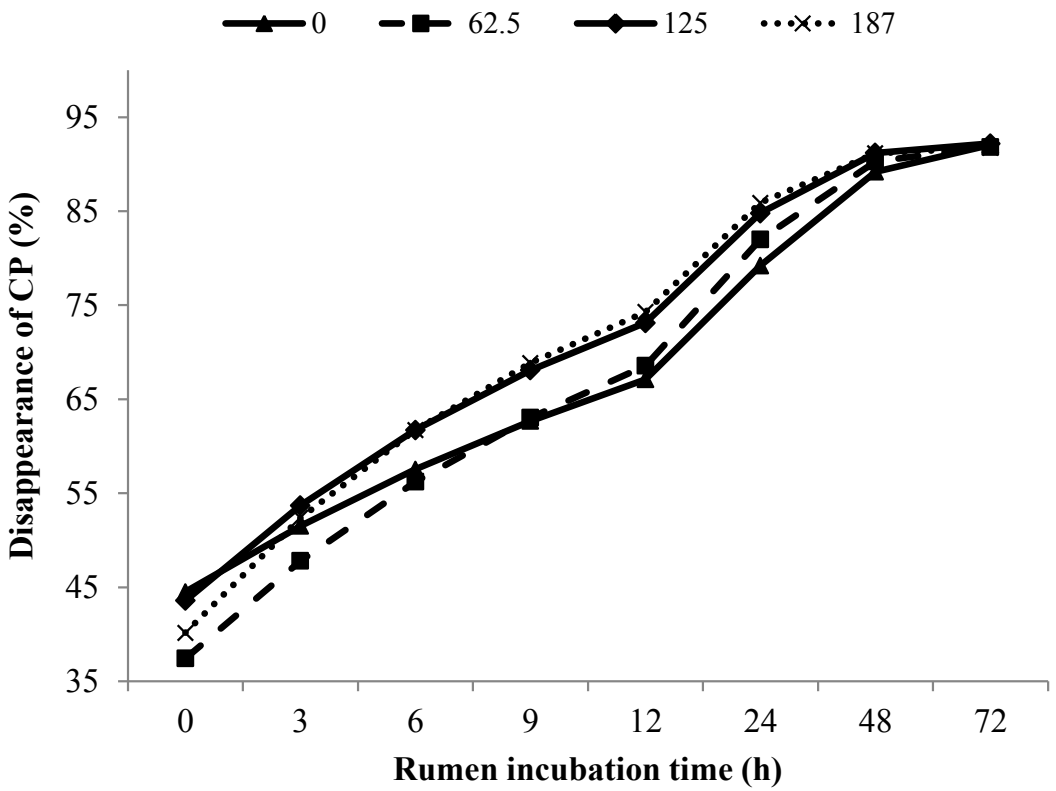

Figure 2. *In situ* crude protein (CP) disappearance rate of the concentrate (g kg^{-1} DM) containing crude glycerin from biodiesel production from frying oils according to rumen incubation time (h).

The downward trend in PD of DM can be attributed to the decrease in the "B" fraction, with a consequent increase in the UF of nutrients in the rumen. This behavior can be explained by the increase in EE levels in the concentrate, which exceeded the recommended level of 6% on a DM basis. This fact may have negatively influenced the action of some species of rumen microorganisms, due to the toxic effect of unsaturated fatty acids and their binding to the surface of the feed, functioning as a physical barrier limiting the adhesion and microbial digestive activity on the feed particles in the rumen (Granja-Salcedo et al., 2016).

The EE content of an incubated feed can clog the pores of nylon bags and slow down degradation (Belato et al., 2013). During incubation, the bags containing the highest percentage of CG were greasy to the touch, which was not the case with the control treatment.

An increase in the "A" fraction indicates that glycerol can stimulate enzymes and other ruminal microorganisms. Higher NFC levels in the concentrate also contribute to the increase in this fraction. Part of the "B" fraction can be solubilized and incorporated into the "A" fraction with increasing CG levels; therefore, the highest percentage of this fraction is observed at the highest CG level (Gomes et al., 2015).

The downward trend in PD may indicate a lower nutritional value of the feed, so it is possible that nutrient utilization was low (Belato et al., 2013). This may have been due to the low amount of glycerol and high content of unsaturated fatty acids present in the CG used in this study, since the amount of protein remained constant with the increase in CG levels. Gomes et al. (2015) observed an increase in PD of DM with the inclusion of up to 20% CG; however, the amount of glycerol (82.54%) present in the glycerin was much higher than that of this study.

The average DM lag time of 7.7 h explains the significant increase in ruminal disappearance of DM only after 12 h of incubation, with ED at 5% and 8% h^{-1} also increasing. This shows that lag time was not affected despite the low pH values resulting from the incorporation of high levels of unsaturated fatty acids in the incubated material. This is believed to be the main factor explaining the degradability data found in this experiment.

Despite the lower degradation of DM after the inclusion of CG, the pattern of the degradation curve was similar in all treatments, indicating that, from 12 h of incubation, there was high energy availability in the rumen (Figure 1). This may reinforce the positive aspect of including CG in this experiment, since ruminal degradation can be improved by greater energy availability, given that nutrient digestibility is directly related to the energy content of feedstuffs (Alves et al., 2016).

The crude protein PD rates above 90% in all treatments and the increasing "c" fraction with increasing CG content in the concentrate contributed to the microorganisms taking less time to colonize the feed particles, resulting in a greater efficiency of ED. Lag time decreased as the amount of CG was increased due to the high solubility of glycerol, which facilitates the colonization and use of substrates.

Belato et al. (2013) studied the kinetics of ruminal degradation in sheep diets with different levels of CG and found that lag time was longer in diets containing higher levels of glycerin, which differs from the findings of the present study. This occurrence can be explained by the higher concentration of readily available carbohydrates in our experiment. These results corroborate data found by several authors on the positive aspects of including CG in ruminant diets.

Van Cleef et al. (2018) investigated the effects of partial and total replacement of grain corn with up to 30 g CG kg^{-1} of total diet DM on the rumen metabolism of crossbred sheep and observed changes in rumen fermentation parameters, a decrease in volatile fatty acid production, and reduced *in vitro* methane gas production. Furthermore, the potential effective degradation rates and *in vitro* digestibility of dietary DM were improved, whereas fiber digestibility was impaired. Similarly, Van Cleef et al. (2014) evaluated high concentrations of CG (30 g kg^{-1} DM) in the diet of feedlot Nellore cattle and found that despite the negative effect on the digestibility of the fiber fraction of the diet, there were no significant differences in the performance or carcass yield of animals, suggesting that crude glycerin can be a good energy source in animal feed.

For Beserra et al. (2016), the use of CG in ruminant feed is advantageous in that it increases the speed of ruminal degradation and leads to greater production

of propionate. This culminates in lower rates of methane production and eructation, which is fundamental to reducing the greenhouse effect.

The differences found between studies regarding the inclusion of glycerin in the diet of ruminants are mainly a result of its composition, which is altered both by the method of obtaining it and the intrinsic effects of glycerol on ruminal and animal metabolism (Santos et al., 2018).

Although the CG inclusion levels tested in this study reveal that it can be used as an energy source, it is important to emphasize that the deleterious effects of glycerin may vary according to the concentration used as well as the presence of unsaturated lipids generated from various thermal reactions, salt, and methanol. However, as the animals adapt to the diet, the rates of digestion and disappearance of glycerin in the rumen are maximized (Dias et al., 2016). The variation in CG composition is attributed to the facts that it is a byproduct and that there is no concern by the Brazilian industry to separate the remaining material (glycerol) from the reagents used in the production of biodiesel, which results in excess impurities in this compound (Santos et al., 2018).

Therefore, considering that the glycerin used in the present study was low-purity, we believe that the levels of glycerol and EE in the material incubated alone or together influenced the ruminal degradability of DM and CP by increasing propionate production, indicating that glycerol can stimulate enzymes and other rumen microorganisms (D'Aurea et al., 2017). Thus, we assume that the rapid CP degradation rate was synchronized with the degradation of DM, allowing for greater efficiency in the fermentation process through the release of short chain fatty acids to be used by rumen microorganisms.

Conclusion

Supplementing sheep feed with crude glycerin as a source of glycerol did not change dry matter intake. However, it induced changes in rumen degradation and fermentation parameters without negatively affecting the rumen environment. In this context, we recommend the inclusion of crude glycerin (306.2 g kg^{-1} DM glycerol; 29.43 g 100^{-1} g fat) at up to 75 g kg^{-1} of the total DM in the diet of hair sheep.

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