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The crude glycerin reduces losses fermentative and improves the nutritional value of marandu grass silage in a semiarid region

A glicerina bruta reduz perdas fermentativas e melhora o valor nutricional da silagem de capim marandu em uma região semiárida

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ABSTRACT
The ensiling of marandu grass at the recommended time of management results in low dry matter (DM) content and nutritional value, but the addition of crude glycerin can compensate for these deficits if used during ensiling. Thus, the aim of this study was to evaluate the best level of inclusion of crude glycerin that can improve fermentation and the nutritional value of silages prepared with *Urochloa brizantha* cv. Marandu. The treatments consisted of five levels of inclusion of crude glycerin (0, 7.5, 15, 22.5, 30% of fresh forage) during ensiling of marandu grass with eight replications following the completely randomized design. For the evaluation of ruminal kinetics, four crossbred steers were used, cannulated in the rumen, following a randomized block design in a split plot scheme. For the percentage unit of inclusion of glycerin, there was a linear reduction of 0.34% in gas losses and increase of 0.45% and 0.55% in the recovery of DM (P <0.01) and in the DM content (P <0.01), respectively. The inclusion of up to 22.5% of crude glycerin in the silage of marandu grass is recommended to reduce losses during fermentation and improve the recovery of dry matter and the nutritional value of silage.

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RESUMO

A ensilagem do capim-marandu no momento recomendado para o manejo resulta em baixo teor de matéria seca (MS) e valor nutricional, mas a adição de glicerina bruta pode compensar esses déficits se utilizada durante a ensilagem. Assim, o objetivo deste estudo foi avaliar o melhor nível de inclusão de glicerina bruta que pode melhorar a fermentação e o valor nutricional das silagens preparadas com Urochloa brizantha cv. Marandu. Os tratamentos consistiram de cinco níveis de inclusão de glicerina bruta (0, 7,5, 15, 22,5, 30% de forragem fresca) durante a ensilagem do capim-marandu com oito repetições, seguindo o delineamento inteiramente casualizado. Para a avaliação da cinética ruminal, foram utilizados quatro novilhos mestiços, canulados no rúmen, seguindo um delineamento de blocos casualizados em esquema de parcelas subdivididas. Para a unidade percentual de inclusão de glicerina, houve uma redução linear de 0,34% nas perdas de gases e um aumento de 0,45% e 0,55% na recuperação do DM (P <0,01) e no teor de MS (P <0,01), respectivamente. . Recomenda-se a inclusão de até 22,5% de glicerina bruta na silagem do capim-marandu para reduzir as perdas durante a fermentação e melhorar a recuperação da matéria seca e o valor nutricional da silagem.

Palavras chave: biodiesel; *Urochloa brizantha*; conservação de forragem; glicerol; ruminantes

1 INTRODUCTION

In Brazil, the production of ruminants is dependent on native or cultivated forage plants as the main source of nutrients due to the low food cost in relation to intensive production systems (MONÇÃO et al., 2019). In the country, it is estimated that 167 million hectares are cultivated with tropical and subtropical forages for animal feed, 80% of which belong to the Urochloa genus (FERRAZ and FELÍCIO, 2010). However, due to the effects of forage seasonality caused by climatic variations, animal production can be compromised throughout the year, especially in the semi-arid region whose drought duration is longer compared to other regions of Central Brazil. In the semi-arid region of Northern Minas Gerais, forage production is limited by low soil moisture, since temperature and solar radiation have little fluctuation during the winter period (ALVALÁ et al., 2019; MONÇÃO et al., 2020). Tropical grasses belonging to the *Urochloa* genus, also called *Brachiaria*, present, when well managed, green mass productivity between 100-120 t/hectare per year, 85-100% being produced in the rainy season (summer season), generating excess mass because animals cannot consume all the forage produced. Therefore, conserving, in the form of silage, the excess forage produced during the summer climatic season is essential to maintain or increase the production of ruminants throughout the year. Currently, there are machines on the Brazilian market that are efficient in harvesting grasses, which has favored the production of low-cost silage. However, at the time of cutting management (40 cm) of *Brachiaria brizantha*, the DM below 250 g/kg DM, low content of soluble carbohydrates and high buffering power are limiting factors for ensiling, being important insert additives in order to adjust the fermentative capacity of the dough (RIGUEIRA et al., 2018; KUNG JR et al., 2018; MUCK et al., 2018). Among the additives with the potential to be used as a moisture scavenger in

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grass silage stands out the crude glycerin, which is a by-product obtained from oil processing in the biodiesel industry and contains 900 g DM, 108 g/kg DM of crude fat , 800–880 g glycerol/kg DM (ORRICO JR et al., 2017; RIGUEIRA et al., 2017; RIGUEIRA et al., 2018).

According to the National Petroleum Agency (ANP, 2019), in 2018 440,600 m^3 of glycerin were generated as a by-product of biodiesel production (B100), 17.6% more than in 2017. According to the ANP, the largest generation of glycerin occurred in the South Region (40.7% of the total), followed by the Midwest (39.7%), Southeast (9%), Northeast (7.7%) and North (2.9%). The excess of raw glycerin in the biodiesel industries can pose an environmental risk if handled incorrectly due to its pollutant content (considerable levels of glycerol and residual lipids). Thus, the alternative use of this by-product as an additive during grass ensiling has the potential to improve the fermentation of the ensiled mass, as it contains glycerol in its composition. In addition, glycerol is a rich source of energy for anaerobic microorganisms (Carvalho et al., 2017), which can favor microbial growth and improve the quality of fermentation and forage conservation. Several researches were conducted using crude glycerin in the silage of tropical grasses (ORRICO JR et al., 2017; RIGUEIRA et al., 2017; RIGUEIRA et al., 2018). However, the inclusion of crude glycerin has not been tested in the ensilage of *Urochloa brizantha* cv. Marandu. Due to the variations in the management and nutritional value of forages, it is necessary to know the best level of inclusion of glycerin during silage of marandu grass.

Based on the above, the aim of this study was to evaluate the best level of inclusion of crude glycerin that can improve fermentation and the nutritional value of silages prepared with *Urochloa brizantha* cv Marandu.

2 MATERIAL AND METHODS

2.1 ANIMAL CARE

The study was approved by the Ethics and Welfare Committee of the Montes Claros State University (Protocol No. 167/2018).

2.2 SITE

The experiment was carried out at the Experimental Farm of the State University of Montes Claros, Campus Janaúba, MG, Brazil (15º 52 '38" South and 43º 20 '05" West). The average annual precipitation is 800 mm with an average annual temperature of 28 ° C, relative humidity around 65% and, according to the climatic classification of Koppen and Geiger (1948), the predominant climate type in the region is Aw.

2.3 TREATMENTS AND PROCESSING

The treatments consisted of the inclusion of crude glycerin during ensiling of marandu grass in five levels (0, 7.5, 15, 22.5, 30% of fresh forage) with eight replications. The levels were defined according to Rigueira et al., (2018). The forage was collected in a pre-installed area at the Unimontes Experimental Farm after 60 days of uniform cutting. The grass was cut manually and then crushed in a shredder-chopper (JF, Model Z - 6; Itapira, SP, Brazil) coupled to the TL75, 4x4 tractor (New Holland, Curitiba, PR, Brazil). The machine's knives were adjusted to crush the forage and obtain a particle size of 2 cm.

Five heaps were made with the chopped forage, the additive was added in the respective proportions and homogenized before ensiling. For silage, experimental polivinil chloride (PVC) silos, of known weights, 50 cm long and 10 cm in diameter were used. At the bottom of the silos, they contained 10 cm of dry sand (300g), separated from the forage by a foam to quantify the effluent produced. After the complete homogenization of the forage with the additives, it was deposited in the silos and compacted with the aid of a wooden plunger. For each treatment, the silage density was quantified and approximately 3 kg of the chopped material from each fresh forage was ensiled. After filling, the silos were closed with PVC caps with a "Bunsen" type valve, sealed with adhesive tape and weighed afterwards. The silos were stored in a covered place, kept at room temperature and opened 60 days after ensiling according Orrico Junior et al. (2017). After opening, samples were collected in the middle of each silo.

2.4 EVALUATIONS

2.4.1 Ph And Dry Matter Losses During Fermentation

To determine the pH of the silages, a peameter (digital; model MA522, Marconi Laboratory Equipment, Piracicaba, SP, Brazil) was used according to the methodology described by Silva and Queiroz (2006). Losses of dry matter in silages in the form of gases and effluents were quantified by weight difference. The loss of dry matter in the form of gases was calculated by the difference between the gross weight of the initial and final ensiled dry matter, in relation to the amount of ensiled dry matter, discounting the weight of the silo and dry sand set. The dry matter recovery was calculated by the difference between the initial and final dry matter content of the silage. All formulas can be found in the methodology described by Jobim et al. (2007).

2.5 CHEMICAL COMPOSITION

The marandu grass {*Urochloa brizantha* (Hoschst.Ex. A. Rich) R. D. Webster cv. Marandu Syn. *Brachiaria brizantha* (Hochst. Ex A. Rich) Stapf cv. Marandu}, *in natura*, crude glycerin and

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silage samples were analyzed for dry matter content (DM; 934.05), ash (942.05), ether extract (EE; 920.39), crude protein (PB; 978.04), as described by AOAC (1995). The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the sequential method, according to procedures described by Robertson and Van Soest (1991) using the TECNAL® TE-149 fiber determiner (Piracicaba, SP, Brazil) using alpha amylase. Cellulose was solubilized in 72% sulfuric acid and lignin content was obtained as the difference (GOERING; VAN SOEST, 1970). The content of total carbohydrates (TC) was estimated by the equation: TC (%) = 100 [CP (%) + EE (%) + ash (%)] and those of non-fibrous carbohydrates (NCF) according to Sniffen et al. (1992). The total digestible nutrients (TDN) were estimated using the formula according to the NRC (2001). The chemical-bromatological composition of crude glycerin and marandu grass before of ensiling can be seen in Table 1.

Table 1. Chemical composition of crude glycerin and marandu grass (*Urochloa brizantha*)

Item (g/kg)	Crude Glycerin	Marandu grass [1]
Total Glycerol	869.0	-
Dry matther	895.0	239.1
Methanol	<1.0	-
pH	5.3	-
Moisture	92.0	-
Crude protein	4.0	122.6
Ether extract	100.8	18.2
Neutral detergent fiber	-	643.3
Acid detergent fiber	-	349.5
Lignin		42.5
Ash	32	119
Sodium	13	-
Chlorine	19	-
Potassium	<1	-

[1] 60 days old regrowth and 40 cm height

2.5.1 Ruminal kinetics

For the evaluation of ruminal degradation kinetics, four crossbred steers, cannulated in the rumen, with an average weight of 530 ± 50 kg were used. The animals received 3.0 kg of concentrate mixed with 200 mL of crude glycerin divided in two equal, morning and afternoon. In addition to the concentrate, the animals received roughage, in the same proportion, based on marandu grass and elephant grass silage (*Pennisetum purpureum* Schum.) For 14 days before the experiment. The in situ degradability technique was used using non-woven synthetic fiber type bags (NWS, weight 100), with porosity of 50 μm according to Casali et al. (2009), with a quantity of samples following a ratio of 20 mg of cm cm-² of bag surface area (NOCEK, 1988). The bags were placed in filo bags, along

with 100 grams of lead. The bags were tied with nylon thread, leaving a free length of 1 m so that they could move freely in the solid and liquid phases of the rumen. The bags were deposited in the ventral sac region of the rumen for 0, 3, 6, 12, 24, 48, 72, 96, 120 and 144 hours, with the end of the nylon thread tied to the cannula. The bags are placed in reverse order, starting with 144 hours. The samples referring to time zero were washed in running water (20ºC) together with the other samples. Subsequently, the samples were placed in a forced ventilation oven, at 55°C until reaching constant weight.

The remaining residues in the NWS bags, collected in the rumen, were analyzed for the levels of DM, NDF and ADF. The data obtained were adjusted for non-linear regression by the Gauss-Newton method (NETER et al., 1985), using the SAS software (SAS Institute Inc., Cary, NC), according to the equation proposed by Ørskov and McDonald (1979): $Y = a + b (1-e^{-ct})$, where: Y = accumulated degradation of the analyzed nutritional component, after time t; a = intercept of degradation curve when t = 0, which corresponds to the water-soluble fraction of the analyzed nutritional component; b = potential for degradation of the water-insoluble fraction of the analyzed nutritional component; a + b = potential degradation of the nutritional component analyzed when time is not a limiting factor; c = rate of degradation by fermentative action of b; t = incubation time.

Once calculated, the coefficients a, b and c were applied to the equation proposed by Ørskov and Mcdonald (1979): $ED = a + (b \times c / c + k)$, where: ED = effective ruminal degradation of the analyzed nutritional component; k = rate of passage of the food. Rumen passage rates estimated at 2, 5 and 8% h^{-1} were assumed, as suggested by the AFRC (1993).

The degradability of the NDF and ADF were estimated using the model Mertens and Loften (1980): $Rt = B \times e^{-ct} + I$ where R = the fraction degraded at time t; B = potentially degradable insoluble fraction; and I_p = the indigestible fraction. After adjusting the NDF and ADF degradation equation, the fractions were standardized as proposed by Waldo et al. (1972) using the equations: $B_p = B / (B + I) \times 100$ and $Ip = I / (B + I) \times 100$, where B_P = the potentially degradable fraction pattern (%); I_p = the standardized indigestible fraction (%); B = the potentially degradable insoluble fraction; and I = the indigestible fraction. The effective degradability of NDF and ADF was calculated using the ED $= B_p \times c / (c + k)$ model, where B_p is the standardized potential of the degradable fraction (%).

2.5.2 Experimental Designs And Statistical Analyzes

For evaluations of pH, losses by gases and effluents, recovery of dry matter and chemical composition, a completely randomized design was used with five levels of inclusion of crude glycerin with eight replications (experimental unit). The variables were analyzed according to the mathematical model:

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$Y_{ij} = \mu + Trat\ i + e_{ij}$

On what:

Y_{ijk} = The observation regarding the level of glycerin "i", in repetition "j";

μ = constant associated with all observations;

Trat i = Effect of glycerin level "i", with i = 1, 2, 3, 4 and 5;

e_{ij} = experimental error associated with all observations (Y_{ij}) independent which by definition has a normal distribution with zero mean and variance δ_2.

The ruminal degradability test was carried out in a randomized block design in a split plot scheme, with the treatments being the plots and the incubation times the subplots. The variation in cattle weight was the blocking factor. The following statistical model was used:

$Y_{ijk} = \mu + T_i + B_j + e_{ij} + P_k + T_i\ x\ P_{ik} + e_{ijk}$

On what:

$Y_{k(ij)}$ = The observation regarding the time (P) in subparcel k of treatment (T) i in block j;

μ = constant associated with all observations;

T_i = Effect of treatment "i", with i = 1, 2, 3, 4 and 5;

B_j = Block effect j, with j = 1, 2, 3 and 4;

e_{ij} = experimental error associated with plots that hypothetically have normal distribution with zero mean and variance δ_2;

P = Effect of k incubation time, with k = 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10;

TP_{ik} = Effect of the interaction of level i of treatment with level k of incubation time;

e_{ijk} = experimental error associated with all observations that, by hypothesis, have normal distribution with zero mean and variance δ_2.

The collected data were subjected to analysis of variance and, when the "F" 'test was significant, the inclusion levels of crude glycerin were analyzed using orthogonal polynomials, where linear and quadratic regression models were tested. For all statistical procedures, $\alpha = 0.05$ was adopted as the maximum tolerable limit for type I error.

3 RESULTS

The inclusion of crude glycerin in the silage of marandu grass modified the pH values (P <0.01) of the silage, and it was verified that the means adjusted to the quadratic regression model with a minimum point at the level of 25%. Gas losses (P = 0.04) decreased from 17.6% in the control silage (without glycerin) to 4.10% at the level of 30%, and for the percentage unit of inclusion of glycerin, there was a linear reduction of 0.34 % in gas losses (Table 2).

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Table 2. Chemical composition of marandu grass silages with inclusion of crude glycerin

| Item (%) | Inclusion of crude glycerin (% of fresh forage) | | | | | SEM | P-value | |
	0.0	7.5	15.0	22.5	30.0		Linear	Quadratic
Dry matter	23.96	26.10	32.18	36.73	39.63	0.38	<0.01	0.99
Ash	10.79	9.22	9.69	8.15	7.09	0.70	<0.01	0.71
Crude protein	5.34	4.78	3.78	2.90	2.63	0.12	<0.01	0.15
Ether extract	1.88	3.53	5.29	7.03	8.69	0.17	<0.01	1.00
Neutral detergent fiber	71.86	56.97	45.14	35.73	29.19	1.60	<0.01	0.01
Acid detergent fiber	48.81	35.62	29.11	23.16	18.47	1.50	<0.01	0.01
Hemicellulose	23.05	21.34	16.03	12.57	10.72	0.48	<0.01	0.40
Cellulose	40.76	34.50	25.60	20.66	16.87	0.54	<0.01	0.00
Lignin	8.05	5.02	3.51	2.50	1.60	0.20	<0.01	<0.01
Total carbohydrates	82.37	84.50	85.03	87.45	88.78	0.72	<0.01	0.91
Non-fibrous carbohydrates	10.51	27.53	39.88	51.72	59.59	1.71	<0.01	0.01
Total digestible nutrientes	42.39	54.09	63.12	71.85	78.78	0.92	<0.01	0.01

SEM – Standard error of the means; P – Probability

The inclusion of crude glycerin in the marandu grass silage reduced effluent losses (P = 0.01), with the lowest values (minimum point) verified at the level of 20.75% of glycerin inclusion. There was a linear increase in the recovery of silage matter with the inclusion of crude glycerin. For each 1% inclusion of glycerin, there was an increase of 0.45% in the recovery of dry matter.

The DM content (P <0.01) of the silage increased 39.54% with the inclusion of crude glycerin during silage, varying from 23.06% in the control silage to 39.63% at the maximum level of inclusion (0.55% increase for each percentage unit inclusion of crude glycerin) (Table 3).

Table 3. Regression equations for the variables of the chemical composition of marandu grass silages with the inclusion of crude glycerin

| Item (%) | Inclusion of crude glycerin (% of fresh forage) | | | | | R^2 | P-value |
	0.0	7.5	15.0	22.5	30.0		
Dry matter			$\hat{y}= 23.32 + 0.5596*X$			0.9803	<0.01
Ash			$\hat{y}= 10.68 - 0.1129*X$			0.8859	<0.01
Crude protein			$\hat{y}= 5.346 - 0.0973*X$			0.9735	<0.01
Ether extract			$\hat{y}= 1.86+0.2283*X$			0.9999	<0.01
Neutral detergent fiber			$\hat{y}= 69.09 - 1.4211*X$			0.9775	<0.01
Acid detergent fiber			$\hat{y}= 45.66 - 0.9752*X$			0.9543	<0.01
Hemicellulose			$\hat{y}= 23.42 - 0.4457*X$			0.9709	<0.01
Cellulose			$\hat{y}= 40.00 - 0.8216*X$			0.9785	<0.01
Lignin			$\hat{y}= 7.22 - 0.2056*X$			0.9288	<0.01
Total carbohydrates			$\hat{y}= 82.47 + 0.2103*X$			0.9753	<0.01
Non-fibrous carbohydrates			$\hat{y}= 13.37 + 1.6313*X$			0.9828	<0.01

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| Total digestible nutrientes | $\hat{y}= 43.93 + 1.2072*X$ | 0.9913 | <0.01 |

P – Probability; R² - Determination coefficient. X is the percentage of inclusion of crude glycerin; * significant by the t test

The ash content (P <0.01), crude protein (P <0.01), neutral detergent fiber (NDF; P <0.01), acid detergent fiber (ADF; P <0.01), hemicellulose (P <0.01), cellulose (P <0.01), lignin (P <0.01) of marandu grass silage reduced linearly with the inclusion of crude glycerin. There was an increase from 0.22% to 1% of inclusion of crude glycerin in silage of marandu grass on the levels of ether extract (EE; P <0.01). The levels of total carbohydrates (TC; P <0.01), non-fibrous carbohydrates (NFC; P <0.01) and total digestible nutrients (TDN; P <0.01) increased by 0.21%, 1.63% and 1.20% for each percentage unit of inclusion of crude glycerin.

The inclusion of crude glycerin in the marandu grass silage modified the readily soluble fraction (fraction a; P <0.01), potentially degradable insoluble fraction (fraction b; P <0.01), microbial colonization time (*lag time*; P = 0.02) and potential degradability (PD; P <0.01) of the dry matter of the produced silage. The means adjusted to the quadratic regression model, with the maximum point for the fraction "a", lag time and PD of 16.70%, 17.50% and 21.07%, respectively (Table 4). The rate of degradation of fraction b (c; P <0.01) of DM increased 0.0289%/hour for each 1% inclusion of crude glycerin.

Table 4. Ruminal degradability of dry matter of marandu grass silages with inclusion of crude glycerin

Item	Inclusion of crude glycerin (% of fresh forage)					SEM	P-value	
	0.0	7.5	15.0	22.5	30.0		Linear	Quadratic
Fraction a, %[1]	14.43	25.01	42.74	55.55	11.52	0.84	<0.01	<0.01
Fraction b, %[2]	58.85	49.52	41.47	30.08	68.20	0.89	0.80	<0.01
c, %/h[3]	1.44	2.20	2.12	2.05	2.60	<0.01	<0.01	<0.01
Colonization time, h [4]	4.68	6.36	13.59	24.96	4.94	1.10	0.02	<0.01
Potential degradability, %[5]	73.28	74.53	84.22	85.64	79.72	1.20	<0.01	<0.01
Effective degradability, 2%[6]	39.07	50.76	63.89	70.64	74.89	0.56	<0.01	<0.01
Effective degradability, 5%[7]	27.59	40.04	54.98	64.22	68.81	0.56	<0.01	<0.01
Effective degradability, 8%[8]	23.41	35.63	51.36	61.63	63.80	0.59	<0.01	<0.01
Undegradable fraction, %[9]	26.72	25.47	15.78	14.36	20.28	1.20	<0.01	<0.01

Regression equation: [1] $\hat{y}= 8.60 + 4.678*X -0.1449*X^2$, $R^2 = 0.6965$; [2] $\hat{y}= 62.85 – 3.4979*X + 0.1163*X^2$, $R^2 = 0.6816$; [3] $\hat{y}= 1.64 + 0.0289*X$, $R^2 = 0.6757$; [4] $\hat{y}= 1.47 + 1.7503*X – 0.0498*X^2$, $R^2 = 0.4893$; [5] $\hat{y}= 71.45 +1.18*X – 0.0287*X^2$, $R^2 = 0.7622$; [6] $\hat{y}= 41.54 + 1.2203*X$, $R^2 = 0.9590$; [7] $\hat{y}= 29.80 + 1.4216*X$, $R^2 = 0.9668$; [8] $\hat{y}= 25.81 + 1.4237 *X$, $R^2 = 0.9494$; [9] $\hat{y}= 28.55 – 1.1812*X + 0.0287*X^2$, $R^2 = 0.7622$. X is the percentage of inclusion of crude glycerin; * significant by t test.
SEM – Standard error of the means

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3.1 P – PROBABILITY

The effective degradability (ED; P <0.01) of DM of marandu grass silage at 2, 5 and 8%/hour passage rates increased linearly with the inclusion of crude glycerin during ensiling. The means observed for the indigestible fraction (UF; P <0.01) of DM were adjusted to the quadratic regression model with a minimum point at the level of 21.09% of inclusion of crude glycerin.

For the standardized insoluble fraction (B_p fraction; P <0.01) of the neutral detergent fiber (NDF) of the silage, the means adapted to the quadratic regression model (Table 5). The rate of degradation of the B_p fraction there was not modified with the inclusion of crude glycerin (mean 1.4%/hour).

Table 5. Rumen degradability of neutral detergent fiber from marandu grass silages with inclusion of crude glycerin

Item	Inclusion of crude glycerin (% of fresh forage)					SEM	P-value	
	0.0	7.5	15.0	22.5	30.0		Linear	Quadratic
Neutral detergent fiber								
Fraction Bp, %	75.2	78.36	80.95	88.06	53.1	2.05	<0.01	<0.01
c, %/h	1.00	1.75	1.75	1.25	1.00	0.1	0.42	0.42
Effective degradability, 2% h^{-1}	30.65	34.81	36.43	33.51	17.7	1.19	<0.01	<0.01
Effective degradability, 5% h^{-1}	16.23	19	20.1	17.47	8.85	0.87	<0.01	<0.01
Effective degradability, 8% h^{-1}	11.04	13.06	13.89	11.82	5.9	0.65	<0.01	<0.01
Undegradable fraction Ip, %	24.8	21.65	19.05	11.94	46.9	2.05	<0.01	<0.01
Acid detergent fiber								
Fraction Bp, %	61.18	61.08	64.68	63.16	45.83	4.09	0.04	0.02
c, %/h	1.32	1.50	1.90	1.47	2.00	<0.01	0.84	0.84
Effective degradability, 2% h^{-1}	23.02	25.84	30.73	26.37	22.91	0.63	<0.01	<0.01
Effective degradability, 5% h^{-1}	12.05	13.86	17.36	14.14	13.09	0.56	<0.01	<0.01
Effective degradability, 8% h^{-1}	8.17	9.47	12.11	9.67	9.16	0.45	<0.01	<0.01
Undegradable fraction Ip, %	38.81	38.91	35.31	36.83	54.16	4.09	0.04	0.02

SEM – Standard error of the means; P – Probability

The ED of NDF of the marandu grass silage was modified with the inclusion of crude glycerin, with a quadratic behavior of the means in the passage rates of 2, 5 and 8%/hour. The maximum point of the standardized undegradable fraction (I_p fraction; P <0.04) of the NDF was at the level of 12.61% inclusion of crude glycerin (Table 6). There was no difference in the rate of degradation (P = 0.84) of the fraction B_p of the acid detergent fiber (ADF) with the inclusion of crude glycerin (mean

1.63%/hour). The fraction B_p of the ADF showed the maximum value at the inclusion level of 11.20% crude glycerin. The means verified for the ED of the ADF adjusted to the quadratic regression model.

Table 6. Regression equations for the ruminal kinetics variables of the fibrous fraction of marandu grass silages with the inclusion of crude glycerin

Item	Inclusion of crude glycerin (% of fresh forage)					R²	P-value
	0.0	7.5	15.0	22.5	30.0		
Neutral detergent fiber							
Fraction Bp, %	$\hat{y}= 71.78 + 2.272*X – 0.0911*X^2$					0.6981	<0.01
c, %/h	$\hat{y}= 0.01$						<0.01
Effective degradability, 2% h^{-1}	$\hat{y}= 29.70 +1.33*X-0.0565*X^2$					0.9502	<0.01
Effective degradability, 5% h^{-1}	$\hat{y}=15.80 + 0.7927*X -0.033*X^2$					0.9762	<0.01
Effective degradability, 8% h^{-1}	$\hat{y}=10.76+0.5618*X-0.0238*X^2$					0.9817	<0.01
Undegradable fraction Ip, %	$\hat{y}= 28.21 – 2.2719*X+0.0911*X^2$					0.6979	<0.01
Acid detergent fiber							
Fraction Bp, %	$\hat{y}= 59.25 + 1.12*X - 0.0503*X^2$					0.8357	<0.01
c, %/h	$\hat{y}=0.012$						<0.01
Effective degradability, 2% h^{-1}	$\hat{y}=22.59 + 0.83*X – 0.027*X^2$					0.8349	<0.01
Effective degradability, 5% h^{-1}	$\hat{y}= 11.85 + 0.5054*X – 0.015*X^2$					0.7298	<0.01
Effective degradability, 8% h^{-1}	$\hat{y}=8.03 + 0.3605*X – 0.011*X^2$					0.6925	<0.01
Undegradable fraction Ip, %	$\hat{y}=40.73 – 1.12*X+0.0503*X^2$					0.8357	<0.01

P – Probability. R² - Determination coefficient. X is the percentage of inclusion of crude glycerin; * significant by the t test

4 DISCUSSION

The chemical-bromatological characteristics of marandu grass before silage (Table 1) are within the values observed in the literature for this forage (SILVA et al., 2018). The inclusion of crude glycerin in the marandu grass silage reduced the pH of the silage because the crude glycerin is acidic (pH = 5.3) and the lactic acid bacteria use the soluble carbohydrates present in the forage to produce lactic acid, mainly favoring the reduction of the pH of the ensiled mass (KUNG JR. et al., 2018). Crude glycerin is a by-product of the biodiesel industry and some variables such as pH and methanol concentration are not constant. Carvalho et al., (2017) evaluated the inclusion of crude glycerin in the sugarcane (*Saccharum officinarum* L.) ensilage and observed that the pH of the glycerin ranged from 6.5–7.5 and did not change the pH value of the silage. Orrico Jr et al. (2017)

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also did not find a reduction in the pH of the silage of piatã grass (*Brachiaria brizantha*). The acid character of the crude glycerin used in this research and its effects on pH values was a novelty.

In this research, it was found that the methanol content in glycerin was less than 1 g kg-1 of DM, indicating no possibility of animal poisoning. With 12% inclusion of fresh forage of sugar cane, Carvalho et al. (2017) found a concentration of 1.9 g kg^{-1} of DM and pointed out that it does not harm the health of animals. The use of 30% in the MN of crude glycerin in silage of marandu grass reduced 76.70% of the losses by gases and minimized the effluent losses in the level of 20.75%. This is justified due to the high DM content of the crude glycerin (895 g/kg) favoring the recovery of dry matter. Thus, the relevance of the additive crude glycerin in the most efficient fermentation during the ensiling of marandu grass is highlighted. Carvalho et al. (2017) observed that the crude glycerin used for silage is not consumed by microorganisms during fermentation and thereby reduces DM losses and improves the recovery of dry matter, which justifies the results obtained in this research. According to Orrico Jr et al. (2017), gas losses during fermentation are related to the type of fermentation during ensiling, which tends to decrease when homofermentative bacteria predominate. In this case, the bacterial metabolism of soluble carbohydrates leads to the production of lactic acid, which is important to decrease the pH of the silage, justifying the behavior of this variable in this research. According to Pasteris and Strasser Saad (2009), strains of *Lactobacillus* break down glycerol from crude glycerin and use it as an energy source for the synthesis of organic acids. Therefore, fermentation efficiency was better at higher levels of inclusion of crude glycerin due to the lower pH value (Orrico Jr. et al., 2017). However, the final pH of the silage is related to the fermentation quality, but it does not necessarily explain the speed at which the pH drops, neither the type of fermentation (populations of microorganisms), nor the nutritional value of the silage (Orrico Jr. et al., 2017). Even so, the lower gas losses and better dry matter recovery observed in this research are suggestive of a rapid decline in pH during fermentation as a result of the inclusion of crude glycerin.

The high DM content of glycerin increased the silage DM content from 23.96% (control silage) to 39.63% (silage with 30% inclusion). As the glycerin was possibly not consumed by the microorganisms inside the silo, there was a dilution effect on the contents of ash, crude protein and fibrous fraction. Although crude glycerin contains minerals and crude protein in its composition (Table 1), these concentrations were not sufficient to reduce the effects of the dilution. This dilution behavior was also verified in other studies with tropical forages (CARVALHO et al., 2017; ORRICO JR et al., 2017; RIGUEIRA et al., 2017; RIGUEIRA et al., 2018; SILVA et al., 2020). In contrast, at the maximum level of inclusion of crude glycerin, there was an increase of 78.36% in the crude fat content, justified by the concentration of lipids in this by-product (108 g/kg). This is important

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because it increased the TDN content of the silage by 46.19% compared to the control silage (42.39%). Marandu grass is one of the most cultivated forages in Brazil and the low TDN content for animal feed is one of the factors that most limit the silage of surplus production during the rainy season. Compared to corn or sorghum silages, the energy content of marandu grass silage can be considered low (CARVALHO et al., 2016), which can be compensated by offering higher proportions of concentrate to guarantee the weight gain of the animals. However, this can lead to considerable increases in production costs. Therefore, the inclusion of crude glycerin contributes to increase the energy levels of the silage produced due to the increase in the content of ether extract and nonfibrous carbohydrates. It is worth mentioning that energy additives (i.e., ground corn, molasses and pure glycerol), would probably allow superior results in terms of energy levels. However, the low cost of acquisition of the crude glycerin by-product seems to be the differential in decision making to make the inclusion of this additive in grass silage economically viable.

Regarding the ruminal kinetics of DM, the inclusion of 16.7% of crude glycerin in the silage of marandu grass maximized the fraction "a". The fraction "a" of DM includes carbohydrates from feeds that are readily soluble when in contact with water and available to microorganisms in the rumen, and when solubilized and fermented, it produces short-chain fatty acids, ammonia and microbial proteins, which are sources of energy and amino acids for ruminants (RIGUEIRA et al., 2018). The use of crude glycerin modified the degradation rate of fraction "b", maximizing the time of microbial colonization up to the level of 17.50%, but the PD of DM was greater when using a maximum of 21.07% of crude glycerin. The ED of DM was higher when it included 30% of crude glycerin in the silage of marandu grass. This increase in PD and ED is associated with the effects of glycerin on fractions "a" and "b" and the rate of degradation "c" of DM. Specifically, ED was higher at the level of 30% of inclusion due to the high rate of degradation "c" of DM (average of 2.6% / hour) in relation to the other levels of inclusion. During silage of Napier grass and BRS capiaçu grass (*Pennisetum purpureum* Schum.), Rigueira et al. (2018) and Silva et al. (2019) recommended 15% inclusion crude glycerin in fresh forage. Jenkins and Palmquist (1984), Firkins et al. (2007) and Abubakr et al. (2013) observed that in some studies, the addition of saturated lipids in the diet reduces the degradability of the fibrous fraction of foods, depending on the level of inclusion. In this research, the addition of crude glycerin by up to 20.74% increased the fraction B_p AFD of the silage compared to control silage. Levels above 12.62% of inclusion of crude glycerin reduced the fraction B_p of the NDF. At this level of inclusion, it is estimated that 85.93% of the fraction B_p of the NDF has potential for degradation in the rumen, being a valuable contribution of crude glycerin as a source of glycerol for cellulolytic bacteria, since there was an increase of 16.47% in fraction B_p compared to control silage (71.78%).

Regarding the use of silage containing crude glycerin to feed ruminants, there is still little information on intake, digestibility, ingestive behavior and animal performance. Most studies with crude glycerin for ruminants deal with the use of this direct by-product in the diet and not in silage. Therefore, a consistent contribution to livestock on animal performance is incipient. However, the results reported in this research are convincing of the potential for the inclusion of crude glycerin by reducing losses during fermentation and producing marandu grass silage with superior nutritional and nutritional value.

5 CONCLUSION

The inclusion of up to 22.5% of crude glycerin in the silage of marandu grass is recommended to reduce losses during fermentation and improve the recovery of dry matter and the nutritional value of silage.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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