

## Effects of the inclusion of glycerin in diets containing 30 or 70% roughage on feed disappearance and digestibility

### Efeito da inclusão de glicerina em dietas contendo 30 ou 70% de forragem sobre o desaparecimento e a digestibilidade de alimentos

Antonio Carlos Homem Junior<sup>1\*</sup>; Jane Maria Bertocco Ezequiel<sup>2</sup>; Leonardo Fernandes Cremasco<sup>3</sup>; Marco Túlio Costa Almeida<sup>4</sup>; Josimari Regina Paschoaloto<sup>1</sup>; André Pastori D'Áurea<sup>1</sup>; Henrique Leal Pérez<sup>5</sup>

#### Abstract

The objective of this study was to evaluate *in vitro* nutrient disappearance and *in vivo* nutrient digestibility of cattle diets containing 70 or 30% roughage, with and without glycerin supplementation. Four Nelore cattle were used in the study based on a 4 × 4 Latin Square design in a 2 × 2 factorial arrangement. Inclusion of glycerin and roughage proportion did not affect the nutrient disappearance of each ingredient ( $P > 0.05$ ). Regardless of the inclusion of glycerin, a reduction in the proportion of roughage led to an increase ( $P < 0.05$ ) in DMDIs (64 vs. 72%), NDFDis (41 vs. 54%), and ADFDis (31 vs. 44%) of the total rations. Inclusion of glycerin resulted in an increase ( $P < 0.05$ ) in DMD (70 vs. 62%) and a reduction ( $P < 0.05$ ) in CPD (32 vs. 38%) and STD (82 vs. 74%). The diet containing 30% roughage led to higher ( $P < 0.05$ ) DMD (67 vs. 65%) and CPD (35 vs. 33%) in comparison with the 70% roughage diet, but did not differ ( $P > 0.05$ ) in STD (78%). Inclusion of glycerin at 20% of the total DM increased the utilisation of dietary DM without affecting the fibrous fraction of the diet. Glycerin supplementation in diets containing 70% roughage improves neutral detergent fibre digestibility.

**Key words:** INDF. Glycerol. Ingredients. *In vitro*. *In vivo*. Total ration.

#### Resumo

O objetivo deste estudo foi avaliar o desaparecimento *in vitro* de nutrientes e a digestibilidade *in vivo* de alimentos para bovinos contendo 70 ou 30% de forragem, com e sem suplementação de glicerina. Foram utilizados quatro bovinos Nelore num delineamento quadrado latino 4 × 4 em um esquema fatorial 2 × 2. A inclusão de glicerina e forragem não afetou o desaparecimento de nutrientes dos ingredientes ( $P > 0,05$ ). Independentemente da inclusão de glicerina, uma redução na proporção de forragem provocou um aumento ( $P < 0,05$ ) na DesMS (64 vs. 72%), DesFDN (41 versus 54%) e DesFDA (31 vs. 44%) das rações totais. A inclusão de glicerina resultou em um aumento ( $P < 0,05$ ) na DMS (70 contra 62%) e uma redução ( $P < 0,05$ ) na DPB (32 vs. 38%) e DAM (82 versus 74%). A dieta contendo 30% de forragem proporcionou maior ( $P < 0,05$ ) DMS (67 vs. 65%) e DPB (35 versus 33%) em comparação à

<sup>1</sup> Drs. em Zootecnia, Universidade Estadual Paulista, FCAV, Jaboticabal, SP, Brasil. E-mail: achomemj@hotmail.com; jpaschoaloto@yahoo.com.br

<sup>2</sup> Profª, FCAV, Jaboticabal, SP, Brasil. E-mail: janembe\_fcav@yahoo.com.br

<sup>3</sup> Zootecnista, FCAV, Jaboticabal, SP, Brasil. E-mail: leonardocremasco@yahoo.com.br

<sup>4</sup> Discente, Curso de Doutorado do Programa de Pós-Graduação em Zootecnia, FCAV, Jaboticabal, SP, Brasil. E-mail: marco695@yahoo.com.br

<sup>5</sup> Prof., Universidade Estadual de Maringá, UEM, Maringá, PR, Brasil. E-mail: hlperez@hotmail.com

\* Author for correspondence

dieta com 70% de forragem, mas não diferiu ( $P > 0,05$ ) na DAM (78%). A inclusão de 20% de glicerina aumentou a utilização de MS sem afetar a da fração fibrosa da dieta. A suplementação de glicerina em dietas contendo 70% de forragem melhora a digestibilidade de fibra de detergente neutro.

**Palavras-chave:** FDNi. Glicerol. Ingredientes. *In vitro*. *In vivo*. Ração total.

## Introduction

Currently, the use of low-roughage diets for feedlot beef cattle has increased. According to the literature, a reduction in the proportion of dietary roughage improves nutrient digestibility (VÉRAS et al., 2000; RESENDE et al., 2001; ÍTAVO et al., 2002; SILVA et al., 2005; PEREIRA et al., 2007). Many alternative ingredients are available on the market for formulating different animal feeds. Soybean hulls are a roughage ingredient and crude glycerin a concentrate; both are high-energy ingredients and are increasingly used in ruminant feed, minimising the use of corn grain.

Glycerin supplementation can bring benefits to rumen health, as it minimises the sharp drop in pH that frequently occurs when a high concentration of corn is used in diets. The inclusion of glycerol at a concentration higher than 5% of dietary dry matter (DM) may inhibit the *in vitro* growth of cellulolytic bacteria (ROGER et al., 1992), but studies reported that the inclusion of glycerol at 15% of dietary DM did not affect *in vivo* neutral detergent fibre digestibility (DONKIN, 2008; AVILA-STAGNO, 2013).

Some methods used for the evaluation of feed for ruminants, such as the total collection of faeces, involve greater operational difficulties when compared to *in vitro* or *in vivo* methods that use internal indicators. *In vitro* and *in vivo* methods that use indicators have the advantage of allowing separate analysis of the performance of total rations and ration ingredients.

The present study was conducted to evaluate the *in vitro* nutrient disappearance and *in vivo* nutrient digestibility of cattle diets containing 30 or 70% of roughage, with and without the inclusion of glycerin.

## Material and Methods

Four ruminally cannulated Nellore cattle, weighing approximately 450 kg each and housed individually in 12 m<sup>2</sup> semi-covered stalls, were used in this study. The animals received one of four diets, two containing 70% roughage and two containing 30% roughage, with and without the inclusion of glycerin (Table 1).

**Table 1.** Ingredient percentage and nutritional composition of total rations (%DM).

Ingredient	Diet <sup>1</sup>			
	70RG	70R	30RG	30R
Corn silage	70.0	70.0	30.0	30.0
Sunflower meal	5.5	5.5	5.5	5.5
Soybean hulls	2.75	2.75	21.7	21.3
Corn grain	-	20.85	21	42
Urea	1.0	0.35	0.75	0.15
Crude glycerin	20.0	-	20.0	-
Calcitic limestone	0.4	0.3	0.45	0.45
Mineral mix <sup>2</sup>	0.35	0.25	0.6	0.6

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<i>Nutritional Composition</i>				
Crude protein	12.0	12.7	12.0	12.3
Neutral detergent fiber	31.2	46.9	43.9	47.0
Acid detergent fiber	19.8	27.4	23.2	26.2
Starch	23.0	41.6	28.9	42.3
Ether extract	1.1	3.7	1.7	4.1
Metabolizable energy (Mcal kg <sup>-1</sup> )	2.6	2.6	2.7	2.7

<sup>1</sup>70RG, 70% roughage with glycerin; 70R, 70% roughage without glycerin; 30RG, 30% roughage with glycerin; 30R, 30% roughage without glycerin. <sup>2</sup>Mineral supplement for bovine diets - guaranteed levels per 1,000 g of product: phosphorus 40 g, calcium 80 g, sodium 195 g, chlorine 300 g, magnesium 5 g, sulfur 26 g, zinc 2,000 mg, copper 1,000 mg, manganese 500 mg, cobalt 100 mg, iodine 100 mg, selenium 5 mg, fluorine (max.) 400 mg, excipient q.s.p. 1,000 g.

Meals were given twice a day at a 12-h interval, and the daily feeding rations were adjusted to 2% of the body weight. Glycerin was weighed separately and added to the concentrate and silage at the time of feeding.

The *in vitro* disappearance of DM, crude protein (CP), and neutral detergent fibre (NDF) from the total rations and ration ingredients was assessed using the DAISY<sup>II</sup> fermenter (ANKOM<sup>®</sup>). Samples were ground to 1.0 mm, weighed (0.5 g), and placed into F57 filter bags (ANKOM<sup>®</sup>). The bags were incubated in digestion jars containing 1330 mL of buffer A (containing 10.0 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 0.5 g L<sup>-1</sup> MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g L<sup>-1</sup> NaCl, 0.1 g L<sup>-1</sup> CaCl<sub>2</sub>·2H<sub>2</sub>O and 0.5 g L<sup>-1</sup> urea), 266 mL of buffer B (containing 15.0 g L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> and 1.0 g L<sup>-1</sup> Na<sub>2</sub>S·9H<sub>2</sub>O), and 400 mL of rumen fluid. CO<sub>2</sub> was added to the jars at the time of incubation to promote anaerobic conditions. Jars were kept in the fermenter under constant rotation for 48 h at 39 °C.

Samples and residues from the incubation were analysed to determine DM at 105 °C, CP (AOAC, 1995), starch (HENDRIX, 1993), and NDF content (VAN SOEST; WINE, 1967). Nutrient (DM, CP, NDF, and acid detergent fibre; ADF) disappearance was calculated according to the equation  $Dis = (MI - MR)/MI \times 100$ , where Dis is the disappearance, MI is the mass of nutrient incubated, and MR is the mass of nutrient in the residue.

*In vivo* digestibility was estimated using the indigestible neutral detergent fibre (INDF) as an internal indicator (CASALI et al., 2008). After the 10-day diet adaptation period, faeces samples were collected every 8 h for 3 days and composited for later analysis. Samples were pre-dried (55 °C) and ground to 2 mm. Then, samples were weighed (5 g), placed in nylon bags, and incubated in the rumen of each animal for 12 days, after which the bags were washed, dried, and weighed.

NDF analysis was performed using the undegraded residue to determine the INDF. The percentage of INDF in each sample was calculated using the equation: %iNDF = incubated sample mass (g of DM) × %NDF of the residue/mass of the residue (g of DM). Digestibility was calculated using the following equations: DMD (%) = 1 - (%INDF feed/%INDF faeces) × 100; DNutrient (%) = 1 - [(%INDF feed/%INDF faeces) × (%Nutrient in faeces/%Nutrient in feed)] × 100.

A 4 × 4 Latin Square design was used in a 2 × 2 factorial arrangement (two roughage proportions × glycerin inclusion). Each experimental period consisted of 10 days for diet adaptation and 5 days for sample collection. SAS 9.0 PROC GLM was used for data analysis.

## Results and Discussion

The inclusion of glycerin and the proportion of roughage did not influence ( $P > 0.05$ ) nutrient disappearance of ration ingredients; therefore, only mean values and standard deviations are presented in Table 2. The ingredient with the greatest ( $P < 0.05$ ) DM disappearance (DMDis) was corn grain, followed by soybean hulls, sunflower meal, and corn silage. Sunflower meal and corn silage did not differ from each other ( $P > 0.05$ ). Sunflower meal showed

higher ( $P < 0.05$ ) CP disappearance (CPDis) than the other ingredients, which showed no difference between each other ( $P > 0.05$ ). NDF digestibility (NDFD) of soybean hulls was higher ( $P < 0.05$ ) than that of other ingredients. Corn silage had the second highest ( $P < 0.05$ ) NDFD, followed by corn grain and sunflower meal, which did not differ ( $P > 0.05$ ) from each other (Table 2). These results clearly show the superior quality of soybean hulls and corn silage in comparison to the other ingredients.

**Table 2.** *In vitro* dry matter (DMDis), crude protein (CPDis), and neutral detergent fiber (NDFDis) disappearance of each ration ingredient.

Variable, %	Ingredient				P	SE
	Corn grain	Soybean hulls	Sunflower meal	Corn silage		
DMDis	81.4 ± 2.7 a	71.6 ± 4.7 b	54.1 ± 2.1 c	56.7 ± 3.3 c	***	3.0
CPDis	73.4 ± 10.9 b	67.8 ± 4.7 b	88.7 ± 2.0 b	72.6 ± 11.5 b	***	9.4
NDFDis	33.9 ± 8.4 c	73.1 ± 4.9 a	35.6 ± 5.2 c	61.9 ± 8.0 b	***	5.7

Means followed by the same letter in a line do not differ significantly according to Tukey's test ( $P > 0.05$ ); P, probability; SE, standard error.

The results of nutrient disappearance of ration ingredients, after analysis of variance, revealed interaction ( $P < 0.05$ ) solely between the main factors; thus, only these results are presented. Regardless of the inclusion of glycerin, reduction in the proportion of roughage in the diet caused an increase ( $P < 0.05$ ) in DMDis and in NDF disappearance (NDFDis) of the total rations. Glycerin inclusion provided greater nutrient disappearance in the diet containing

30% roughage. In the case of the diets containing 70% roughage, glycerin supplementation increased ( $P < 0.05$ ) CPDis and NDFDis without affecting ( $P > 0.05$ ) DMDis and ADF disappearance (ADFDIs) (Table 3). The associative effect of the ingredients in the diets may alter their utilisation, independent of diet composition and the proportion of concentrate (MCDONALD et al., 1993).

**Table 3.** *In vitro* dry matter (DMDis), crude protein (CPDis), neutral detergent fiber (NDFDis), and acid detergent fiber (ADFDIs) disappearance of total rations.

Variable, %	Glycerin	%Roughage		Mean	SE	Effect		
		70R	30R			R	G	R×G
DMDis	Included	65.8 ± 3.6 Ab	75.0 ± 3.2 Aa	70.4 ± 5.7 A	3.2	***	***	*
	Not included	63.5 ± 4.3 Ab	68.6 ± 4.5 Ba	66.0 ± 5.0 B				
	Mean	64.6 ± 4.0 b	71.8 ± 5.0 a					
	Included	76.4 ± 2.4 Ab	82.7 ± 2.1 Aa	79.6 ± 3.9 A				

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CPDis	Not included	72.9 ± 3.3 Ba	73.5 ± 4.1 Ba	73.2 ± 3.7 B	2.7	***	***	***
	Mean	74.7 ± 3.4 b	78.1 ± 5.6 a					
	Included	43.5 ± 7.1 Ab	58.2 ± 6.6 Aa	50.9 ± 10.1 A				
NDFDis	Not included	39.4 ± 5.7 Bb	49.6 ± 8.7 Ba	44.5 ± 8.9 B	5.0	***	***	ns
	Mean	41.4 ± 6.7 b	53.9 ± 8.8 a					
	Included	32.8 ± 7.0 Ab	47.7 ± 6.2 Aa	40.3 ± 9.9 A				
ADFDIs	Not included	30.2 ± 4.0 Ab	40.1 ± 7.5 Ba	35.1 ± 7.8 B	5.6	***	***	ns
	Mean	31.5 ± 5.7 b	43.9 ± 7.8 a					

Within rows, means followed by the same lowercase letter do not differ significantly according to Tukey's test ( $P > 0.05$ ); within columns, means followed by the same uppercase letter do not differ significantly according to Tukey's test ( $P > 0.05$ ); SE, standard error; R, roughage; G, glycerin; RxG, interaction.

In the literature, there are conflicting results for DMDIs from glycerin-containing feeds. Avila-Stagno et al. (2011) reported a linear increase in DMDIs when barley grain was substituted by glycerol at increasing concentrations (7 to 14 to 21% of total DM). Corroborating the results of the present study, Hampy et al. (2007) reported a linear increase in DM digestibility (DMD) of forage by increasing levels of glycerol from 5 to 10 to 20% of total DM. However, Ramos and Kerley (2012) demonstrated a linear reduction in DMD of total rations with a high proportion of roughage.

In this study, 20% crude glycerin was used which contains 83% glycerol, corresponding to an inclusion of glycerol at approximately 16% of total DM. Glycerin did not affect the disappearance of the nutritional components of the total ration, especially that of fibre. This finding is in contrast to the results of a study by Roger et al. (1992), in which the inclusion of 5% glycerol drastically reduced growth and cellulolytic activity of two bacterial species. In the present study, glycerol at 16% of total DM had positive effects on the disappearance

of total ration fibre, contrary to a report that 7.2 or 10.8% of glycerol reduced fibre digestibility (ABO EL-NOR et al., 2010). The benefits of glycerin supplementation may be related to its high digestibility; 80% digestibility was observed after 24 h of *in vitro* incubation (TRABUE et al., 2007).

No interaction ( $P > 0.05$ ) between factors was observed for DMD, CP digestibility (CPD), and starch digestibility (STD); therefore, only the main factors are shown in Table 4. Inclusion of glycerin increased ( $P < 0.05$ ) DMD, but caused a reduction ( $P < 0.05$ ) in CPD and STD. According to Wang et al. (2009), the inclusion of 1.1 or 2.2% of glycerol in diets of cattle promoted an increase in nutrient digestibility, whereas glycerol at 3.3% of DM resulted in a reduction. According to Farias et al. (2012), the inclusion of approximately 3, 6, or 9% of glycerin did not affect nutrient digestibility of diets fed to heifers supplemented on pasture. In a study by Borges et al. (2013), no changes in nutrient digestibility were observed with the inclusion of 5, 10, or 15% of crude glycerin in the diet of meat goats fed 45% of Tifton 85 bermudagrass.

**Table 4.** Apparent digestibility of dry matter (DMD), crude protein (CPD), and starch (STD) of the diets.

Variable, %	%Roughage		Glycerin		SE	Effect		
	70R	30R	Included	Not included		R	G	R×G
DMD	64.7 ± 5.3	67.4 ± 7.3	70.3 ± 4.9	61.8 ± 4.8	2.82	**	***	ns
CPD	33.2 ± 9.2	37.3 ± 7.5	32.2 ± 6.7	38.3 ± 9.2	6.5	*	***	ns
STD	75.1 ± 14.9	81.1 ± 4.6	74.4 ± 3.9	81.9 ± 17.3	12.2	ns	*	ns

SE, standard error; R, roughage; G, glycerin; RxG, interaction.

The diet containing 30% roughage led to higher ( $P < 0.05$ ) DMD and CPD but did not differ ( $P > 0.05$ ) in STD in comparison with the diet containing 70% roughage (Table 4). The higher utilisation of the 30% roughage diet may be due to differences in the DMDs of the ingredients, as corn silage had lower utilisation than soybean hulls and corn grain (Table 2). This study corroborates published data, showing that a reduction in dietary roughage promotes an increase in the digestibility of dry matter and crude protein (VERÁS et al., 2000;

RESENDE et al., 2001; PEREIRA et al., 2007). However, some authors reported an increase in DMD but no increase in CPD (ÍTAVO et al., 2002; SILVA et al., 2005).

Data on the interaction between the main factors of NDFD are presented in Table 5. Glycerin supplementation improved ( $P < 0.05$ ) NDFD of the 70% roughage diet, whereas for the 30% roughage diet, it had no effect ( $P < 0.05$ ). These results suggest that glycerin improves the utilisation of the fibrous fraction of high-roughage diets.

**Table 5.** Effect of the interaction of roughage and glycerin on neutral detergent fiber digestibility (NDFD) of the diets.

Variable, %	Glycerin	%Roughage		Mean	SE	Effect		
		70R	30R			R	G	R×G
	Included	73.1 Aa	57.4 Ab	65.2 A				
NDFD	Not included	56.9 Ba	56.3 Aa	56.6 B	8.9	**	**	**
	Mean	65.0 a	56.9 b					

Within rows, means followed by the same lowercase letter do not differ significantly according to Tukey's test ( $P > 0.05$ ); within columns, means followed by the same uppercase letter do not differ significantly according to Tukey's test ( $P > 0.05$ ); SE, standard error; R, roughage; G, glycerin; RxG, interaction.

A reduction in NDFD was observed when the proportion of roughage in diets containing glycerin was reduced from 70 to 30%, but this effect was not observed for diets with no glycerin supplementation ( $P > 0.05$ ; Table 5). This effect may be due to the absence of corn in the diet containing glycerin and 70% roughage, as the pH of the ruminal environment is probably higher and thus favours the performance of cellulolytic microorganisms responsible for cellulose degradation (DIJKSTRA et al., 2012).

## Conclusions

Inclusion of glycerin at 20% of the total DM increased the utilisation of dietary DM without affecting the utilisation of the fibrous fraction of the diet. Glycerin supplementation in diets containing 70% roughage improves neutral detergent fibre digestibility.

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## Ethics and Biosafety Committee

This work was conducted in accordance with the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) and approved by the Ethics Committee on Animal Use (CEUA) of the São Paulo State University (protocol no. 028066/12).

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