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The Effect of Graded Levels of Crude Glycerin in BRS Capiaçu Grass Silage: Fermentation Profile and Bromatological Composition

Research Article

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ABSTRACT

The objective of this study was to evaluate the effect of crude glycerin inclusion levels on BRS Capiaçu grass (*Pennisetum purpureum*) silage on the parameters of fermentation and chemical-bromatological composition. Experimental treatments consisted of BRS Capiaçu grass silage with five levels of inclusion of crude glycerin (0, 1, 5, 10 and 15% inclusion in the natural matter) during ensiling. In this study, a completely randomized design was used along with five treatments and six replicates. The inclusion of crude glycerin in BRS capiaçu grass silage linearly reduced pH (P<0.01) and effluent losses (P<0.01). For each percentage unit of glycerin addition, there was a reduction of 0.013 pH units and 0.0485% of the effluent losses. There was no effect of crude glycerin on the ammoniacal nitrogen content (P=0.82, an average of 6.65% of the total nitrogen). The variation in the dry matter content between the highest glycerin inclusion dose (15% of natural matter) and the control group (without glycerin) was 30.5%. The crude protein content (P=0.01), ash (P<0.01), neutral detergent fiber (NDF; P<0.01), neutral detergent fiber corrected for ash and crude protein (NDFap; P<0.01) and acid detergent fiber (ADF; P<0.01) linearly reduced with the inclusion of crude glycerin compare to the control group. In the same order, there was a reduction of 0.115%, 0.08%, 1.95%, 1.84% and 1.06%, respectively, for each percentage unit of glycerin added. The addition of crude glycerin in elephant grass silage *vs*. BRS Capiaçu up to 15% of the natural material had improved the fermentation profile and the chemical-bromatological composition.

KEY WORDS dry matter, effluent loss, glycerol, *Pennisetum purpureum*, total digestible nutrients.

INTRODUCTION

The production of cattle in Brazil is based on the use of forage crops as the main source of nutrients. However, due to the seasonality of production of the plants, the availability of feed for animals is not constant, which modifies the supply of milk and meat in the market (Sampaio *et al.* 2017; Rigueira *et al.* 2018). One of the strategies to maintain the production is the conservation of forages by the technique of silage and / or fenation.

For ensiling purposes, elephant grass (*Pennisetum purpureum*) is one of the most studied forage plants in several parts of the world due to high mass production per unit area and animal acceptability (Santos *et al.* 2013; Negawo *et al.* 2017; Sirait, 2017; Rigueira *et al.* 2018). Among the cultivars of elephant grass, the BRS capiaçu was recently launched by Embrapa (Pereira *et al.* 2017). BRS capiaçu produces about 30% more mass in relation to the best cultivars of elephant grass (*Pennisetum purpureum vs.* Cameeron and Mineiro; 30 t/ha), make it a suitable forage for animal production.

However, BRS capiaçu is characterized by low dry matter (DM) content (<20% of the green mass; Pereira *et al.* 2017) and buffering power, essential factors in the adequacy of fermentation processes (Borreani *et al.* 2018; Ferraretto *et al.* 2018), which lead to several efforts for ensiling. Thus, the use of moisture sequestering additives is essential in adjusting the DM content (Muck *et al.* 2018).

The high content of DM is the fundamental factor in the characterization of the additive. The use of agroindustrial by-products such as crude glycerin presents a potential as silage additive due to the high content of DM (above 85%) and ethereal extract of 10%, which can increase the energy levels of the ensiled mass. In addition, the growing demand for biofuels in the world reinforces the large availability of crude glycerin with a tendency to reduce the acquisition value.

Rigueira *et al.* (2018) evaluated crude glycerin levels in Napier grass ensilage (*Pennisetum purpureum*) and recommended 15% inclusion in the natural matter. For cane sugar (*Saccharum officinarum*), Rigueira *et al.* (2017) recommended the inclusion of 15% in the natural matter in order to reduce losses during fermentation. In *Brachiaria brizantha vs.* Piatã, Orrico Júnior *et al.* (2017) verified that the crude glycerin can be used in the dose of 30% of the DM. Dias *et al.* (2014) studying sugarcane silage suggested the inclusion of 4% of crude glycerin in the natural matter to avoid losses and improve nutritional value. Consistently, the dose of glycerin addition can vary in relation to forages' DM variation. Therefore, it is essential to know the best inclusion dose of glycerin for ensiling BRS capiaçu grass on fermentation parameters and bromatological composition.

Based on the above, the objective of this study was to evaluate the effect of crude glycerin inclusion levels on BRS Capiaçu grass silage on the parameters of fermentation and chemical-bromatological composition.

MATERIALS AND METHODS

The experiment was conducted at the State University of Montes Claros - UNIMONTES, Campus Janaúba-MG, in the North of Minas Gerais. The geographical coordinates are Latitude: 15˚ 48 '09 "S, Longitude: 43˚ 18' 32" W and Altitude: 533 m 15˚ 47` 50``. The mean annual precipitation of the region is 700 mm with an average annual temperature of 28 ˚C, relative humidity of about 65%, with the predominant climate type Aw (Antunes, 1994).

Experimental treatments consisted of BRS Capiaçu grass silage with five levels of inclusion of crude glycerin (0, 1, 5, 10 and 15% inclusion in the natural matter) during ensiling.

We used a completely randomized design with five treatments and six replicates. The composition of the crude glycerin commercial and BRS Capiaçu grass, in natura, at 70 days of age, used in the experiment can be observed in Table 1.

The forage was collected in a pre-installed area at the UNIMONTES experimental farm. The cutting of BRS Capiaçu grass was performed manually on April 17, 2018 (70 days regrowth), and crushed in a chopper crushing machine coupled to an electric motor. The machine knives were set to grind the forage to a particle size of 1.5 cm. After grinding and homogenization of all the material, five mounds were formed and the additive (crude glycerin) added in the respective proportions of 0, 1, 5, 10 and 15% of natural matter.

For silage, experimental silos of polyvinyl chloride (PVC) were used, with 50 cm of length and 10 cm of diameter. In the bottom of the silos they contained 10 cm of dry sand, separated from the forage by foam to quantify the produced effluent. After complete homogenization of the forage with the additive, the material was deposited in the silos and compacted with the aid of a wooden plunger. After filling, the silos were closed with PVC caps containing "bunsen" valve. After the process of ensilage, the silos were sealed with tape properly, weighed and stored at room temperature. The silos were opened 90 days after silage. Samples were collected (replicates) in the middle of the silo after discarding the top of the silages. The samples were pre-dried in a forced ventilation oven with a temperature of 55 ˚C for 72 hours. After this period, the pre-dried silage was milled in a Willey-type mill in 1 mm diameter sieves for the chemical-bromatological analysis. Samples were collected to extract the juice using a hydraulic press for pH analysis using a digital potentiometer and ammoniacal nitrogen ($N-NH_3$), using distillation using magnesium oxide and calcium chloride, using a solution containing boric acid and titration with 0.1 N hydrochloric acid (Bolsen *et al.* 1992). The losses of DM in the silages in the form of effluents were quantified by weight difference, according to Schmidt *et al.* (2011):

$$E= ((Pab–Pen)/(MVfe)) \times 1000$$

Where:
E: effluent production (kg t^{-1} green mass).
Pab: weight of the PVC and cover assembly (silo+cover+sand+foam) at the opening (kg).
Pen: weight of the PVC and cover assembly (silo+cover+sand+foam) at the ensilage (kg).
MVfe: green forage ensiled mass (kg).

Table 1 Levels of crude glycerin guarantee and chemical composition of BRS Capiaçu in natura

Item (%)	Crude glycerin	BRS Capiaçu[1]
Total glycerol	86.9	-
Dry matter	89.5	17.5
Metanol	< 0.01	-
pH	5.3	-
Moisture	9.2	-
Crude protein	4.0	74.4
Neutral detergent fiber	-	67.1
Acid detergent fiber	-	46.2
Lignin	-	4.5
Total digestible nutrients	-	46.2
Ether extract	10.0	-
Ash	3.2	-
Sodium	1.3	-
Chlorine	1.9	-
Potassium	< 0.01	-

[1] 70 days of regrowth (Monção *et al*. 2019).

After drying, the silage corresponding to each treatment was analyzed for DM, ash, organic matter (OM), ether extract (EE), crude protein (CP), lignin (LIG), neutral detergent fiber corrected for ash and protein (NDFap), hemicellulose (HEM), cellulose (CEL), and neutral detergent insoluble nitrogen (NIDN) according to procedures described by Detmann *et al*. (2012). The total carbohydrate (TC) content was estimated by the equation: TC (%)= 100 - [% Moisture + CP (%) + EE (%) + ashes (%)] and those of non fibrous carbohydrates (NFC) according to Sniffen *et al.* (1992). Total digestible nutrients (TDN) were estimated using the formula (Weiss, 1998):

$$TDN= 40.2625 + 0.1969\ CP + 0.4028\ NFC + 1.903\ EE - 0.1377\ ADF$$

The data were submitted to statistical analysis using PROC GLM and PROC REG (SAS, 2008), and when the variables were significant by the F test, the inclusion rates of crude glycerin were submitted to analysis regression analysis.

The regression equations were selected based on the trend of the data and a higher coefficient of determination (R^2). The probability was 5%.

RESULTS AND DISCUSSION

The inclusion of crude glycerin in the BRS capiaçu grass silage linearly reduced pH (P<0.01) and effluent losses (P<0.01; Table 2) compare to control group. For each percentage unit of glycerin addition, there was a reduction of 0.013 pH units and 0.0485% of the effluent losses (Table 3). There was no effect of crude glycerin on the ammoniacal nitrogen content among treatment groups (P=0.82, an average of 6.65% of the total nitrogen).

The variation in the DM content between the highest glycerin inclusion dose (15% of natural matter) and the group control (without glycerin) was 30.5%.

The CP content (P=0.01), ash (P<0.01), NDF (P<0.01), NDFap (P<0.01) and ADF (P<0.01) were linearly reduced with the inclusion of crude glycerin. In the same order, there was a reduction of 0.115%, 0.08%, 1.95%, 1.84% and 1.06%.

The hemicellulose content (P<0.01), cellulose (P<0.01) and lignin (P<0.01) reduced the silage 0.88%, 0.88% and 0.28% for each 1% addition of glycerin, respectively, compare to control group. There was a linear increase of OM (P<0.01), EE (P<0.01), TC (P<0.01), NFC (P<0.01) and TDN (P<0.01) of the silage as the inclusion of crude glycerin compare to control group. For each 1% of inclusion of crude glycerin, there was an increase of 0.08%, 0.38%, 0.24%, 2.04% and 0.95%, respectively. With the production of 50 t/ha of dry mass or 200 t/ha of green mass (Pereira *et al*. 2017), BRS Capiaçu elephant grass silage has been quite attractive to the producers.

However, the DM content at 70 days of regrowth (2.5 meters high) was 17.5% (Table 1), which is below the recommended DM to obtain adequate fermentability of the ensiled material (Borreani *et al*. 2018); Ferraretto *et al.* 2018). Thus, the use of additives with high DM content is necessary to increase the DM content of the ensiled mass (Rigueira *et al*. 2018). The pH is one of the fermentative profile indicators (Kung Jr *et al*. 2018; Grant and Ferraretto, 2018), our study showed a decrement of 6.25% of pH with the inclusion of crude glycerin.

The decrement of pH is related to the content of glycerol of the additive used. Glycerol is a source of energy for the lactic acid bacteria (LAB) to produce lactic and acetic acids that are responsible for the reduction of the pH of the ensiled mass (Borreani *et al*. 2018).

Table 2 Effect of crude glycerin on fermentative profile and chemical composition of BRS Capiaçu grass silage

Item, %	Levels of crude glycerin (%)					SEM	P-value	
	0	1	5	10	15		L	Q
pH	3.99	3.82	3.76	3.75	3.74	0.05	< 0.01	0.08
$N\text{-}NH_3$, % total nitrogen	6.7	6.25	7.03	6.62	6.68	0.52	0.82	0.69
Effluent losses, kg/t	38.45	33.63	32.00	32.46	28.55	1.87	< 0.01	0.65
DM (%)	18.77	20.26	22.04	24.33	27.01	0.34	< 0.01	0.58
CP (%)	7.95	8.39	7.96	7.26	6.58	0.37	0.01	0.33
OM (%)	90.2	90.06	90.82	91.22	91.37	0.22	< 0.01	0.23
Ash (%)	9.8	9.93	9.18	8.78	8.63	0.22	< 0.01	0.23
EE (%)	1.96	3.24	5.98	7.77	7.78	0.53	< 0.01	< 0.01
NDF (%)	72.48	71.11	58.3	51.21	43.6	0.85	< 0.01	< 0.01
NDFap (%)	65.22	61.17	50.42	43.2	37.07	0.67	< 0.01	< 0.01
ADF (%)	38.5	36.49	28.48	26.23	19.62	3.35	< 0.01	0.88
Hemicellulose (%)	37.31	34.62	29.81	24.98	23.98	2.76	< 0.01	0.23
Cellulose (%)	27.92	25.48	21.33	19.8	14.93	2.99	< 0.01	0.31
Lignin (%)	7.25	11.01	7.15	6.43	4.69	1.40	0.01	0.82
NDIP (%)	1.11	1.46	1.12	0.95	0.74	0.11	< 0.01	0.55
TC (%)	80.09	80.58	81.71	82.81	83.79	0.44	< 0.01	0.83
NFC (%)	15.86	19.35	31.29	39.61	46.72	0.86	< 0.01	< 0.01
TDN (%)	45.58	46.88	52.71	56.23	59.85	0.56	< 0.01	< 0.01

DM: dry matter; CP: crude protein; OM: organic matter; EE: ether extract; NDF: neutral detergent fiber; NDFap: neutral detergent fiber corrected for ash and protein; ADF: acid detergent fiber and neutral detergent insoluble nitrogen (NIDN). The total carbohydrate (TC) content was estimated by the equation: TC (%)= 100 - [% moisture + CP (%) + EE (%) + ashes (%)] and those of non fibrous carbohydrates (NFC)= TC - NDFap according to Sniffen *et al.* (1992). Total digestible nutrients (TDN) were estimated using the formula: TDN= 40.2625 + 0.1969 CP + 0.4028 NFC + 1.903 EE - 0.1377 ADF (Weiss, 1998).
SEM: standard error of the means.
P: probability; L: linear equation and Q: quadratic equation.

Table 3 Regression equations for variables on BRS capiaçu silages with increasing levels of glycerin crude

Item	Regression equation[1]	R^2
pH	$\hat{Y}$= 3.89-0.013X	0.59
Effluent losses, kg/t	$\hat{Y}$= 36.02-0.485X	0.73
DM (%)	$\hat{Y}$= 19.27+0.517X	0.99
CP (%)	$\hat{Y}$= 8.31-0.115X	0.89
OM (%)	$\hat{Y}$= 90.19+0.080X	0.91
Ash (%)	$\hat{Y}$= 9.81-0.08X	0.91
EE (%)	$\hat{Y}$= 2.93+0.389X	0.87
NDF (%)	$\hat{Y}$= 71.47-1.957X	0.97
NDFap (%)	$\hat{Y}$= 62.84-1.84X	0.91
ADF (%)	$\hat{Y}$= 35.82-1.068X	0.78
Hemicellulose	$\hat{Y}$= 35.65-0.888X	0.92
Cellulose	$\hat{Y}$= 27.38-0.883X	0.77
Lignin	$\hat{Y}$= 9.06-0.284X	0.59
NDIP (%)	$\hat{Y}$= 1.29-0.035X	0.73
TC (%)	$\hat{Y}$= 80.71+0.204X	0.95
NFC (%)	$\hat{Y}$= 17.87+2.04X	0.97
TDN (%)	$\hat{Y}$= 46.36+0.95X	0.97

[1] X: level of crude glycerin.
DM: dry matter; CP: crude protein; OM: organic matter; EE: ether extract; NDF: neutral detergent fiber; NDFap: neutral detergent fiber corrected for ash and protein; ADF: acid detergent fiber and neutral detergent insoluble nitrogen (NIDN). The total carbohydrate (TC) content was estimated by the equation: TC (%)= 100 - [% moisture + CP (%) + EE (%) + ashes (%)] and those of non fibrous carbohydrates (NFC)= TC - NDFap according to Sniffen *et al.* (1992). Total digestible nutrients (TDN) were estimated using the formula: TDN= 40.2625 + 0.1969 CP + 0.4028 NFC + 1.903 EE - 0.1377 ADF (Weiss, 1998).
R^2: coefficient of determination.

According with Kung Jr *et al.* (2018) during ensiling, lactic acid (*pKa* of 3.86), is usually the acid found in the highest concentration in silages, and it contributes the most to the decline in pH during fermentation because it is about 10 to 12 times stronger than any of the other major acids [e.g., acetic acid (p*K*a of 4.75) and propionic acid (p*K*a of 4.87)] found in silages.

Rigueira *et al.* (2018) evaluated the inclusion of glycerin (0, 1, 5, 10 and 15% of natural matter) in Napier grass ensilage and did not observe changes in pH value (mean 3.9). In the Piatã grass silage, Orrico Júnior *et al.* (2017) did not verify the effect of the crude glycerin on the pH values, an average of 3.97. In grasses, Kung Jr *et al.* (2018) reported that pH values ranged between 4.3 and 4.7% (25%-35%

DM) are adequate for well-preserved silages since this range restricts the proteolytic enzymes of the plant as well as the enterobacteria and clostridia, resulting in appropriate fermentation of all silages.

Thus, it is suggested that the observed pH values, in this study, are within the range recommended by the literature to obtain quality silage even with lower DM contents in the silage with less than 10% glycerin inclusion. It is worth mentioning that the pH of crude glycerin is acid (5.3%) which also contributes to the acidification of the medium.

The N-NH_3 content is widely used in the evaluation of silages because it indicates the degree of proteolysis that occurs during fermentation, and together with the pH value, it provides an indication of how the fermentation process performed (Kung Jr *et al.* 2018).

The same authors inferred that a lack of stability during silage fermentation results in the extensive degradation of amino acids in ammonia, carbon dioxide (CO_2) and amines, and increasing the N-NH_3 content, which was not verified in this research.

In this research, the N-NH_3 content, mean 6.65, expressed as a percentage of the total nitrogen, indicates that there was no proteolysis of the CP. In sugarcane silage, Dias *et al.* (2014) also did not verify the effect of crude glycerin on ammoniacal nitrogen values. Rigueira *et al.* (2018) observed a reduction of 11.9% in the ammoniacal nitrogen content in the silages with 15% inclusion of crude glycerin (mean 7.3% TN).

Kung Jr *et al.* (2018) reported that changes in ammoniacal nitrogen content occur from increases in dry matter content above 50%. Therefore, the inclusion of glycerin in BRS capiaçu grass silage was not modified at the doses studied.
The inclusion of 15% of crude glycerin in the silage of BRS capiaçu grass reduced effluent losses by 25.74%. Effluent losses is strictly linked to the DM content, which was increased by 30.5% due to the high DM content of crude glycerin (89.5%). Normally, silage with DM content below 25% can presents high effluent losses, as observed in this research mainly for the group control (silage without additive).
The losses effluents reduction in the nutritional value of the ensiled mass because the effluents are also composed of cellular content, rich in energy. Rigueira *et al.* (2018) found a 44.44% reduction in effluent losses when using 15% of crude glycerin inclusion in Napier grass ensilage. In this research, the inclusion of 15% glycerin increased the DM content and reduced effluent losses. In addition, 74.80% of the EE content, 66.05% of the NFC content and 23.84% of the TDN content of the silage in relation to the control silage, highlighting the potential of using crude glycerin as an additive in grass silage. This increase in EE content occurs due to the high (10.5%) fat content in the crude glycerin.

It is important to emphasize that there is a limit of EE content in the diet for ruminants. As negative aspect, values above 7% of DM of diet may limit DM consumption and degradability of the fibrous fraction (Buccioni *et al.* 2012; Manso *et al.* 2016). In this study, even using only 15% glycerin silage, the EE content is within the limit. Orrico Júnior *et al.* (2017) also verified increases in EE content and DM digestibility of piatã grass silage with the inclusion of crude glycerin in the order of 30% of DM. As positive aspect, the EE content increases the energy levels of the diet, which is important for CP synthesis, as long as there is available nitrogen (Van Soest, 1994) and also for nutrient degradation of the diet in the rumen.

The reduction on CP, ash, NDF, NDFap, ADF, hemicellulose, cellulose and lignin content by crude glycerin inclusion could be explained by a dilution effect. Dias *et al.* (2014), Orrico Júnior *et al.* (2017) and Rigueira *et al.* (2018) working with ensiled tropical forages also verified dilution effect with the inclusion of crude glycerin. According to Van Soest (1994), it is desirable to have a minimum CP content in ruminant diet in the order of 7% for ruminal microorganism activity. In this study, the need to include a source of nitrogen to supplement in the nitrogen of the silage with 15% inclusion of crude glycerin. Most of the studies using glycerin for ruminants are the direct use of this additive in the diet, which highlights the importance of this study in the knowledge gaps on the effects of glycerin on silage and ruminant nutrition, and further studies are needed.

CONCLUSION

The crude glycerin in elephant grass silage cv. BRS Capiaçu up to 15% of the natural material improves the fermentation profile and the chemical-bromatological composition.

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