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Ingestive behavior, volatile fatty acids, blood biochemical and hormonal variables of dairy goats supplemented with glycerin

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ABSTRACT. The objective of this research was to determine the ingestive behavior, volatile fatty acids, and blood biochemical and hormonal variables of goats consuming a diet with 15% glycerin. Feed efficiency (FE) and rumination (ER) of dry matter intake (DMI) and neutral detergent fiber (NDFI) of dairy goats supplemented with glycerin (0 and 15%) were not influenced by treatments ($p > 0.05$). The specific activities of defecation, urination and drinking had a significant effect ($p < 0.05$) in relation to the treatments with glycerin in the diet of dairy goats. The occasional activities (defecation, urinating and drinking water) decreased with the addition of 15% of glycerin in the goats' diet. Lactic acid had a significant effect ($p < 0.001$) with the addition of 15% glycerin. Diets for dairy goats with 15% glycerin did not change the consumption of dry material, neutral detergent fiber, nor did they change the ingestive behavior of these animals. The levels of globulin, protein, albumin/globulin, glucose, cholesterol, urea, triglycerides, cortisol, and T4 variables were significantly influenced ($p < 0.05$) by the addition of glycerin in the diet.

Keywords: Agv's glycerin; rumination; idleness; defecation; water.

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Introduction

Animal behavior is a way of looking at the entire production system, including the individual activities of the animal in its social and physical environment (Custodio et al., 2017). Small ruminants have the ability to adapt to different environmental and feeding conditions, modifying their behavioral parameters to reach and maintain a certain level of consumption compatible with nutritional requirements (Cirne et al., 2014). Intake behavior estimates are reported as relevant tools in dietary assessments, allowing the adjustment of administration of small ruminant feed to obtain the best productive performance (Silva et al., 2016).

As feed costs are high, alternative food sources such as glycerin (rich in glycerol) have become a major focus for the livestock industry (Chanjula, Pakdeechanian, & Wattanasit et al., 2015). Crude glycerin (GC) is a by-product of biodiesel production resulting from the formation of methyl esters of triglyceride fatty acids. The addition of 20% crude glycerin had no significant effect on performance, carcass characteristics and meat quality of goats (Chanjula et al., 2015). Freire et al. (2022) observed that the use of 15% double-distilled glycerin in the feeding of lactating goats increased milk production, the amount of fat, and also increased the size of the milk fat globule. However, regarding the saturated and polyunsaturated fatty acids no changes were shown comparing the control group and the level of 15% of glycerin.

Thus, glycerin can be effectively used as an alternative source of energy to replace cereals in the diets of finishing goats. The use of alternative products in the sheep diet did not affect the consumption of DM and NDF nor did they modify the ingestive behavior of these animals. Sá et al. (2015), rumination (358.67 min.) and chewing time (530 min.) were not influenced by babassu pie levels. The addition of glycerol in the diet has been reported (Gunn, Neary, Lemenager, & Lake et al., 2010) and different outcomes have been reported for glycerol when entering the rumen: passage to the lower gut, absorption through the rumen wall and conversion to glucose in the liver, or fermentation to propionate.

In a study carried out with dairy goats using in their feed the addition of four increasing levels of glycerin (0, 6, 12, and 18%), it was observed that the physicochemical characteristics of the milk did not show any

significant difference ($p > 0.05$) except for fat, which showed low concentration at levels of 12 and 18%. Thus, we used the same animals in a separate experiment to analyze the 15% level of glycerin addition (Lima et al., 2021). There was an increase of 18%, which was one of the reasons that led us to test the level of 15% glycerin in the feeding in our study.

Therefore, the objective of this research was to determine the ingestive behavior, volatile fatty acids and blood biochemical and hormonal variables of goats consuming a diet with 15% glycerin.

Material and methods

Experimental site

The experiment was conducted at the Federal University of Paraíba, Campus at Bananeiras-Paraíba, Brazil (altitude 552m, latitude 6° 41' 11'', longitude 35° 37' 41''). Air temperature was 24.97ºC and relative humidity was 76.48% in the stalls.

Animals and management

This project was submitted to the Ethics Committee on Animal Use (CEUA) of the Federal University of Paraíba and approved according to protocol no. 052/2017. Twelve multiparous Saanen goats weighing 47.07 ± 2.41 kg and at 90 ± 5 days of lactation. The experimental design was completely randomized with two treatments (0 and 15% of glycerin).

The diets were adjusted to meet the requirements of the National Research Council (NRC, (2007) for lactating goats producing 2.0 kg of 4% fat milk day^{-1}, with a bulk ratio of 55:45 forage: concentrate. The experimental diet was offered ad libitum as a complete mixture at 07:30 a.m. and 04:30 p.m. The ingredients were: Tifton hay, milled corn, soybean meal, vitamin/mineral supplement and urea, along with the following levels of CG (99.66% glycerol): 0% (control) and 15% to corn in the diets (Table 1). The crude energy contents of the corn and CG were 3.50 and 3.71 Mcal kg^{-1}, respectively.

Table1. Percentage and bromatological composition of experimental diets.

Ingredient (g kg^{-1} DM)	Levels of addition (%)	
	0.00	15.0
Glycerin	0.00	15.0
Soybean meal	9.50	9.50
Ground corn	33.5	18.0
Tifton hay	55.0	55.0
Urea	0.00	0.50
Mineral supplement[1]	1.50	1.50
Calcitic limestone	0.50	0.50
Chemical composition		
Dry matter, DM (g kg^{-1} as fed)	882	836
Crude protein. CP (g kg^{-1} DM)	114	98.7
Ethereal extract. EE (g kg^{-1} DM)	12.8	18.8
Neutral detergent fiber. NDF (g kg^{-1} DM)	905	674
Fiber in acid detergent. FAD (g kg^{-1} DM)	282	267
Metabolizable energy. ME (Mcal kg^{-1} DM)	3.62	3.58

[1]Composition of mineral supplement. per kg: P: 70 g; Ca: 140 g; Na: 148 g; S: 12 g; Mg: 1.320 mg; F: 700 mg; Zn: 4.700 mg; Mn: 3.690 mg; Fe: 2.200 mg; Co: 140 mg; I: 61 mg; Se: 15 mg; Sodic Monensin: 100 mg

The animals went through a period of 15 days to adapt to the diet and three days to collect data. During the adaptation and collection periods, daily weighing of food supply and leftovers were carried out to calculate the voluntary consumption and adjust the feed supply, so as to guarantee leftovers of 10% based on dry matter (DM). Water for animal consumption was offered ad libitum, and consumption was quantified daily during the data collection period.

Behavioral

During the period in the feedlot (34 days), three visual evaluations were performed (for the last four weekends of the confinement in the period i.e., 7 and 14 days). The animals were evaluated 24hours (08:00 to 08:00), with an interval of 5 min in a direct fashion. The behavioral variables were: feeding (chewing of starter in mouth), ruminating (chewing regurgitated food, either in standing or in lying position), idleness (standing

without any movement or behavior), drinking (swallow water), mastication (the sum of feeding times and the time of rumination), others (defecation, urination, drinking) activities (Nicory et al., 2015).

The results referring to the efficiency of the ingestive behavior, obtained by the relations: (a) FE = DMI/FT; (b) FE = NDFI/FT; (c) RE = DMI/RT; (d) RE = NDFI/RT; (e) TCT = FT + RT, in which: FE = feeding efficiency (g DM $min.^{-1}$); DMI = dry matter intake (g DM $min.^{-1}$); FT = feeding time (min. day^{-1}); RE = rumination efficiency (g DM $min.^{-1}$); NDFI = Neutral detergent fiber intake (g NDF $min.^{-1}$); RT = rumination time (min. day^{-1}); TCT = total chewing time (min. day^{-1}).

There was also continuous observation of the number of times the animal defecated, urinated and sought water, through adopting visual observation of the animals for 24 hours, which was performed by trained observers in an alternation system, strategically positioned so as not to promote changes in the routine of the animals.

Volatile fatty acids

For the analysis of volatile fatty acids (VFA), 2.0 ml of culture medium sample was removed from all experimental units after 48 hours of incubation (Kaffarnik, Kayadermi, Heid, & Vetter, 2014) , and placed in Eppendorf tubes which were centrifuged at 5,200 x g for 10 minutes; the supernatant was then frozen for analysis of volatile fatty acids (VFA) on a High Performance Liquid Chromatograph (HPLC), brand SHIMADZU, model SPD-10A VP coupled to the Ultra Violet (UV) Detector using a wavelength of 210 nm. A SHIMADZU C18 column with a diameter of 30 cm x 7.9 mm was used, with a flow in the column of 0.6 mL $minute^{-1}$. At a pressure of 69 kgf, 20 µL of the mobile phase of water in 1% orthophosphoric acid was injected. Concentrations of VFA acetate, propionate and butyrate were analyzed.

Biochemical and hormonal variables

To collect blood samples, the jugular vein was punctured after disinfection with iodine alcohol. For analysis of biochemical and hormonal parameters, blood was collected in 7 mL vacuum tubes containing separating gel and sodium fluoride (used for glucose analysis). The samples were homogenized, promptly refrigerated and taken to the laboratory for processing. All samples were centrifuged at 1,100 × g and 4°C for 15 minutes. After centrifugation, the supernatants were collected and separated into 1.5 mL aliquots for biochemical and hormonal tests. Samples were stored at −20°C until analysis (Ribeiro, Costa, Pimenta, Filho, Ribeiro, & Bozzi, 2018), which was performed on the day following collection. A UV-Vis spectrophotometer (Thermo Scientific GENESYS 10S Vis, USA) was used to measure the following biochemical parameters: total protein (TP), albumin (ALB), Globulin (TP-ALB), ratio albumin/globulin (A/G), glucose (GLU), triglycerides (TRI), cholesterol (CHO), urea (URE), Gamma SL GT (GGT) and aspartate aminotransferase (AST). All tests were performed by using commercially available kits (Labtest, Lagoa Santa, Minas Gerais State, Brasil).

The plasma concentrations of cortisol (COR), total thyroxine (T_4) and total triiodothyronine (T_3) were measured in duplicate using an xMark™ Microplate Absorbance Spectrophotometer (BIO-RAD, Hercules, CA, USA) and quantified by enzyme linked immunosorbent assay (ELISA by competition) using a commercial kit (In Vitro diagnostic Ltd, Belo Horizonte, Minas Gerais State, Brasil) developed for the quantitative analysis of hormones.

Statistical analysis

The animals were distributed in a crossover experimental design in a scheme of subdivided parcels 2 x 2 (two levels of dietary glycerin addition x two periods) with the addition of glycerin in the main portion diet. Data were analyzed by the MIXED procedure of SAS 9.13 software (Statistical Analysis Systems Institute [SAS], 2001), considering the effect within period as random, as well as carryover effect between the two periods. Means, when significant, were compared by Tukey test ($p < 0.05$).

Results and discussion

The initial and final weight, DM, NDF, rumination, idle, feeding and drinking were not influenced by the treatments ($p > 0.05$) (Table 2).

As glycerin did not interfere with the consumption of DM and NDF, this shows us that the animals did not reject glycerin. Although there was no difference between treatments for DMI, it is observed that according to the NRC (2007) the DMI should be 1.67 kg for goats producing 2 kg of milk and this value was higher in the treatment with 15% glycerin (1.76 kg). These values for DM intake suggest that glycerol increased the energy

efficiency of the diet used by microorganisms in the rumen of the animals. Furthermore, this suggests that 15% crude glycerin did not cause severe damage or changes in the rumen environment that impaired ingestion.

Table 2. Mean occurrences and SEM of performance and ingestive behavior of dairy goats supplemented with glycerin.

Variable	Levels of addition of glycerin %		SEM	*P* Value
	0.00	15.0		
Performance measure				
Initial Weight (Kg)	47.10	47.03	3.19	0.971
Final live weight (Kg)	47.15	47.23	3.09	0.643
Dry matter intake (Kg day^{-1})	1.64	1.76	0.46	0.545
NDFI (Kg day^{-1})	1.34	1.36	0.48	0.939
Behavioral measure				
Rumination (min. day^{-1})	737	697	126.65	0.452
Idle (min. day^{-1})	447	464	114.26	0.724
Feeding (min. day^{-1})	232	252	65.59	0.444
Drinking (min. day^{-1})	23	25	7.80	0.519
Total chewing time (min. day^{-1})	679	717	123.23	0.463

[a,b] Means followed by distinct letters on the same line differ from each other by the Tukey test; SEM= standard error means; NDFI= neutral detergent fiber intake

The rumen environment and the simultaneous decrease in DMI may not be affected until crude glycerin concentrations exceed 10 to 20% of the dietary MD. Gunn et al. (2010) reported no changes in DMI when increasing crude glycerin concentrations (0 to 20% DM) was used to replace dry-rolled corn in lamb diet. Terré, Nudda, Bach, and Casado et al. (2011) and Avila-Stagno et al. (2013) found no difference in DM consumption with increasing levels of glycerin in the diet of lambs. Chanjula et al. (2015) studied the supplementation of crude glycerin (0, 5, 10 and 20%) in the diet of finishing goats and observed that there was no significant difference attributable to dietary treatment in the consumption of DM and NDF, although the average intake of MS was numerically higher in the glycerin-fed groups. Andrade et al. (2018) observed that the intake of DM decreased, so they concluded that up to 18% of crude glycerin (80.5% of glycerol) could be used in the diet of Santa Ines sheep without compromising the animal's performance.

The feeding efficiency (FE) and rumination efficiency (RE) of dry matter intake (DMI) as well as neutral detergent fiber intake (NDFI) of dairy goats supplemented with glycerin were not influenced by the treatments ($p > 0.05$) (Table 3).

Table 3. Mean occurrences and SEM of feeding efficiency (FE) and rumination efficiency (RE) of dry matter intake (DMI) and neutral detergent fiber intake (NDFI) of dairy goats supplemented with glycerin.

Efficiency	Levels of addition of glycerin (%)		SEM	*P* Value
	0.00	15.0		
FE (g DMI h^{-1})	427.24	438.67	180.26	0.877
RE (g DMI h^{-1})	217.50	236.13	73.56	0.541
FE (g NDFI h^{-1})	303.42	342.26	191.36	0.624
RE (g NDFI h^{-1})	161.16	178.32	82.94	0.617

[a,b] Means followed by distinct letters on the same line differ from each other by the Tukey test; SEM= standard error means

The specific activities of defection and urination had a significant effect ($p < 0.05$) in relation to the addition of glycerin in the diet of dairy goats (Figure 1)

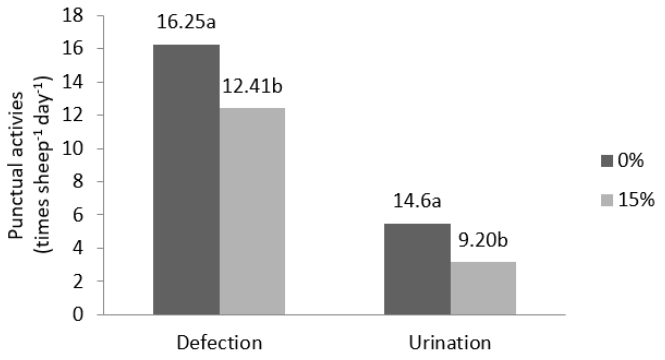

Figure 1. Distribution of the punctual activities of defection, urination and drinking in number of times goat^{-1} day^{-1} comparing to dairy goats supplemented with glycerin (0 and 15%). [a,b] Means followed by distinct letters on the same line differ from each other by the t test.

We observed that the values of punctual activities were lower with 15% of glycerin addition.

Rumen volatile fatty acids showed a significant effect ($p < 0.05$) with the addition of glycerin in the diet (Table 4).

Table 4. Volatile fatty acids from the rumen of dairy goats supplemented with glycerin.

Variables	Levels of inclusion glycerin %		SEM	*P* value
(m Mol 100 mL^{-1})	0.00	15.00		
Acetic	15.26	12.84	1,28	0,0456
propionic	7.53	8.64	0,61	0,0022
Butyric	2.54	2.78	0,19	0,0014

[a,b] Means followed by distinct letters on the same line differ from each other by the Tukey test; SEM= standard error means

The proportions of the individual VFA measured were affected by feeding glycerin. Feeding glycerin reduced the proportion of acetic acid and increased the proportions of propionic and butyric acids. Acetic and propionic acid presented averages of 60 and 30%, respectively, within the normal range presented by Wang, Zhang, and Zhang et al. (2020).

The levels of globulin, protein, albumin/globulin, glucose, cholesterol, urea, triglycerides, cortisol, and T_4 (Table 5) variables were significantly influenced ($p < 0.05$) by the addition of glycerin in the diet. The levels of blood albumin, Gamma SL GT (GGT), aspartate aminotransferase (AST) and T3 were not significantly ($p > 0.05$) influenced by the addition of glycerin in the diet, whereas the levels of thyroid hormones (T4) and cortisol decreased significantly when glycerin was included in the diet.

Table 5. Biochemical and hormonal parameters of dairy goats submitted to addition of glycerin in the diet.

Parameters	Levels of addition (%)		SEM	*P* value
	0.00	15.0		
Biochemical				
Total Protein (g dL^{-1})	7.96	10.21	0.143	<.0001
Albumin (g dL^{-1})	3.81	3.68	0.201	0.6524
Globulin (g dL^{-1})	4.15	6.52	0.228	<.0001
Albumin/Globulin	0.98	0.58	0.086	0.0032
Glucose (g dL^{-1})	77.19	97.59	2.901	<.0001
Cholesterol (g dL^{-1})	178.71	151.26	3.745	<.0001
Triglycerides (g dL^{-1})	20.58	22.55	0.412	0.0029
Urea (g dL^{-1})	59.39	67.97	1.080	<.0001
GGT (UI L^{-1})	29.00	32.40	2.790	0.6953
AST (UI L^{-1})	77.96	79.06	1.810	0.8939
Hormonal				
T3 (ng mL^{-1})	1.15	0.81	0.240	0.3276
T4 (µg dL^{-1})	4.10	3.27	0.144	0.0005
Cortisol (ng mL^{-1})	5.47	7.50	0.237	<.0001

Standard error means = SEM

Globulin, protein, albumin/globulin, glucose, urea and triglyceride levels increased with the inclusion of 15% CG in the diet. However, cholesterol levels decreased with the addition of CG. The thyroid hormones decreased with the addition of CG in the diet, while cortisol levels increased with the addition of CG (Table 5).

The values of globulin, protein, glucose and urea that were recorded following the addition of CG in the diet were higher. The results showed that the albumin levels for all treatments that received crude glycerin were above the 2.4 g mL^{-1} minimum threshold (Kaneko, Harvey, & Bruss et al., 2009).

Most glycerol not converted to propionate in the rumen is directly absorbed either by the rumen epithelium or small intestine. It is then carried to the liver, where it is converted by the glycerol kinase enzyme to glycerol-3-phosphate and drives gluconeogenesis, which may also have contributed to the highest amount of free glucose in the blood of animals fed crude glycerin (Rojek, Praetorius, Frokiaer, Nielsen, & Fenton, 2008). It implies that glycerol fermentation promoted an increase in propionate synthesis, and consequently, glucose synthesis in the livers of animals fed crude glycerin. The synthesis of glucose from volatile fatty acids is dependent on liver function – as the only site of gluconeogenesis, this organ regulates blood glucose levels and the supply of glucose to tissues (Chedea et al., 2016). Therefore, the energy metabolism of the animals should be evaluated primarily by studying the liver. Our results of AST and GGT levels suggest that the liver was not affected by the addition of CG in the diet.

Animals fed a 15% CG diet had high levels of blood glucose, possibly derived from ruminal propionate or glycerin directly absorbed by the ruminal wall. According to Ezequiel et al. (2015) a 30% CG diet causes blood glucose levels to return to the control level. This may be a result of changes in the complex endocrine system responsible for glucose regulation.

The lower amounts of cholesterol present in ruminants' blood may be due mainly to its synthesis in gonads, intestine and liver (Fernandes et al., 2012). In line with this hypothesis, it was observed that goats fed a 15% CG diet presumably with a lower acetate: propionate ratio had average cholesterol levels, indicating lower availability of acetate for cholesterol synthesis. Plasma cholesterol levels correspond to approximately 30% of the total plasma lipids, and have a direct relationship with the diet (Ohlsson, 2010).

High plasma levels of urea are indicative of an inadequate balance between protein and energy levels in the diet, either a high protein intake or energy deficit, which leads to accumulation of ammonia in the rumen and increased urea formation by the liver (Vieira et al., 2008). According to the recommendations of the NRC (2007), daily requirements of dry matter, crude protein and metabolizable energy of dairy goats weighing 40 kg and producing 2.0 kg of milk day^{-1} are 1.670 g day^{-1}, 89 g day-1, 3.19 Mcal day^{-1}, respectively. The plasmatic urea for all treatments was not high enough to promote damage on the liver or other organs of the animals in the study, because they were within the reference interval considered normal for dairy goat.

The greatest value found for the GGT enzyme was 32.40 U L$^{-1,}$ i.e., lower than the reference of 32 U L^{-1}. Levels greater than this value indicate lesions on the liver, and these results therefore indicate no hepatic alterations in animals, even at the high level of crude protein inclusion. The greatest value for AST of 79.06 U L^{-1} is lower than the 132 U L^{-1} critical value (Kaneko et al., 2009) for goat, which also indicates the animals had no hepatic lesions.

Levels of ethereal extract increased with the addition of CG in the diet (Table 1). It is likely that this led to increased metabolism of lipoproteins in the intestine and liver. However, there is no information in the literature concerning the effect of dietary CG on goats' blood or hormonal parameters.

Conclusion

Diets for dairy goats with 15% glycerin did not alter the intake of dry matter, neutral detergent fiber, or the ingestive behavior of these animals. As it also does not change its ingestive behavior.

Diets for dairy goats with 15% glycerin did not change the consumption of dry material, neutral detergent fiber, nor did they change the ingestive behavior of these animals.

Occasional activities (defecation, urinating and drinking water) decreased with the addition of 15% of glycerin in the goat's diet.

The production of acetic acid decreased and propionic and butyric acid increased with the addition of 15% glycerin.

The levels of GGT and AST unchanged by the addition of CG in the diet, suggesting that this by-product of biodiesel may be a useful alternative to include in the diets of dairy goats.

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