



Glycerin levels in the diets for crossbred bulls finished in feed-lot: ingestive behavior, feeding and rumination efficiency

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ABSTRACT. This work was carried out to study corn substituting by glycerin levels on animal behavior, feeding and rumination efficiency of Purunã young bulls finished in feed-lot. It was utilized 40 bulls Purunã breed with 208.8 ± 33.3 kg and eight months old. The diets were: without glycerin - G00, 6% of glycerin - G06, 12% of glycerin - G12 and 18% of glycerin - G18. Dry matter intake was similar among diets. On the other hand, NDF intake decreased linearly with glycerin levels supplementation in the diets. Feeding and rumination efficiency DM and NDF were similar among diets. Glycerin changed activities durations of bulls. Glycerin did not affect feed frequency. At contrary, rumination frequency was reduced linearly with glycerin inclusion. Others activities frequencies showed a quadratic effect with the glycerin addition. Glycerin inclusion in the diet reduced the time duration for feed frequency, but had no effect on the time spent for rumination frequency. However, the frequency duration for other activities increased linearly with glycerin inclusion.

Keywords: behavior, cattle, feed intake, feed-lot, glycerol.

Níveis de glicerina na dieta de bovinos mestiços terminados em confinamento: comportamento ingestivo, eficiência de alimentação e ruminação

RESUMO. Este trabalho foi realizado para avaliar a substituição do milho por níveis de glicerina sobre o comportamento animal, eficiências de alimentação e ruminação de novilhos precoces Purunã terminados em confinamento. Foram utilizados 40 novilhos Purunã com $208,8 \pm 33,3$ kg e oito meses de idade. As dietas utilizadas foram sem glicerina - G00, 6% de glicerina - G06, 12% de glicerina - G12 e 18% de glicerina - G18. A ingestão de MS foi semelhante em todas as dietas. Por outro lado, a ingestão de FDN diminuiu linearmente com a suplementação de diferentes níveis de glicerina nas dietas. As eficiências de alimentação e ruminação de MS e FDN foram semelhantes em todas as dietas. A glicerina alterou a duração das atividades comportamentais. A suplementação da dieta com glicerina não afetou a frequência de alimentação. De outro modo, a frequência de ruminação foi reduzida linearmente com a inclusão de glicerina. As frequências de outras atividades apresentaram efeito quadrático com a adição de glicerina. A inclusão de glicerina à dieta reduziu o tempo de duração das frequências de alimentação, mas não afetou o tempo despendido nas frequências de ruminação. Entretanto, a duração das frequências de outras atividades aumentou linearmente com a inclusão de glicerina à dieta.

Palavras-chave: comportamento, bovinos, consumo, confinamento, glicerol.

Introduction

Factors that regulate dry matter intake by ruminants are complex and not understood fully. Nevertheless, accurate estimates of feed intake are vital to predicting rate of gain of animals. Previous research has established relationships between dietary energy concentration and dry matter intake by beef cattle based on the concept that consumption of less digestible, low-energy (often

high-fiber) diets is controlled by physical factors such as rumen fill and digest passage, whereas consumption of highly digestible, high-energy (often low-fiber, high-concentrate) diets is controlled by the animal's energy demands and by metabolic factors (NRC, 2000).

The feeding behavior is related of intake, obtaining data to improve animal performance by feed intake (ALBRIGHT, 1993). Thus, the problems

related to declining intake in critical times, management practices, quality and quantity of diet offered can be improved by changing the feeding behavior (MARQUES et al., 2008). Animal performance is mainly influenced by dry matter intake that can be affected by the amount of fiber (MISSIO et al., 2010) and energy content in the diet (FREITAS et al., 2010). Feed intake of diet with high concentration of NDF, increases the number and chewing duration and rumination duration due to fill the rumen-reticulum (DADO; ALLEN, 1995). According to Van Soest (1994) rumination duration is influenced by the nature of the diet and seems to be proportional to the cell wall content of forages. Thus, intake of fiber is highly correlated with rumination time, and in general, the nutritional quality of the diet may determine changes in food intake, modifying the ingestive behavior and animal performance (SIGNORETTI et al., 1999). According to Forbes (1988), ruminants can modify in part the ingestive behavior minimizing the effects of unfavorable dietary conditions, reaching their nutritional requirements for maintenance and growth. Bürger et al. (2000) found that period feeding duration of animal finished in feed-lot may vary from one to six hours, depending directly of the energy levels in the diet. The decrease of NDF caused by increased levels of concentrate (energy) in the diet reduces feeding and rumination duration, providing more time to animal performance and other activities that require lower energy expenditures, improving the animal performance (SOUZA et al., 2007).

The use of glycerin replacing corn as an energy source in diets for feedlot bulls can change the feeding behavior. Elam et al. (2008) and Farias et al. (2012) observed that animals supplemented with glycerin in the diet needed more time to consume food than control group. The use of glycerin as a corn substitute, an energy source, determines fast rumen fermentation (TRABUE et al., 2007) which modifies intake behavior to the point that animals require more time to consume feed when compared to glycerin-less diets.

This study was conducted to evaluate the effects of different glycerin levels as corn substitution in the diets on ingestive behavior, feed intake and rumination efficiency of young bulls breed Purunã finished in feed-lot.

Material and methods

Animals, housing and diets

This experiment was approved by Department of Animal Production at the State University of

Maringá (CIOMS, 1985). It was conducted at the Experimental Station of Farm Modelo at Institute Agronomic of Paraná – IAPAR in Ponta Grossa, Paraná State, Brazil.

Forty Purunã bulls breed ($\frac{1}{4}$ Aberdeen Angus + $\frac{1}{4}$ Caracu + $\frac{1}{4}$ Charolais + $\frac{1}{4}$ Canchim) were used in a complete randomised design. Bulls were weighed and distributed in four diets with ten replications per group. After an 11-day diet adaptation period, the bulls were weighed and started the study with an average initial BW of 208.8 ± 33.3 kg and average age of 8 months. The bulls' BW and concentrate and corn silage intakes were recorded monthly until day 229 of the experiment when the bulls reached a final BW of 471.7 ± 57.3 kg.

The glycerin was produced in a soy-diesel facility (BIOPAR, Rolândia, Paraná State, Brazil). Glycerin fed in the current study was used as an energetic ingredient; therefore, to obtain four isoenergetic diets, the increase in glycerin level was counterbalanced, mainly by a decrease in corn grain content (Table 1). All diets were formulated to be isonitrogenous (Table 2).

Table 1. Ingredients and percent composition (% DM) of the diet treatments.

Ingredients %	Glycerin levels % DM			
	Go0 ¹	Go6 ²	G12 ³	G18 ⁴
Corn silage	53.00	53.00	53.00	53.00
Soybean meal	11.78	13.39	14.99	16.87
Corn grain	34.40	26.77	19.14	11.38
Glycerin	0.00	6.00	11.99	17.99
Mineral salt ⁵	0.83	0.83	0.83	0.76

¹Diet without glycerin; ²6% glycerin; ³12% glycerin; ⁴18% glycerin; ⁵Guarantee levels (per kg): calcium - 175 g; phosphorus - 100 g; sodium - 114 g; selenium - 15 g; magnesium - 15 g; zinc - 6.004 mg; manganese - 1.250 mg; copper - 1.875; iodine - 180 mg; cobalt - 125 mg; selenium - 30 mg; fluorine (maximum) - 1.00 mg.

Table 2. Chemical composition of the base diets (% DM).

Ingredients %	DM ¹	%DM								
		OM ²	Ash	CP ³	EE ⁴	TC ⁵	NFC ⁶	NDF ⁷	ADF ⁸	TDN ⁹
Corn silage	29.11	97.27	2.73	6.06	3.36	87.85	51.41	36.44	19.16	62.20
Soybean meal	81.50	92.86	7.14	48.89	2.50	41.47	23.40	18.07	11.65	78.03
Corn grain	81.76	97.68	2.32	10.32	5.93	81.43	64.15	17.28	4.77	81.64
Glycerin	94.27	95.24	4.76	0.07	0.12	-	-	-	-	80.61
Mineral salt	98.00		96.00							
Diets %										
Go0 ¹⁰	53.96	87.94	2.79	10.81	3.69	73.44	47.53	25.91	12.61	70.24
Go6 ¹¹	54.69	88.45	3.00	10.81	3.36	68.91	43.83	25.07	12.47	70.10
G12 ¹²	55.42	88.96	3.22	10.81	3.03	64.37	40.14	24.23	12.32	69.96
G18 ¹³	56.18	89.56	3.45	10.91	2.70	59.84	36.43	23.40	12.20	69.92

¹Dry matter; ²Organic matter; ³Crude Protein; ⁴Ether extract; ⁵Total carbohydrates; ⁶Non-fibre carbohydrates; ⁷Neutral detergent fibre; ⁸Acid detergent fibre; ⁹Total digestive nutrients; ¹⁰Diet without glycerin; ¹¹6% glycerin; ¹²12% glycerin; ¹³18% glycerin.

The bulls were randomly assigned to 1 of 4 diets containing 0, 6, 12 or 18% glycerin in DM basis. The bulls were fed concentrate and corn silage in separate troughs, both for *ad libitum*. Bulls were fed twice a day (8:00 am and 3:00 pm). The diets were weighed daily, so that the refusals represented 5% of the total. The concentrate intake was fixed in 1.2%

of BW and adjusted every 28-day. The diets formulation and quantity supplied were designed to provide a weight gain of 1.2 kg day⁻¹, according to NRC (2000) recommendations.

Samples collection

There were two visual assessments of behavioral activities interval of 56 days between observations ones. The data collections were realized during 48 consecutive hours, with a record of activities in specific ethogram every five minutes (SILVA et al., 2006). The behavioral activities were collected by eight observers, divided into four teams who alternated every two hours (SILVA et al., 2006).

Data were collected to estimate the duration and numbers of the periods spent feeding, ruminating and others activities. The total time of each activity was determined by the sum of repetitions, while the number of periods was accounted for in accordance with the number of consecutive repetitions of each activity. The times of each activity were determined by the ratio between the length and the number of periods for each activity.

The efficiencies of feeding and rumination of dry matter and neutral detergent fiber were determined and adapted the methodology proposed by Bürger et al. (2000), according to the formulas described below:

$$\begin{aligned} FE_{DM} &= DMI \text{ FD}^{-1} \\ FE_{NDF} &= NDFI \text{ FD}^{-1} \\ RE_{DM} &= DMI \text{ RUD}^{-1} \\ RE_{NDF} &= NDFI \text{ RUD}^{-1} \end{aligned}$$

where:

FE_{DM} – Feeding efficiency of dry matter (kg DM h⁻¹);
 DMI – Dry matter intake (kg DM day⁻¹);
 FD – Feeding duration (h day⁻¹);
 FE_{NDF} – Feeding efficiency neutral detergent fiber (NDF kg h⁻¹);
 NDFI – Neutral detergent fiber intake (NDF kg day⁻¹);
 RE_{DM} – Rumination efficiency of dry matter (kg DM h⁻¹);
 RUD – Rumination duration (h day⁻¹);
 RE_{NDF} – Rumination efficiency of neutral detergent fiber (NDF kg h⁻¹).

Chemical analyses

Dry matter content of the ingredients (silage, concentrate mix) was determined by oven-drying at 105°C for 24h (AOAC, 1990)(method 930.15).

The OM content was calculated as the difference between DM and ash contents, with ash determined by combustion at 550°C for 5h. The NDF and ADF contents were determined using the methods described by Van Soest et al. (1991) with heat stable alpha-amylase for solubilization the amylaceous compound (MERTENS, 2002) and sodium sulfite used in the NDF procedure, and expressed inclusive of residual ash. Content of N in the samples was determined by the Kjeldahl method (AOAC, 1990) (method 976.05). The total carbohydrates (TC) were obtained by using the following equation: TC = 100 - (% CP + % EE + % Ash) (SNIFFEN et al., 1992). Non-fiber carbohydrates (NFC) were determined by the difference between TC and NDF. Total digestible nutrients (TDN) content of diets was obtained by the methodology described by Kears (1982): silage = -17.2649 + 1.2120 (% CP) + 0.8352 (% ENN) + 2.4637 (% EE) + 0.4475 (% CF); energetic foods = 40.2625 + 0.1969 (% CP) + 0.4228 (% ENN) + 1.1903 (% EE) + 0.1379 (% CF) and protein foods = 40.3227 + 0.5398 (% CP) + 0.4448 (% ENN) + 1.4218 (% EE) - 0.7007 (% CF). The samples were analyzed in triplicate at the Laboratory of Feed Analyses and Animal Nutrition at the State University of Maringá.

Statistical analysis

The experimental design was completely randomized with four treatments and ten replications. Results were statistically interpreted by regression equations using (SAS, 2004) procedure (PROC REG):

$$Y_{ijk} = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \alpha_{ijk} + \epsilon_{ijk}$$

where:

Y_{ijk} = dependents variables;
 β_0 = regression coefficient;
 X_{ijk} = independents variables;
 α_{ijk} = regression deviations;
 ϵ_{ijk} = residual error.

Results and discussion

Total dry matter intake (7.9 kg day⁻¹) was similar ($p > 0.05$) among diets (Table 3). Similarly, Mach et al. (2009) reported no changes in DMI when glycerin was included at 0, 4, 8 or 12% in the diet (8.3 kg day⁻¹) of Holsteins bulls fed high-concentrate diets. Likewise, some others studies conducted with lactating cows that were fed high-forage diets (CHUNG et al., 2007; DEFRAIN et al., 2004) have reported no negative effects on feed intake when supplementing the diets with glycerin at inclusion

rates similar to the present study. On the other hand, Ogborn (2006) reported that 5% glycerin increased DMI in prepartum dairy cows. In contrast, Parsons et al. (2009) reported a 13% reduction in DMI when glycerin was added at 16% to a steam-flaked corn fed to heifers for the final 85 days before slaughter. Thus dry matter intake can be dependent glycerin quality (DONKIN, 2008).

On the other hand, NDF intake ($p < 0.05$) decreased linearly with glycerin levels supplementation in the diets, which can be explained by the lower content of NDF in the glycerin of the diet offered for bulls (Table 2). However, the reduced NDF intake did not reduce DM intake depending glycerin levels in the diets.

FE_{DM} and FE_{NDF} were similar ($p > 0.05$) among diets (Table 3). Farias et al. (2012) observed the negative quadratic effect on FE_{DM} and FE_{NDF} with replacing corn by glycerin in the diets of heifers supplemented in pasture system.

Table 3. Glycerin levels on feed intake, feed efficiency and rumination efficiency of Purunã bulls finished in feed-lot

Item	Glycerin levels, % of DM				Regression equation ⁵	SEM ⁶	r ²
	Goo ¹	Go6 ²	G12 ³	G18 ⁴			
DMI ⁷ kg d ⁻¹	8.27	8.47	7.29	7.46	$\hat{Y}=7.87$	0.27	-
NDFI ⁸ kg d ⁻¹	2.67	2.64	2.35	2.31	$\hat{Y}=2.699 - 0.019x$	0.07	0.11
FE_{DM} ⁹ kg h ⁻¹	2.36	2.42	2.15	2.59	$\hat{Y}=2.38$	0.09	-
FE_{NDF} ¹⁰ kg h ⁻¹	0.76	0.75	0.69	0.81	$\hat{Y}=0.75$	0.02	-
RE_{DM} ¹¹ kg h ⁻¹	1.12	1.10	1.08	1.14	$\hat{Y}=1.11$	0.04	-
RE_{NDF} ¹² kg h ⁻¹	0.36	0.34	0.34	0.35	$\hat{Y}=0.35$	0.01	-

¹Diet without glycerin; ²6% glycerin; ³12% glycerin; ⁴18% glycerin; ⁵Effect of glycerin level; ⁶Standard error of mean; ⁷Dry matter intake; ⁸Neutral detergent fiber intake; ⁹Dry matter feeding efficiency; ¹⁰Neutral detergent fiber feeding efficiency; ¹¹Dry matter rumination efficiency; ¹²Neutral detergent fiber rumination efficiency.

RE_{DM} and RE_{NDF} were not changed due to replacement levels, demonstrating that glycerin was effective to replace corn without affecting the performance characteristics. Moreover, Farias et al. (2012) observed a negative quadratic effect on RE_{DM} and RE_{NDF} due to the inclusion of glycerin in the diet.

Replacing corn by glycerin as energy source in the diets changed ($p < 0.01$) activities durations of bulls finished in feed-lot (Table 4). The time spent for feeding and ruminating decreases linearly as a function of replacing corn by glycerin in the diets. The behavioral characteristics are mutually excluding, so there was a linear increase ($p < 0.01$) in the time spent for other activities.

Table 4. Glycerin levels on duration (minutes) behavior intake of Purunã bulls finished in feed-lot.

Item	Glycerin levels, % of DM				Regression equation ⁵	SEM ⁶	r ²
	Goo ¹	Go6 ²	G12 ³	G18 ⁴			
Feeding	217.37	212.75	203.00	177.62	$\hat{Y}=222.037 - 1.842x$	5.06	0.21
Rumination	445.87	463.50	414.37	400.00	$\hat{Y}=458.950 - 2.667x$	8.57	0.15
Other activities	776.75	763.50	822.62	862.37	$\hat{Y}=758.91 + 4.514x$	11.42	0.25

¹Diet without glycerin; ²6% glycerin; ³12% glycerin; ⁴18% glycerin; ⁵Effect of glycerin level;

⁶Standard error of mean.

Glycerin supplementation in the diet for bulls finished in feed-lot reduced feeding duration by 18.2%. According to Bergner et al. (1995) the increased of rumen fermentation of glycerin allows its transformation into volatile fatty acids, especially propionate (RÉMOND et al., 1993; TRABUE et al., 2007). The rapid metabolism of this compound into energy for maintenance and growth of the animal promotes satiety. Besides that, glycerin can be absorbed by the ruminal epithelium and metabolized into glucose by the liver (DONKIN, 2008). According to Trabue et al. (2007) inclusion of high levels of glycerin levels can inhibit the feed intake for a certain time due to the amount of energy delivered to the animal by the possible presence of salts, impurities, and high methanol levels resulting from the transesterification process (CHUNG et al., 2007; PARSONS et al., 2009). The data in this study corroborate to those of Missio et al. (2010) when evaluating the influence of concentrate levels (22, 40, 59 and 79%) on the ingestive behavior of young bull sin feed-lot. The authors obtained a linear decrease on feed intake due a higher intake (energy) in less time, reaching the nutritional requirements of animals. Likewise, Silva et al. (2005) evaluated the feeding behavior of cattle fed different levels of concentrate and observed a linear reduction on feeding duration due to the lower NDF and higher energy intake in the diet. Corroborating to above authors, Farias et al. (2012) observed a reduction in feeding duration depending on glycerin levels (2.8, 6.1 and 9.1%) in the diet for heifers supplemented in pasture system.

Replacing corn by different glycerin levels in the diets reduced the NDF content in the diets (Table 2). The reduction of NDF in the diet decreased the rumination duration (Table 4). Mendes Neto et al. (2007) observed differences between the rumination duration for roughage and concentrate depending the type and fiber content in the food. According to Kijora et al. (1998), 85% of ingested glycerin may disappear within the first two hours after feeding, agreeing with Bergner et al. (1995) stated that when levels of 15 to 25% of glycerin in the diet of ruminant is modified into six hours. Therefore, the glycerin is rapidly metabolized by bacteria in the rumen or volatile fatty acids, can be absorbed by the ruminal epithelium and promote a negative feedback on the necessary rumination duration (DONKIN, 2008). Farias et al. (2012) observed no difference for rumination duration (382.86 min. day⁻¹) for heifers fed different glycerin levels in the diets (2.8, 6.1 and 9.1%). According to Missio et al. (2010) decreasing rumination duration, the increased the rest time of animals imply a decrease in physical activity,

contributing thus for increases on animal performance.

The time spent for other activities were 11% higher for bulls fed with inclusion of glycerin in the diets. The time utilized for other activities (862 min. day⁻¹) was higher than those found by Bürger et al (2000) when evaluated concentrate levels (30, 45, 60 and 75%) in the diet of steers (655, 701, 795 and 841 min. day⁻¹) and Farias et al. (2012) evaluating the levels of crude glycerin (2.8, 6.1 and 9.1%) in the diets for heifers (575, 547 and 623 min.). Glycerin of high purity can be better utilized by the body when compared to ruminant diets supplemented with concentrated crude glycerin or not. Fatty acid resulting from ruminal fermentation of glycerin can

be metabolized by the gastrointestinal tract into energy, or absorbed through the portal vein and sent to the liver (DONKIN, 2008). Subsequently, propionate derived from the biohydrogenation or glycerin absorbed by the ruminal epithelium is converted into glucose (PLUSKE, 2007).

Glycerin supplementation in the diet did not affect ($p > 0.05$) feed frequency (18 visits day⁻¹) for bulls finished in feed-lot (Table 5). At contrary, rumination frequency was reduced linearly ($p < 0.01$) with the substitution of corn by glycerin. Others activities frequencies showed a quadratic effect ($p < 0.01$) with

Table 5. Glycerin levels on frequency and duration frequency per activity of Purunã bulls finished in feed-lot

Item	Glycerin levels, % of DM				Regression equation ⁵	SEM ⁶	r ²
	G00 ¹	G06 ²	G12 ³	G18 ⁴			
FF ⁷ (visits day ⁻¹)	17.55	18.62	18.42	18.07	$\hat{Y}=18.16$	0.35	-
RF ⁸ (visits day ⁻¹)	17.47	16.85	15.70	14.55	$\hat{Y}=17.632 - 0.141x$	0.33	0.28
OAF ⁹ (visits day ⁻¹)	27.47	30.02	28.17	27.15	$\hat{Y}=27.736 + 0.342x - 0.018x^2$	0.36	0.17
FDF ¹⁰ (min.)	12.48	11.52	11.07	9.85	$\hat{Y}=12.480 - 0.118x$	0.29	0.26
RDF ¹¹ (min.)	25.77	27.84	26.53	27.81	$\hat{Y}=26.99$	0.64	-
ODF ¹² (min.)	28.29	25.70	29.32	31.89	$\hat{Y}=26.637 + 0.206x$	0.58	0.19

¹Diet without glycerin; ²6% glycerin; ³12% glycerin; ⁴18% glycerin; ⁵Effect of glycerin level; ⁶Standard error of mean; ⁷Feeding frequency; ⁸Rumination frequency; ⁹Other activities frequency; ¹⁰Feeding duration frequency; ¹¹Rumination duration frequency; ¹²Others activities duration frequency.

the glycerin addition, being 9.5% level glycerin showed higher frequency number (29 visits day⁻¹).

Glycerin inclusion in the diet of bulls reduced ($p < 0.01$) the time duration for feed frequency, but had no effect ($p > 0.05$) on the time spent for rumination frequency.

However, the frequency duration for other activities increased linearly ($p < 0.01$) with glycerin inclusion.

Glycerin inclusion in the diets reduced the fibrous portion due to the substitution of corn by glycerin. Allowances energy requirements, either by ruminal fermentation or by hepatic metabolism of glycerin, allowed greater availability of cattle to perform other activities during the evaluation period. Thus, the frequency of reduced rumination is related to the rapid disappearance of glycerin in the gastro intestinal tract of ruminants and lower NDF content in bolus regurgitated food by animals (MISSIO et al., 2010).

Conclusion

Corn partial replacement by glycerin in the diets for bulls finished in feed-lot and fed with 53% corn silage and 47% concentrate can be an alternative due your availability on market and feed utilization for animals.

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References

- ALBRIGHT, J. L. Nutrition, feeding and calves: feeding behaviour of dairy cattle. **Journal of Dairy Science**, v. 76, n. 2, p. 485-498, 1993.
- AOAC-Association of Official Analytical Chemists. **Official Methods of Analysis of AOAC International**. 14th ed. Arlington: AOAC International, 1990.
- BERGNER, H.; KIJORA, C.; CERESNAKOVA, Z.; SZAKACS, J. *In vitro* studies on glycerol transformation by rumen microorganisms. **Archiv für Tierernaehrung**, v. 48, n. 3, p. 245-256, 1995.
- BÜRGER, P. J.; PEREIRA, J. C.; QUEIROZ, A. C. D.; SILVA, J. F. C.; VALADARES FILHO, S. C.; CECON, P. R.; CASALI, A. D. P. Comportamento ingestivo em bezerros holandeses alimentados com dietas contendo diferentes níveis de concentrado. **Revista Brasileira de Zootecnia**, v. 29, n. 1, p. 236-242, 2000.

- CHUNG, Y. H.; RICO, D. E.; MARTINEZ, C. M.; CASSIDY, T. W.; NOIROT, V.; AMES, A.; VARGA, G. A. Effects of feeding dry glycerin to early postpartum Holstein dairy cows on lactational performance and metabolic profiles. **Journal of Dairy Science**, v. 90, n. 12, p. 5682-5691, 2007.
- CIOMS-Council for International Organizations of Medical Services. **International guideline principles for biomedical research involving animals**. Geneva: WHO, 1985.
- DADO, R. G.; ALLEN, M. S. Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary or inert bulk. **Journal of Dairy Science**, v. 78, n. 1, p. 119-133, 1995.
- DEFRAIN, J. M.; HIPPEN, A. R.; KALSCHUR, K. F.; JARDON, P. W. Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance. **Journal of Dairy Science**, v. 87, n. 12, p. 4195-4206, 2004.
- DONKIN, S. S. Glycerol from biodiesel production: the new corn for dairy cattle. **Revista Brasileira de Zootecnia**, v. 37, n. spec., p. 280-286, 2008.
- ELAM, N. A.; ENG, K. S.; BECHTEL, B.; HARRIS, J. M.; CROCKER, R. **Glycerol from biodiesel production: considerations for feedlot diets**. Las Cruces: New Mexico State University, 2008.
- FARIAS, M. S.; PRADO, I. N.; VALERO, M. V.; ZAWADZKI, F.; SILVA, R. R.; EIRAS, C. E.; RIVAROLI, D. C.; LIMA, B. S. Níveis de glicerina para novilhas suplementadas em pastagens: desempenho, ingestão, eficiência alimentar e digestibilidade. **Semina: Ciências Agrárias**, v. 33, n. 3, p. 1177-1188, 2012.
- FORBES, T. D. A. Researching the plant-animal interface: the investigation of ingestive behavior in grazing animal. **Journal of Animal Science**, v. 66, p. 2369-2379, 1988.
- FREITAS, L. S.; SILVA, J. H. S.; SEGABINAZZI, L. R.; SILVA, V. S.; FILHO, D. C. A.; BRONDANI, I. L. Substituição da silagem de milho por silagem de girassol na dieta de novilhas em confinamento: comportamento ingestivo. **Revista Brasileira de Zootecnia**, v. 39, n. 1, p. 225-232, 2010.
- KEARL, L. C. **Nutrient requirements of ruminants in developing countries**. Logan: International Feedstuffs Institute, 1982.
- KIJORA, C.; BERGNER, H.; GÖTZ, K. P.; BARTELT, J.; SZAKACS, J.; SOMMER, A. Research note: investigation on the metabolism of glycerol in the rumen of bulls. **Arch Tierernähr**, v. 51, n. 4, p. 341-348, 1998.
- MACH, N.; BACH, A.; DEVANT, M. Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets. **Journal of Animal Science**, v. 87, n. 2, p. 632-638, 2009.
- MARQUES, J. A.; PINTO, A. P.; SANTOS, A. J. J.; NASCIMENTO, W. G. Intervalo de tempo entre observações para avaliação do comportamento ingestivo de tourinhos em confinamento. **Semina: Ciências Agrárias**, v. 29, n. 4, p. 955-960, 2008.
- MENDES NETO, J.; CAMPOS, J. M. S.; VALADARES FILHO, S. C.; LANA, R. P.; QUEIROZ, A. C.; EUCLYDES, R. F. Comportamento ingestivo de novilhas leiteiras alimentadas com polpa cítrica em substituição ao feno de capim-tifton 85. **Revista Brasileira de Zootecnia**, v. 36, n. 3, p. 618-625, 2007.
- MERTENS, D. R. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. **Journal of AOAC International**, v. 85, n. 6, p. 1217-1240, 2002.
- MISSIO, R. L.; BRONDANI, I. L.; ALVES FILHO, D. C.; SILVEIRA, M. F.; FREITAS, L. S.; RESTLE, J. Comportamento ingestivo de tourinhos terminados em confinamento, alimentados com diferentes níveis de concentrado na dieta. **Revista Brasileira de Zootecnia**, v. 39, n. 7, p. 1571-1578, 2010.
- NRC-National Research Council. **Nutrient requirements of Beef Cattle**. 7th ed. Washington, D.C.: National Academy Press, 2000.
- OGBORN, K. L. **Effects of method of delivery of glycerol on performance and metabolism of dairy cows during the transition period**. Ithaca: Cornell University, 2006.
- PARSONS, G. L.; SHELOR, M. K.; DROUILLARD, J. S. Performance and carcass traits of finishing heifers fed crude glycerin. **Journal of Animal Science**, v. 87, n. 2, p. 653-657, 2009.
- PLUSKE, J. Evaluation of glycerine as co-product of biodiesel production for the pig industry. **Subiaco: Pork Co-operative Research Center**, v. 1, suppl., p. 1-47, 2007.

- RÉMOND, B.; SOUDAY, E.; JOUANY, J. P. *In vitro* and *in vivo* fermentation of glycerol by rumen microbes. **Animal Feed Science and Technology**, v. 41, n. 2, p. 121-132, 1993.
- SAS-Statistical Analysis System. **SAS/STAT**, version 9.1. User's guide. Cary: SAS Institute, 2004.
- SIGNORETTI, R. D.; SILVA, J. F. C.; VALADARES FILHO, S. C.; PEREIRA, J. C.; CECON, P. R.; QUEIROZ, A. C.; ARAUJO, G. G. L.; ASSIS, G. M. L. Consumo e digestibilidade aparente em bezerros da raça holandesa alimentados com dietas contendo diferentes níveis de volumoso. **Revista Brasileira de Zootecnia**, v. 28, n. 1, p. 169-177, 1999.
- SILVA, R. R.; SILVA, F. F.; CARVALHO, G. G. P.; FRANCO, I. L.; VELOSO, C. M.; CHAVES, M. A.; BONOMO, P.; PRADO, I. N.; ALMEIDA, V. S. Comportamento ingestivo de novilhas mestiças de Holandês x Zebu confinadas. **Archivos de Zootecnia**, v. 54, n. 205, p. 75-85, 2005.
- SILVA, R. R.; SILVA, F. F.; PRADO, I. N.; CARVALHO, G. G. P.; FRANCO, I. L. F.; MENDES, F. B. L.; OLIVEIRA, A. P.; PINHEIRO, A. A. Metodologia para o estudo do comportamento de bezerros confinados na fase pós-aleitamento. **Archivos Latinoamericanos de Producción Animal**, v. 14, n. 4, p. 135-138, 2006.
- SNIFFEN, C. J.; O'CONNOR, J. D.; VAN SOEST, P. J.; FOX, D. G.; RUSSELL, J. B. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. **Journal of Animal Science**, v. 70, n. 11, p. 3562-3577, 1992.
- SOUZA, S.; ÍTAVO, L. C. V.; RÍMOLI, J.; ÍTAVO, C.; DIAS, A. M. Comportamento ingestivo diurno de bovinos em confinamento e em pastagens. **Archivos de Zootecnia**, v. 56, n. 213, p. 67-70, 2007.
- TRABUE, S.; SCOGGIN, K.; TJANDRAKUSUMA, S.; RASMUSSEN, M. A.; REILLY, P. J. Ruminal fermentation of propylene glycol and glycerol. **Journal of Agricultural and Food Chemistry**, v. 55, n. 17, p. 7043-7051, 2007.
- VAN SOEST, P. J. **Nutritional ecology of the ruminant**. 2nd ed. Ithaca: Cornell University Press, 1994.
- VAN SOEST, P. J.; ROBERTSON, J. B.; LEWIS, B. A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v. 74, n. 10, p. 3583-3597, 1991.

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