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Feeding behavior, microbial efficiency, and nitrogen balance of Nellore heifers supplemented with crude glycerin

Comportamento ingestivo, síntese microbiana e balanço de nitrogênio de novilhas Nelore suplementadas com glicerina bruta

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Abstract

This study aimed to evaluate the inclusion of crude glycerin in diets for Nellore heifers grazing on a *Brachiaria brizantha* pasture, during the dry season, on urine and plasma urea concentrations, feeding behavior, and microbial protein synthesis. Sixty Nellore heifers with an average initial weight of 285.89 ± 18.74 kg, at approximately 19 ± 2 months of age, were distributed, in a completely randomized design, into the following five treatments with twelve replicates: 0.00, 4.00, 8.00, 12.00,and 16.00% inclusion of crude glycerin in the diet they were fed. Grazing time decreased linearly (P<0.05) by 7.44 min with every percent of crude glycerin included in the diet. Microbial efficiency was not affected (P>0.05), averaging 113.73g CP per kg TDN ingested. Plasma nitrogen concentration did not show any effects (P>0.05), averaging 13.11 mg dL^{-1}. Supplementing heifers during the dry season, at 0.7% BW, using up to 16% crude glycerin in the diet composition, did not elicit positive responses from feeding behavior and had little influence on microbial synthesis.
Key words: Efficiency. Glycerol. Grazing. Rumination.

Resumo

Objetivou-se avaliar a inclusão de glicerina bruta na dieta de novilhas Nelore em pastejo de *Brachiaria brizantha* no período da seca, sobre as concentrações de ureia na urina e no plasma, o comportamento ingestivo e a sínteses de proteína microbiana. Foram utilizadas 60 novilhas da raça Nelore, com peso médio inicial 285,89 ± 18,74 kg e aproximadamente 19 ± 2 meses de idade, distribuídas em um delineamento inteiramente casualizado, sobre 5 tratamentos e 12 repetições: 0,00; 4,00; 8,00; 12,00 e 16,00% de inclusão de glicerina bruta na dieta das novilhas. O tempo de pastejo apresentou efeito linear decrescente (P<0,05), para cada porcentagem de glicerina bruta inclusa na dieta, foi observada uma redução de 7,44 minutos. A eficiência microbiana não apresentou efeito (P>0,05), com valor médio de 113,73g de PB por kg de NDT ingerido. A concentração de nitrogênio no plasma não apresentou efeito (P>0,05), tendo assim, valor médio de 13,11mg dl^{-1}. A suplementação de novilhas no período seco com 0,7% PC com utilização da glicerina bruta na composição da dieta até 16,00% não proporcionou

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respostas positivas para o comportamento ingestivo e apresentou pouca influência sobre a síntese microbiana.
Palavras-chave: Eficiência. Glicerol. Pastejo. Ruminação.

Introduction

The beef cattle production system in Brazil is basically carried out with animals grazing on grasses of the genera *Brachiaria* and *Panicum*, besides other forage varieties, as is the case of some legume species and native vegetation sought for animal production, which are feed sources for cattle distributed across the regions of the country.

In the dry period of the year, during which rainfall scarcity is present from May to October in a great part of the regions of Brazil, these forages have their production and qualitative potential reduced. This requires the use of supplementation with protein and energy concentrates to maintain animals alive and under production, aiming to lessen the negative effects on the livestock activity.

In the Brazilian cattle farming activity, the use of tropical pastures and supplementation, especially during the dry season, has been exploited so as to make beef cattle rearing systems biologically feasible, as stated by Silva et al. (2009).

According to Silva et al. (2010), the limiting factor in forage intake by grazing animals is related to three groups of specific factors: those affecting the digestion processes, the ingestion processes, and the nutritional requirements. The feeding behavior of cattle on pasture is influenced by both the quantity and the quality of the dry matter produced, generating a positive or negative effect on animal productivity.

However, other also related factors non-inherent to the vegetation, but directly linked to the feeding behavior, such as the use of supplementation, may provide changes in the behavior displayed by animals, as reported by Souza et al. (2011).

Depending on the quantity of the supplement provided to animals, their dry matter (DM) intake from the pasture can be reduced or increased, thereby causing a substitution effect on forage intake via the consumption of concentrate, and in smaller quantities of supplement, an additive effect on pasture intake.

The feeding behavior of grazing cattle is characterized by long feeding periods, from 4 to 12h day^{-1}, for low-energy diets. The time spent grazing and ruminating is influenced by the pasture and by the use of supplements (MARTINS et al., 2012).

Supplementing cattle on a grazing system in the dry season is a practice aimed at maximizing the productive performance of animals. As a strategy in decision-making, producers have sought to insert alternative feedstuffs in animal diets partially or totally replacing traditional ingredients (maize, soybean, and wheat), aiming to reduce effective operating costs (EOC).

Waste from biodiesel industries has been utilized in the composition of ruminant diets aiming to meet the nutritional requirements of grazing animals. Among them, the crude glycerin has stood out as an alternative feedstuff to supplement cattle on grazing systems, especially during the dry season.

Crude glycerin is a by-product from the biodiesel industry obtained from the transesterification reaction for the formation of biodiesel. Its composition contains impurities like water, catalyst (alkaline or acid), non-reacted alcohol, and impurities originating from ester reagents, propanediols, monoesters, glycerin oligomers, and polymers (FERRARI et al., 2005). Being devoid of protein in its composition, crude glycerin in large quantities can influence the growth of rumen microorganisms and promote their activity on the fiber degradation.

Ruminants have the ability to utilize the glycerol present in crude glycerin as a precursor of glycogen, for the maintenance of plasma glucose levels. Glycerol is converted to glucose, which enters in dihydroxyacetone phosphate form to be converted to 3-phosphoglycerate by the action of the glycerol-3-phosphate dehydrogenase enzyme following the gluconeogenic pathway (FARIAS et al., 2012).

Given the importance of the nitrogen balance and the microbial synthesis on the protein metabolism of ruminants, it is important to know the metabolic variations in the urine, feces, and blood, as well as the efficiency of microbial protein production promoted by changes in the animal diets (SCHIO, 2012), mainly when alternative feedstuffs are added to these diets.

This study aimed to test levels of crude glycerin in diets for Nellore heifers grazing on *Brachiaria brizantha* pastures during the dry season and their implications on the feeding behavior, microbial synthesis, and nitrogen balance of these animals.

Materials and Methods

The experiment was conducted on Boa Vista Farm, in Macarani-BA, Brazil, for the field part and data collection; and at the Laboratory of Forage Crops and Pasture of the Southwest Bahia State University (UESB) - Juvino Oliveira campus, in Itapetinga-BA, for chemical analyses of forage, supplements, and feces.

Sixty Nellore heifers at an average age of 19 ± 2 months and with 285.89 ± 18.74 kg initial body weight were used in a randomized complete design, where they were distributed into five treatments and twelve replications. After selection, all animals were allotted at random to the established treatments with 0.00, 4.00, 8.00, 12.00, and 16.00% inclusion of crude glycerin in the heifer diets.

The experiment started on July 15, 2011 and lasted 85 days, which consisted of 15 days for the adaptation of animals to diets and paddocks, and the remaining 70 days for data collection and evaluation of performance, divided into two 35-day experimental periods.

Climatic data referring to the experimental period were obtained using a pluviometer and a digital thermometer. Total precipitation values (51 mm day^{-1}) and minimum and maximum temperatures (18.85 and 31.13 °C) were recorded during the experiment.

Supplements were formulated using the chemical composition data of the forage samples, collected in the week prior to the beginning of the experimental period (Table 1), to provide nutrients to animals with an estimated gain of 0.750 kg day^{-1}, according to NRC (1996).

Table1. Proportion of ingredients in the supplement and chemical composition, on a dry matter basis.

Ingredient	Crude glycerin level (%)				
	0.00	**4.00**	**8.00**	**12.00**	**16.00**
Ground maize	80.86	67.91	54.62	41	27.04
Glycerin	0.00	10.47	21.2	32.20	43.49
Soybean meal	15.30	17.74	20.25	22.81	25.44
Post-weaning salt[1]	1.85	1.87	1.89	1.92	1.94
Urea	1.99	2.01	2.04	2.07	2.09

[1]Mineralmixcontaining: 233 g Ca/kg; 80 g P/kg; 5 g Mg/kg; 48 g Na/kg; 25 mg Co/kg; 380 mg Cu/kg; 25 mg I/kg; 1,080 mg Mn/kg; 3.75 mg Se/kg; 1,722 mg Zn/kg.

To regulate the feed supply to heifers, the animals were weighed at the beginning and in the middle of the experiment. Supplements were supplied daily in the amount of 0.7% body weight (BW) at 08h30, in a collective 4-m-long plastic trough with double access, located 15 m from the water source. All animals had free access to natural shade provided by trees in the pasture between the paddocks, fresh drinking water, and a high-intake supplement during the entire experimental period.

The experiment was established in a 30-ha area formed by *Brachiaria brizantha* cultivar Marandu, divided into 10 paddocks with approximately 3 ha each. The experimental area was closed, surrounded by electric fences, three months before the start of the experiment. The pasture was deferred aiming to increase the green herbage mass existing in the paddocks, which served to calculate the allowance and availability of pasture dry matter to the animals during the experimental period.

During the first experimental period, heifers were rotated across five paddocks in a direction predefined at random for minimizing effects inherent to the paddocks. Meanwhile, five paddocks remained closed to be used in the second experimental period; these were rotated by the heifers in a direction predefined at random for minimizing effects inherent to the paddocks until the end of the experiment.

To determine the qualitative and quantitative characteristics of the *Brachiaria brizantha*, initial, intermediate, and final forage sample collections were carried out during the experimental period. Initially, the dry matter (DM) of the biomass of the sample in the entire experimental area was quantified visually before the animals were placed in the paddock, in order to quantify the existing scores, considering the height as a parameter. Forage with 20 to 30 cm in height was defined as score 1; between 30 and 40 cm, score 2; and from 40 to 50, score 3.

In the same way, after the paddocks were divided, the same methodology described above was employed to visually quantify the biomass of the pasture sample and the existing scores; these assessments were made in the periods the animals entered and exited the paddocks, determined using a 0.25-m^2 square frame and scissors. The frame was thrown over each paddock 40 times and values from the score on which the frame fell were recorded in proper spreadsheets.

After this procedure, four forage collections were performed 5 cm above the soil level. The material was placed in plastic bags and weighed; values were recorded, and a composite sample was made from which the components were separated into leaf, stem, and dead material.

Stocking rate was calculated considering the animal unit (AU) as 450 kg of live weight (BW), as shown in Eq. 1:

$$\text{Eq. 1} = SR = \frac{(tAU)}{Area},$$

where SR = stocking rate, in AU per ha; tAU = total animal unity; and Area = total experimental area, in ha.

Forage allowance was calculated according to Eq. 2:

$$\text{Eq. 2} = FA = \frac{\left\{ {}^{ADM}/_{\left({}^{[SR \times 450]}/_{100} \right)} \right\}}{ND},$$

where FA is the forage allowance in kg DM 100 $kgBW^{-1}day^{-1}$; available dry matter (ADM)is the pasture dry matter availability, in kg $DMha^{-1}day^{-1}$; SR is the stocking rate, in AU ha^{-1}; and number of days per hectare (ND)is the number of days in the experimental period.

From the pasture sample collection procedures, during the entire experimental period, we obtained the mean values for forage DM availability of the respective *Brachiaria brizantha* components, stocking rate, and forage allowance, as presented in Table 2.

Table 2. Forage production in experimental paddocks.

Forage production	Paddocks average
Available dry matter (kg ha^{-1})	9,056.15
Percentage of leaf (%DM)	27.52
Percentage of stem (% DM)	38.46
Percentage of dead material (% DM)	34.02
Stocking rate (AU ha^{-1})	2.37
Forage allowance (kg DM 100 kg BW^{-1})	12.83

Pasture collection was achieved by the hand-plucking (simulated grazing) technique, by collecting the pasture from the consumed stratum, simulating the real composition of the animal's roughage diet. The individual dry weight and percentage of each one of these samples were obtained.

Purified and enriched lignin (LIPE) was used for the estimate of fecal production, according to the methodology utilized by Rodriguez et al. (2006). LIPE was supplied daily at 08h30 as an external marker, in a single dose in a 500-mg capsule, for seven days. Two of these days were intended for adaptation and regulation of the marker excretion flow, and the other five for feces collection.

Approximately 200 g of feces $animal^{-1}$ day^{-1} were collected directly from the rectal ampulla, once daily, for five days, when the marker was administered. Subsequently, they were stored in a freezer at −10 ºC. LIPE was analyzed at the Animal Nutritional Laboratory of EV/UFMG, on a Varian 099-2243 spectrophotometer with an infrared light detector (FTIR). Samples of feces dried and ground to 2mm were KBr-pelleted, and the LIPE concentration was determined.

Dry matter intake from the concentrate was estimated using the titanium dioxide marker, according to the methodology utilized by Titgemeyer (1997). Ten grams of the marker were used per animal per day, directly in the trough, mixed with the concentrate, for 12 consecutive days, following the procedure described by Rodriguez et al. (2006). The first seven days were used for adaptation and regulation of the marker excretion flow, and the other five for feces collections. This procedure applied for the digestibility of nutrients took place in the middle of the experimental period. When animals were already acclimated to the diet, all of them had their feces collected.

Samples of the supplements provided in the beginning and at the end of the experimental periods were collected. Hand-plucked, supplements, and feces samples were dried in a forced-air oven at 55 ºC for 72 h and processed in a Wiley mill with 1-mm sieve. Later, the samples were analyzed chemically to determine the dry matter (OM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, lignin, and mineral matter (MM) contents, as shown in Table 3, according to procedures described by Silva and Queiroz (2002).

Concentrations of neutral detergent fiber corrected for ash and protein were determined following recommendations of Mertens (2002).

Table 3. Chemical composition of the diet.

Component	CG[1]	Pasture[2]	Crude glycerin level (%)				
			0.00	4.00	8.00	12.00	16.00
Dry matter (%)	90.00	59.82	87.02	82.63	78.11	74.04	69.50
Organic matter (% DM)	93.00	92.26	95.29	96.03	94.64	94.22	92.15
Crude protein (% DM)	-	6.17	20.91	21.81	22.64	22.77	23.17
Ether extract (% DM)	36.70	1.36	2.68	6.61	8.94	10.68	12.60
NDFap (% DM)	-	74.45	7.96	9.27	8.24	8.53	5.52
ADF (% DM)	-	46.25	-	-	-	-	-
NFC (% DM)	-	10.28	56.08	55.87	50.01	45.80	51.58
Hemicellulose (% DM)	-	34.96	-	-	-	-	-
Cellulose (% DM)	-	8.73	-	-	-	-	-
Lignin (% DM)	-	5.70	-	-	-	-	-
iNDF (% DM)	-	29.15	2.71	2.97	3.30	2.53	2.71
Methanol	5.73	-	-	-	-	-	
Glycerol	51.84	-	-	-	-	-	-

[1]Crude glycerin; [2]Hand-plucked samples (simulated grazing).

Because of the presence of urea in the diets, the non-fibrous carbohydrates (NFC) were calculated as proposed by Hall (2000), represented by Eq. 3:

$$\textbf{Eq. 3} = \text{NFC} = \{100 - [(\%\text{CP} - \%\text{CP urea} + \%\text{urea}) + \%\text{NDFap} + \%\text{EE} + \%\text{MM}]\},$$

where NFC = non-fibrous carbohydrates; %CP = percentage of crude protein; %CP urea = percentage of crude protein from urea; %urea = percentage of urea; %NDFap = percentage of neutral detergent fiber (corrected for ash and protein); %EE = percentage of ether extract; and %MM = percentage of mineral matter (Table 3).

The total digestible nutrients (TDN) contents were estimated according to Weiss (1999), by the following equation:

TDN (%) = DCP + DNDFap + DNFC + 2.25 DEE,

where DCP = digestible crude protein; DNDFap = digestible neutral detergent fiber corrected for ash and protein; DNFC = digestible non-fibrous carbohydrates; and DEE = digestible ether extract.

To measure the feeding behavior of the animals, 20 trained observers were selected to visually observe the following behavioral variables: grazing time, rumination time, time at the trough, and idle time of the animals, adopting a 5-min interval between observations, with data recorded in specific tables (SILVA et al., 2006).

During the collection period, animals remained on the grazing system in each paddock, keeping the same stocking rate of 12 animals per treatment. Of these, six were selected according to randomization criteria to make up the group of animals that would be subjected to behavioral analyses.

Animals subjected to the feeding behavior analyses were marked with red paint using a baton sold in agriculture shops (typically used in contests and animal judging in livestock fairs). Animals were marked on the day before analysis of the behavioral variables.

Two observers were allocated to each paddock to be in charge of analyzing the behavioral variables of six animals for a period of 2 h, after which they

switched with other observers who had been resting at the farm. In this regard, two collections were made to be used for the analysis of the feeding behavior of animals, which took place in the middle of each experimental period, both lasting 24 h.

The average number of rumination chews per cud and of the time spent to ruminate each cud in the day and nighttime periods was obtained by visual observation and using digital stopwatches.

The total chewing time was determined as the sum of the grazing and rumination times. The discretization of time series was performed directly on the data collection spreadsheets, by counting the discrete periods spent grazing, ruminating, idle, and at the trough (SILVA et al., 2006).

Blood was collected by jugular vein puncture in the beginning of the first and second experimental periods. Approximately 4 h after the morning feed was supplied, the blood was drawn from six heifers from each group referring to a given treatment. Next, blood samples were transferred to the laboratory, centrifuged at 5,000 rpm for 15 min, and the plasma was stored in 5-mL microcentrifuge tubes and kept frozen at −15 ºC until analyses.

A spot urine sample was collected during spontaneous urination, in the middle of the experiment, approximately 4 h after the feed was supplied. Urine samples were collected from six heifers of each group referring to a given treatment. Samples were filtered through gauze, and a 10-mL aliquot was separated and diluted with 40 mL sulfuric acid (0.036 N), used for quantifying the urea, nitrogen, creatinine, allantoin, and uric acid concentrations in the urine. Samples were packed in an universal collector and kept frozen at −15 ºC.

Plasma and urine concentrations of creatinine, uric acid, and urea were estimated using commercial kits. Urea values were converted to urea nitrogen by multiplying the obtained values by 0.4667. Allantoin and uric acid in the urine were estimated by colorimetric methods, as specified by Chen and Gomes (1992), and the total nitrogen content was estimated by the Kjeldahl method (SILVA; QUEIROZ, 2002).

The nitrogen balance (N retained, g day^{-1}) was calculated as follows: N retained = N intake (g) - N in feces (g) - N in urine (g).

Creatinine excretion (mg kg LW^{-1}), utilized to estimate the urinary volume from spot samples, was obtained for each animal, according to Eq. 5, described by (CHIZZOTTI et al, 2006).

Eq. 5 = EC = (32.27 - 0.01093) × (LW),

where EC = daily excretion of creatinine (mg kg LW^{-1}) and LW = live weight (kg).

The urinary volume was estimated as the ratio between the excretion of creatinine (mg kg $LW^{-1}day^{-1}$), obtained in Eq. 5, and the average concentration in urine samples (mg dL^{-1}), multiplying the result by the live weight of the animal.

Excretion of total purines (TP) was estimated as the sum of the amounts of allantoin and uric acid excreted in urine and the amount of microbial purines absorbed (mmol day^{-1}), by the excretion of total purines ($mmolday^{-1}$), as shown in Eq. 6:

$$\textbf{Eq. 6} = \textbf{AP} = \frac{\text{TP - 0.385} \times \text{LW0.75)}}{0.85},$$

where AP = absorbed purines ($mmolday^{-1}$);TP = total purines ($mmolday^{-1}$);0.85 = recovery of absorbed purines as purine derivatives in urine; and 0.385 = endogenous excretion of purine derivatives in urine (mmol) per unit of metabolic weight.

The intestinal flow of microbial nitrogen (g MN day^{-1}) was estimated from the amount of absorbed purines ($mmolday^{-1}$), according to Eq. 7, described by Chen and Gomes (1992).

$$\text{Eq. 7} = \text{MN}\left(\frac{\text{g}}{\text{day}}\right) = \frac{(70 \times \text{AP})}{0.83 \times 0.116 \times 1000},$$

assuming the value of 70 for the nitrogen content in the purines (mg $mmol^{-1}$); 0.83 for the intestinal digestibility of the microbial purines; and 0.116 for the purine-N: total-bacterial-N ratio.

All statistical analyses were evaluated by analyses of variance (ANOVA) and regression, using the System for Statistical and Genetic Analyses (SAEG, 2000). The statistical models were chosen according to the significance of the coefficients of regression, using the t test at 5% probability level, the coefficient of determination (r^2), and the studied phenomena.

Results and Discussion

Grazing and rumination times (Table 4) decreased linearly (P<0.05) by 7.44 and 3.91 min, respectively, with every percent of crude glycerin included in the diet. The effect observed on grazing time can be explained by the greater heat increment provided by inclusion of crude glycerin in the diet, because the energy content (carbohydrates and lipids) of the diet is transformed into propionate, which is the first to signal the end of a meal, through the greater flow towards the liver. As a result, energy production (ATP) is increased through its use in gluconeogenesis, which signals the animal satiety (FARIAS et al., 2012). Thus, in larger quantities, crude glycerin benefits the intake-regulation mechanism of animals grazing on *Brachiaria brizantha* pastures during the dry season.

Table 4. Behavioral activities of Nellore heifers supplemented with different levels of crude glycerin.

Activity (min)	Crude glycerin level (%)					CV (%)[1]	P[2]
	0.00	4.00	8.00	12.00	16.00		
Grazing[3]	496.25	485.83	450.83	415.83	382.50	10.69	0.001
Rumination[4]	455.42	366.25	331.66	419.17	350.83	15.66	0.001
Idleness[5]	448.75	528.75	600.00	551.67	653.33	11.16	0.001
Trough[6]	39.16	57.50	57.50	53.33	52.50	28.15	0.020

[1]Coefficient of variation; [2]Probability of error; [3]$Y = 505.75 - 7.4375x$. $r^2 = 0.98$; [4]$Y = 415.92 - 3.9065x$. $R^2 = 0.23$; [5]$Y = 470.08 + 10.802x$. $r^2 = 0.79$; [6]$Y = 41.423 + 3.5992x - 0.1898x2$. $R^2 = 0.79$.

The effect observed on rumination time, in turn, is explained by the lower NDFap content of the diet with the inclusion of crude glycerin in the concentrate, since it is devoid of fiber in its chemical composition. Thus, as this ingredient was added to the diet, rumination time was reduced, and it is known that the presence and quantity of fiber in the diet is a key factor to trigger the rumination activity. Agreeing with these results, Pereira et al. (2007) observed that the time spent feeding and ruminating decreases proportionally to the reduction of NDF in the diet; as a consequence, the time spent idle was increased.

Crude glycerin inclusion in the heifers diet caused a linear increase (P<0.05) in idle time, which increased by 10.80 with every percent of crude glycerin added. This response can be explained by the results observed for the grazing and rumination activities, with decreased idle time.

The time spent at the trough responded quadratically (P<0.05), with maxima of 58.07 min estimated at the level of 9.48% of inclusion of crude glycerin in the diet, which may be related to the homogenization of crude glycerin with other mesh ingredients in the diet up to this level. As the levels of inclusion in the diet were increased, DM intake decreased, and this effect may be related to the excess crude glycerin in relation to the other ingredients of the diet.

The total DM intake (Table 5) decreased linearly (P<0.05) by 0.097 kg day^{-1}. Likewise, NDFap intake had a decreasing linear response (P<0.05), in

which crude glycerin inclusion caused it to drop by 0.060 kg day^{-1} animal^{-1}. This response was likely due to the high levels of crude glycerin added to the diet, which might have brought about an inhibitory effect on DM and NDFap intake as a result of the ether extract content in crude glycerin.

Table 5. Nutrient intake and feed and rumination efficiencies of Nellore heifers supplemented with different levels of crude glycerin.

Intake	Crude glycerin level (%)					CV (%)[1]	P[2]
	0.00	4.00	8.00	12.00	16.00		
Feed (kg day^{-1})							
DM intake (kg)[3]	6.01	5.36	5.41	4.92	4.30	6.88	0.001
NDFap intake (kg)[4]	3.15	2.76	2.79	2.51	2.06	6.53	0.001
CP intake (kg)[5]	0.89	0.75	0.79	0.78	0.66	10.88	0.001
TDN intake (kg)[6]	3.29	3.06	3.10	2.90	2.39	10.25	0.001
Efficiency							
Pasture DM intake h^{-1}(kg)[7]	0.95	0.66	0.73	0.60	0.63	10.17	0.001
Pasture NDFap intake h^{-1}(kg)[8]	0.49	0.34	0.38	0.31	0.30	10.14	0.001
Dietary TDN intake h^{-1} (kg)[9]	0.52	0.38	0.42	0.35	0.35	10.15	0.001
Rumination, pasture DM h^{-1} (kg)[10]	1.09	0.89	1.00	0.65	0.62	19.62	0.001
Rumination, pasture NDF h^{-1} (kg)[11]	0.57	0.46	0.51	0.33	0.29	19.92	0.001

[1]Coefficient of variation; [2]Probability of error; [3]$Y = 5.976 - 0.0973x$. $r^2 = 0.91$; [4]Neutral detergent fiber corrected for ash and protein;[5]$Y = 3.14 - 0.0608x$. $r^2 = 0.91$; [6]$Y = 3.34 - 0.049x$. $r^2 = 0.82$; [7]$Y = 0.854 - 0.0175x$. $r^2 = 0.64$; [8]$Y = 0.446 - 0.0103x$. $r^2 = 0.71$; [9]$Y = 0.478 - 0.0093x$. $r^2 = 0.68$; [10]$Y = 1.086 - 0.0295x$. $r^2 = 0.81$; [11]$Y = 0.57 - 0.0173x$. $r^2 = 0.84$.

Crude protein (CP) intake decreased linearly ($P<0.05$) by 0.010 kg day^{-1} with every percent of inclusion of crude glycerin in the diet. This response was due to the reduction of mesh concentrate in the composition of the diets for the treatments with higher levels of crude glycerin, which does not have CP in its composition, thereby providing a decrease in intake by animals.

Total digestible nutrients (TDN) intake decreased linearly ($P<0.05$) by 0.049 kg.day^{-1} with every percent of crude glycerin included in the diet. The decreasing linear effect for the intake of the dietary nutrients was caused by the lower DM intake by the heifers, through the use of the crude glycerin in their feeding.

Feed efficiency in DM, NDFap, and TDN showed a linear decrease ($P<0.05$) of 0.02 kg DM h^{-1}, 0.01 kg NDFap h^{-1}, and 0.1 kg TDN h^{-1}, respectively, with every percent of crude glycerin included in the diet. The same effect was observed for rumination efficiency in DM and NDFap, which decreased linearly ($P<0.05$) by 0.03 kg DM h^{-1} and 0.02 kg NDFap h^{-1}, respectively.

This observed decreasing response was as a result of the decreased DM and NDFap intake as crude glycerin was added to the diet, even though a discrepancy was observed in the times spent feeding and ruminating. These findings corroborate Costa et al. (2011), who detected an increase in feed and rumination efficiency with an increase in DM and NDF intake.

Total chewing time (Table 6) decreased linearly ($P<0.05$), from 990.83 to 785.83 min, with inclusion of crude glycerin in the diets. This response follows the trend shown by the times spent feeding and ruminating, which also decreased linearly, although the time spent at the trough had a quadratic effect.

Table 6. Behavioral activities of Nellore heifers supplemented with different levels of crude glycerin.

Behavioral activity	Crude glycerin level (%)					CV (%)[1]	P[2]
	0.00	4.00	8.00	12.00	16.00		
Total chewing time(min)[3]	990.83	909.58	839.99	888.33	785.83	7.06	0.001
Number of cuds ruminated per day[4]	507.62	401.66	352.15	467.99	369.69	14.55	0.001
Time spent per ruminated cud (s)[5]	53.83	54.71	56.51	53.74	56.94	14.16	0.001
Number of chews per ruminated cud[6]	53.46	52.98	56.64	57.90	55.27	14.14	0.001

[1]Coefficient of variation; [2]Probability of error; [3]$Y = 969.16 - 10.781x$. $r^2 = 0.79$; [4]$Y = 487.54 - 18.143x + 0.8066 x^2$. $R^2 = 0.38$; [5]$Y = 54.096 + 0.1313x$. $r^2 = 0.31$; [6]$Y = 52.585 + 0.6921x - 0.0299x^2$. $R^2 = 0.61$.

The number of cuds ruminated per day showed a quadratic effect (P<0.05), with a minimum of 385.97 cuds obtained at 11.25% inclusion of glycerin. This was an expected result, because the same effect was found for the time spent on rumination, which is a natural process performed by cattle that is important to improve the use of the feed, mainly the fiber. Thus, crude glycerin showed to provide a decrease in DM and NDF intake, and consequently the number of cuds ruminated was compromised.

The time spent per ruminated cud increased linearly (P<0.05) by 0.13 s with every percent of crude glycerin added to the diet. The number of chews per ruminated cud, however, had a quadratic response (P<0.05), with maximum value of 56.58 chews for the level of 11.57% of crude glycerin added to the diet. This result is related to the rumination time.

There was no effect (P>0.05) of glycerin inclusion on the number of grazing and rumination periods (Table 7), which averaged 10.01 and 10.00 periods, respectively.

Table 7. Frequencies and duration of behavioral activities of Nellore heifers supplemented with different levels of crude glycerin.

Behavioral activity	Crude glycerin level (%)					CV (%)[1]	P[2]
	0.00	4.00	8.00	12.00	16.00		
Number of grazing periods[3]	9.91	9.75	10.21	9.91	10.25	21.47	0.674
Number of rumination periods[4]	10.17	10.33	9.42	9.83	10.25	18.93	0.906
Number of idle periods[5]	13.75	14.08	16.25	15.17	17.17	18.69	0.024
Number of periods at the trough[6]	2.17	1.83	2.08	1.42	1.42	33.88	0.005
Grazing time per period (min)[7]	50.07	49.83	44.15	41.96	37.32	22.36	0.001
Rumination time per period (min)[8]	44.78	35.45	35.21	42.64	34.23	15.94	0.001
Idle time per period (min)[9]	32.64	37.55	36.92	36.36	38.05	22.97	0.361
Time at trough per period (min)[10]	18.05	31.42	27.64	37.56	36.97	32.93	0.001

[1]Coefficient of variation; [2]Probability of error; [3]$Y = 10.01$; [4]$Y = 10.00$; [5]$Y = 13.698 + 0.1983x$. $r^2 = 0.76$; [6]$Y = 2.166 - 0.0478x$. $r^2 = 0.72$; [7]$Y = 51.34 - 0.8343x$. $r^2 = 0.95$; [8]$Y = 41.244 - 0.3477x$. $r^2 = 0.21$; [9]$Y = 36.30$; [10]$Y = 21.532 + 1.0995x$. $r^2 = 0.80$.

Nevertheless, DM and NDF intake increased linearly with inclusion of crude glycerin. The time per grazing and rumination periods was reduced to 0.83 and 0.35 min, respectively, for every percent of crude glycerin included in the diet. A similar response was found by Almeida (2011), working

with the levels of 0.00, 3.33, 6.66, and 9.99% crude glycerin in diets for heifers on a grazing system, in which the author observed an average number of grazing periods of 15.1 and a 0.68 min decrease with every percent of inclusion of crude glycerin in the diet.

The number of idle periods increased linearly($P<0.05$) by 0.20 periods with each percent of inclusion of crude glycerin in the diet. The idle time per period, however, did not show significant effects, averaging 36.30 min. This result reflects the response observed for idle time, in which the inclusion of crude glycerin in the diet provided a longer time.

The number of periods at the trough decreased linearly ($P<0.05$), with a reduction of 0.05 periodsfor every percent of glycerin inclusion. The animals visited the trough fewer times, but remained there longer as crude glycerin was included in the diet. The time spent at the trough showed an increasing effect ($P<0.05$), increasing by 1.10 min with the inclusion of crude glycerin in the diet. This effect can be explained by the acceptability and difficulty seizing the glycerin by the animals, with elevated levels of crude glycerin in the diet making the supplement less palatable or leading to greater difficulty to seize it; as a consequence, heifers remained at the trough for a longer time with the inclusion of glycerin in the diet.

The urinary volume showed a decreasing linear effect ($P<0.05$), with a 0.22 L reduction observed with every percent unit of crude glycerin added to the diet (Table 8). This effect may be related to the lower DM intake, which then led to a reduction in water intake by the animals during the day, culminating in a lower urinary excretion volume.

Table 8. Urinary volume, excretion of purine derivatives, microbial protein production, and microbial efficiency of Nellore heifers supplemented with different levels of crude glycerin.

Item	Crude glycerin level (%)					CV (%)[1]	P[2]
	0.00	4.00	8.00	12.00	16.00		
Urinary volume (L day^{-1})[3]	13.22	12.79	11.76	10.00	10.26	21.69	0.006
Urinary excretions (mmolday^{-1})							
Allantoin[4]	84.43	71.84	69.48	75.54	79.11	38.27	0.893
Uric acid[5]	16.55	13.38	14.39	8.36	11.31	46.19	0.016
Total purines[6]	100.98	85.22	83.87	83.90	90.41	32.49	0.722
Absorbed microbial purines[7]	87.30	69.13	67.00	67.04	74.87	38.25	0.721
In % total purines							
Allantoin[8]	81.80	77.64	83.12	89.51	87.09	10.64	0.018
Uric acid[9]	18.20	22.36	16.88	10.49	12.91	55.17	0.018
Syntheses of N and microbial protein (g day^{-1})							
Microbial N[10]	63.47	50.26	48.71	48.74	54.43	38.25	0.721
Microbial CP[11]	396.70	314.14	304.46	304.65	340.21	38.25	0.721
Microbial efficiency							
g CP kg TDN^{-1} [12]	120.61	102.72	98.00	105.03	142.31	37.32	0.122

[1]Coefficient of variation; [2]Probability of error; [3]$Y = 13.348 - 0.2178x$. $r^2 = 0.90$; [4]$Y = 76.08$; [5]$Y = 15.898 - 0.3875x$. $r^2 = 0.62$; [6]$Y = 88.88$; [7]$Y = 73.07$; [8]$Y = 79.342 + 0.5613x$. $r^2 = 0.59$; [9]$Y = 20.658 - 0.5612x$. $r^2 = 0.59$; [10]$Y = 53.12$; [11]$Y = 332.03$; [12]$Y = 113.73$.

No significant effects (P>0.05) were observed for the urinary excretion of allantoin, total purines, and absorbed microbial proteins, which averaged 76.08, 88.88, and 73.07 mmol day^{-1}, respectively. Because the excretions of allantoin, total purines, and absorbed microbial purines are related to the intake of crude protein from the diet, a decreasing linear effect was expected, given the lower crude protein intake of the animals resulting from the inclusion of crude glycerin, which provided a decrease in the intakes of DM and respective diet nutrients.

However, the excretion of uric acid decreased linearly (P<0.05), with every percent unit of crude glycerin causing it to decrease by 0.39 mmolday^{-1}. This was an expected result, given the reduction of crude protein intake caused by the inclusion of glycerin, as mentioned previously.

Allantoin excretion as a percentage of total protein showed an increasing response (P<0.05), increasing by 0.56% with every percent unit of crude glycerin included in the diet. The opposite effect (P<0.05) was observed for uric acid excretion as a percentage of the total protein, which decreased by 0.56%with every percent unit of crude glycerin. This result was expected, since the excretion of uric acid showed a decreasing effect and the excretion of total purines was similar.

The microbial nitrogen and microbial crude protein syntheses did not show a significant response (P>0.05), averaging 53.12 and 332.03 g day^{-1}. This response can be explained by the digestibility of CP being 60%. The microbial protein synthesis depends largely on the availability of carbohydrates and nitrogen in the rumen, such that microbial growth is maximized by the synchronism between the availability of fermentable energy and the rumen-degradable nitrogen. Therefore, it is estimated that all tested diets provided similar efficiency and growth to the rumen microorganisms, irrespective of the reduction in DM intake, which possibly occurred due to the satiety mechanism caused by the energy content of the diet.

No significant response was observed for grams of microbial protein per kilogram of total digestible nutrients (g CP kg TDN^{-1}), either, which averaged 113.73 g CP kg TDN^{-1}. This result followed the same effect as CP digestibility, which was not significantly affected, averaging 60%.

Microbial production was lower than the 130 g CP kg TDN^{-1} reported by NRC (1996) and120 g 130 g CP kg TDN^{-1} by Leal et al. (2007).

Nitrogen intake and nitrogen excreted in feces decreased linearly (P<0.05) by 1.74 and 1.22 g day^{-1}, respectively, with every percent unit of glycerin added to the diet (Table 9). This effect can be explained by the lower DM intake of the animals as glycerin was included in the diet.

The nitrogen excreted in the feces consists of the nitrogen consumed from the diet, the desquamation of cells from the gastrointestinal tract, the proteins that move into the duodenum, and microbial proteins. High-protein diets usually provide a greater nitrogen excretion in the feces.

Results for digested nitrogen per day, digested nitrogen as a percentage of nitrogen intake, nitrogen in urine per day, nitrogen retained per day, nitrogen retained as a percentage of nitrogen intake, and nitrogen retained as a percentage of the digested nitrogen did not differ (P>0.05), averaging 74.69 g day^{-1}, 60.00%, 19.10 g day^{-1}, 55.60 g day^{-1}, 43.53%, and 73.34% with inclusion of crude glycerin in the diet. These effects can be explained by the fact that the diet was formulated to be isoproteic, and thus elicited a similar response from the balance of nitrogen compounds.

Urine urea nitrogen decreased linearly (P<0.05) by 3.93 mg dL^{-1} with every percent unit of crude glycerin added to the diet. This result stemmed from the reduction of DM intake from the diet, which decreased with the inclusion of crude glycerin in the diets.

Table 9. Balance of nitrogen compounds of Nellore heifers supplemented with different levels of crude glycerin.

Balance of nitrogen compounds	Crude glycerin level (%)					CV (%)[1]	P[2]
	0.00	4.00	8.00	12.00	16.00		
N intake (g day^{-1})[3]	142.88	119.64	126.93	125.41	105.27	10.88	0.001
N feces (g day^{-1})[4]	60.10	50.56	51.92	46.19	37.88	9.60	0.001
N digested (g day^{-1})[5]	82.79	69.08	75.00	79.22	67.38	17.74	0.243
N digested (% of N intake)[6]	57.85	56.42	59.05	62.63	64.05	9.67	0.149
Urine N (g day^{-1})[7]	26.25	20.77	19.07	16.46	12.93	54.48	0.272
N retained (g day^{-1})[8]	56.53	48.31	55.93	62.76	54.45	32.94	0.888
N retained (% of N intake)[9]	33.80	38.96	43.92	49.18	51.79	26.17	0.223
N retained (% of N digested)[10]	66.89	67.01	74.17	77.88	80.79	23.05	0.665
Concentrations (mg dL^{-1})							
Urine urea N[11]	322.00	285.07	293.68	270.17	250.81	13.15	0.001
Plasma urea N[12]	13.63	13.08	12.35	13.85	12.64	18.42	0.163
Excretions (g day^{-1})							
Urine urea N[13]	41.27	36.03	34.48	27.22	25.68	20.50	0.001
Urine urea[14]	19.23	16.79	16.07	12.68	11.97	20.50	0.001

[1]Coefficient of variation; [2]Probability of error; [3]$Y = 137.92 - 1.7363x$. $r^2 = 0.65$; [4]$Y = 59.092 - 1.2203x$. $r^2 = 0.90$; [5]$Y = 74.69$; [6]$Y = 60.00$;[7]$Y = 19.10$;[8]$Y = 55.60$; [9]$Y = 43.53$; [10]$Y = 73.34$; [11]$Y = 315.8 - 3.932x$. $r^2 = 0.87$; [12]$Y = 13.11$; [13]$Y = 40.934 - 0.9998x$. $r^2 = 0.96$; [14]$Y = 19.074 - 0.4658x$. $r^2 = 0.96$.

Plasma urea nitrogen did not show to be significantly affected (P>0.05) by inclusion of crude glycerin in the heifers diet, averaging 13.11 mg dL^{-1}. Blood urea concentrations have been used for monitoring the intake of dietary protein near the animal requirements, since excess protein consumption may affect the productive and reproductive performance of an animal, as stated by Carvalho et al. (2011), elevating its energy requirements or even increasing the feed cost.

The excretion of urea nitrogen and urea in the nitrogen decreased linearly (P<0.05) by 1.0 and 0.47 g day^{-1}, respectively, with every percent of crude glycerin added to the diet. These results are related to the lower DM intake and consequent lower CP intake from the total diet. Considering the plasma urea nitrogen concentration of 13.11 mg dL^{-1}, we observed that inclusion of glycerin in the heifer diets led to a lower loss of these nitrogen compounds through urinary and fecal excretions, as a result of the CP digestibility not being compromised.

A similar response was described by Teixeira et al. (2007), who did not observe differences in plasma urea concentration, but found a linear effect on urinary excretion of urea, attributed to the increase in total nitrogen intake.

The urine excretion represents a high biological cost and deviation of energy for the maintenance of the body nitrogen concentrations at non-toxic levels to animals. The conversion of ammonia to urea costs the animal 12 kcal per gram of nitrogen (VAN SOEST, 1994).

This study indicated that the dietary CP was directed to the body tissues and converted to muscle gain, even though weight gain was lower with inclusion of crude glycerin in the diet, which was an expected result, since CP intake decreased as the levels of crude glycerin in the nutrition of animals were elevated.

Conclusion

Inclusion of crude glycerin as a by-product in diets for heifers on a grazing system during the dry season alters the feeding behavior and reduces the time and efficiency of feeding and rumination activity, but does not influence the microbial protein synthesis or retained nitrogen of these animals.

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