The use of crude glycerin as an alternative to reduce fermentation losses and enhance the nutritional value of Piatã grass silage

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ABSTRACT - The objective of this study was to evaluate fermentation losses and the nutritional value of silage of *Brachiaria brizantha* cv BRS Piatã ensiled with different levels of crude glycerin. The experiment followed a 4 × 2 completely randomized factorial design with four doses of crude glycerin (0, 100, 200, and 300 g kg^{-1} of DM silage) and two cutting ages (45 and 60 days) with four replicates per treatment (silos). The parameters of dry matter losses, effluents losses, gas losses, pH, microbial population, and nutritional value of the silage were evaluated. There was no difference in pH among the tested doses of crude glycerin. However, reductions in dry matter and gas losses were observed as a function of crude glycerin doses. Bacterial populations of lactic acid and facultative mesophilic anaerobes also increased as a function of crude glycerin inclusion. Crude glycerin increased ether extract levels and reduced neutral detergent fiber and acid detergent fiber concentrations (dilution effect), improving chemical composition of the silage. Crude glycerin can be used in the ensiling of Piatã grass and the dose of 300 g kg^{-1} of DM yields the best results.

Key Words: biodiesel, byproduct, forage conservation, glycerol, ruminants

Introduction

Biodiesel production generates significant amounts of crude glycerin as byproduct. For every ton of biodiesel produced, 100 kg of crude glycerin are generated, configuring a yield of 10% (ANP, 2015). In Brazil, the production of crude glycerin in 2014 was approximately 460,000 tons, greater than the current demand of 40,000 tons (ANP, 2015). Crude glycerin surplus from biodiesel industries represents a hazard risk due to its pollutant content (considerable levels of glycerol and residual lipids). For this reason, alternative usage for this byproduct must be evaluated to reach equilibrium between demand and production ensuring sustainable usage.

Crude glycerin has the potential to be used as an additive to enhance silage fermentation because it has glycerol in its composition. This is a rich source of energy for anaerobic microorganisms (Santos et al., 2014; Carvalho et al., 2017), which can favor microbial growth and improve the quality of fermentation and forage conservation.

The inclusion of crude glycerin in the process of ensiling has been focused on sugar cane. This has been associated to better fermentation, lower production of ethanol, lower dry matter (DM) losses, and silage with superior nutritional value (Santos et al., 2014; Carvalho et al., 2017). The inclusion of crude glycerin has not been tested in the ensiling of grass. In this case, lower levels of soluble carbohydrates and dry matter found in grass seems to be the major limitation point. This is relevant regarding tropical grasses such as *Brachiaria brizantha* (Mendieta-Araica et al., 2010). Grass may present high buffering capacity, which inhibits fermentation. In addition, several types of yeast that support fermentation in sugarcane silages are not present in grass. These factors may restrain acidification, limit the quality of silage, and configure bottlenecks towards the use of crude glycerin as an alternative to reduce fermentation loss and improve the nutritional value of grass silage.

The addition of low-cost sources of soluble carbohydrates may tackle some limitation points. The

challenge would be to make ensiling of grass feasible by increasing the growth rates of lactic acid bacteria, dropping silage pH, and enhancing fermentation (Keady and Kiely, 1998; McDonald and Henderson, 1962; Nishino et al., 2012; Chen et al., 2016). If possible, this would configure alternative usage of a potentially pollutant byproduct from biofuel production.

In this sense, aiming to support livestock production, this study was designed to verify if the inclusion of crude glycerin could improve fermentation and the nutritional value of silages prepared with *Brachiaria brizantha* cv Piatã.

Material and Methods

The research was conducted in Dourados, MS, Brazil (22°13'18"S latitude and 54°48'23"W longitude). The climate, according to the Köppen, is Cwa mesothermal humid, with average annual rainfall ranging from 1,250 to 1,500 mm and temperatures between 20 and 24 °C.

The experiment followed a 4 × 2 completely randomized factorial design with four doses of crude glycerin (0, 100, 200, and 300 g kg^{-1} of DM silage) and two cutting ages (45 and 60 days), with four replicates per treatment (silos). Experimental silos were built with PVC tubes of 50 cm in height and 15 cm in diameter. The silos were sealed at the bottom end with a cap and held approximately 5 kg of grass.

The crude glycerin used in this study was sampled from a biodiesel industry and presented the following chemical composition: 960.00 g kg^{-1} of dry matter, 64.01 g kg^{-1} of ash, 618.70 g kg^{-1} of glycerol, 61.00 g kg^{-1} of methanol, and 18.03 g kg^{-1} of ether extract.

The area planted with Piatã grass was formed five years prior to the research and was fertilized with 100 kg ha^{-1} of nitrogen (urea) 30 days before the beginning of the experiment. On May 25th, 2014 (beginning of the dry season), the grass was evenly cut at a height of 10 cm above the ground. After standardization, the area was divided into three parts and the grass from each segment was cut as grass reached the specific ages defined in the experiment. A clearing saw was used to cut the grass at a height of 15 cm above the ground and the collected material was ground to obtain a particle size of 1.5 cm.

Grass samples and different doses of crude glycerin were mixed in plastic bags to establish the experimental treatments (silos). The material for ensiling was manually compacted with a wood rod and the average density was 566 kg/m³ of fresh matter. After filling, the silos were sealed with plastic sheets and duct tape, weighed, and stored in the laboratory. All components of the silo, as well as the forage material, were weighed to determine the fermentation losses. After 80 days of fermentation, the silo contents were weighed again to determine the gas, effluent, and dry matter losses according to Ping et al. (2016).

Samples of approximately 300 g of forage from each treatment were collected prior to and after ensiling. Subsequently, these samples were dried at 55 °C for 72 h to determine the dry matter content. After pre-drying, the

Table 1 - Chemical composition of Piatã grass with different doses of crude glycerin and two cutting ages

Parameter	Cutting age (days)	Dose (g kg^{-1} of DM)			
		0	100	200	300
Buffering capacity (meq HCl.100 g^{-1} of DM)	45	6.81	6.74	6.48	6.59
	60	5.55	5.66	5.80	5.76
Dry matter (g kg^{-1})	45	251.20	267.90	279.70	291.90
	60	329.50	338.30	350.40	370.40
Ash (g kg^{-1} of DM)	45	82.70	78.20	74.30	64.30
	60	86.30	78.90	76.00	71.60
Crude protein (g kg^{-1} of DM)	45	128.70	111.40	101.90	82.10
	60	80.60	86.50	73.30	79.90
Ether extract (g kg^{-1} of DM)	45	46.00	50.60	60.70	78.90
	60	34.00	37.40	44.90	58.30
Neutral detergent fiber (g kg^{-1} of DM)	45	621.70	553.00	523.50	504.20
	60	641.80	604.20	548.30	571.50
Acid detergent fiber (g kg^{-1} of DM)	45	292.90	289.30	274.90	253.80
	60	325.90	315.30	292.60	284.60
Non-fibrous carbohydrates (g kg^{-1} of DM)	45	160.80	246.80	279.60	340.50
	60	142.00	177.10	230.10	241.10
In vitro dry matter digestibility (g kg^{-1} of DM)	45	797.20	854.30	862.10	893.40
	60	762.10	832.30	826.60	844.30

DM - dry matter.

samples were ground in a Willey mill with 1-mm sieve for further laboratory analysis (Table 1). The concentrations of DM, crude protein (CP), ash, and ether extract (EE) were determined according to the AOAC (1990). The *in vitro* dry matter digestibility (IVDMD) was evaluated according to the method described by Tilley and Terry (1963). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Mertens (2002).

Non-fibrous carbohydrates (NFC) were calculated by the following equation: NFC = 100 − (NDF + CP + EE + Ash) proposed by Sniffen et al. (1992). The total digestible nutrient (TDN) values were estimated according to Cappelle et al. (2001). Measurements of aqueous extract and pH of the material prior to and after ensiling were carried out as proposed by Kung Junior et al. (1984). The buffering capacity was determined in forage samples according to the protocol described by Playne and McDonald (1966).

For microbiological analysis, samples of 25 g were collected from each silo and placed in flasks with 225 mL of sterile 1% peptone water and stirred for 10 min. From the extract obtained, decimal dilutions of 10:1 to 10:7 were prepared to assess the microorganism populations. The number of facultative anaerobic mesophilic bacteria was determined on plates with nutrient agar and incubated at 35 °C. The lactic acid bacteria were numbered on plates with MRS culture medium (Difco) added with 0.4% nystatin (to prevent fungi growth) and incubated in anaerobic jars (AnaeroGen, Oxoid Ltd.) at 35 °C. Dichloran Glycerol (DG18) agar was used for mold count and the YEPD medium was used for yeast count. The plates were incubated at 28 °C for five to seven days for fungi count and 48 h for yeast count (Bravo-Martins et al., 2006).

The results were subjected to analysis of variance, considering the ages of the plants and crude glycerin doses as sources of variation. Orthogonal contrasts were used to assess the linear and quadratic effects of the levels of crude glycerin. Values observed between different plant ages were compared by the F test. All data analyses were performed using the software R (2014).

Results

Dry matter loss was significantly reduced with the inclusion of crude glycerin, 231.03 g kg^{-1} of ensiled DM in the treatment with 0 g kg^{-1} of DM and 72.64 g kg^{-1} of ensiled DM in the treatment with 300 g kg^{-1} of DM (Table 2).

Effluent losses of 34.63 g kg^{-1} of ensiled DM were observed in the treatment with no glycerin inclusion

against 10.36 g kg^{-1} of ensiled DM for the treatment with the highest inclusion level (Table 2). No effluent production was observed in the silage prepared with the 60-day-old grass. With the exception of DM loss ($P>0.05$), the age of the grass significantly influenced fermentation ($P<0.05$). Significant increase of the population of lactic acid and facultative anaerobic mesophilic bacteria was observed with the addition of glycerin to the ensiling of Piatã grass (Table 2). Bacterial populations of lactic acid and facultative mesophilic anaerobes were counted at 6.87 and 6.60 log cfu/g with no glycerin addition and 7.26 and 7.38 log cfu/g with inclusion of 300 g kg^{-1} DM of crude glycerin, respectively.

Populations of molds ($P<0.01$) and yeasts ($P<0.05$) also showed significant differences as a function of the levels of crude glycerin, especially in the two highest inclusion levels (Table 2). The average population of molds and yeast was 2.32 and 2.83 log cfu.g^{-1} for the silage added with 300 g kg^{-1} of DM of crude glycerin, respectively.

In terms of the nutritional value of silage, reductions were observed in the concentrations of NDF and ADF ($P<0.01$) with increasing levels of crude glycerin (Table 3). Moreover, the inclusion of higher levels of crude glycerin was related to higher concentration of IVDMD [769.68 (0 g kg^{-1} of DM of crude glycerin); 841.55 (300 g kg^{-1} of DM of crude glycerin)] and TDN [777.46 (0 g kg^{-1} of DM of crude glycerin); 853.64 (300 g kg^{-1} of DM of crude glycerin)] ($P<0.01$).

Discussion

The age of the grass used for ensiling was associated to different amounts of gas loss, lower with silos prepared with mature grass (Table 2). Likewise, this was reported by Dias et al. (2014), assigning lower gas losses to greater amounts of dry matter present in the silage of mature sugarcane. Younger grass may present excessive moisture, which inhibits effective fermentation (Collins and Owens, 2003). In silages, poor fermentation becomes problematic when moisture levels exceed 70% and soluble carbohydrates are scarce (less than 50g kg^{-1} DM) (Collins and Owens, 2003).

In this study, all levels of crude glycerin inclusion were associated to notable reduction of DM losses, from 231.03 to 72.64 g kg^{-1} of ensiled DM (Table 2). This is probably related to more efficient fermentation during ensiling. Most likely, the inclusion of crude glycerin itself would not explain such a distinguished decrease (68.86% of DM), considering that the maximum level added to the silos was 300 g kg^{-1} of DM (30%). Using 100 g of glycerin kg^{-1} of DM, the level

Table 2 - Fermentative loss and microorganism populations in Piatã grass ensiled with 0, 100, 200, or 300 g kg⁻¹ of DM glycerin and cut at 45 or 60 days

Parameter	Crude glycerin (g kg⁻¹ of DM)				SEM	Age		SEM	P-value			P-value for contrasts[1]	
	0	100	200	300		45	60		Glycerin (G)	Age (A)	G × A	L	Q
pH	3.95	3.94	4.10	3.90	0.21	3.94	4.00	0.14	0.12	0.32	0.31	0.20	0.12
DM loss (g kg⁻¹ of ensiled DM)	231.03	144.34	91.41	72.64	58.10	122.49	147.23	41.00	<0.01	0.09	0.09	<0.01	0.02
Effluent loss (g kg⁻¹ of ensiled DM)	34.63	35.00	16.73	10.36	5.34	48.36	0.00	17.32	<0.01	<0.01	0.08	<0.01	0.03
Gas loss (g kg⁻¹ of ensiled DM)	90.62	37.55	27.58	19.93	5.54	58.03	29.81	16.00	<0.01	0.03	0.06	<0.01	0.04
LAB (log cfu.g⁻¹ silage)	6.87	7.30	7.49	7.26	0.38	7.23	7.23	0.22	0.02	0.87	0.34	0.03	<0.01
FAMB (log cfu.g⁻¹ silage)	6.60	7.09	6.67	7.38	0.32	6.71	7.16	0.21	0.04	0.12	0.54	0.04	<0.01
Molds (log cfu.g⁻¹ silage)	2.10	2.16	2.34	2.32	0.11	2.23	2.23	0.08	<0.01	0.69	0.23	0.02	<0.01
Yeast (log cfu.g⁻¹ silage)	1.79	1.38	1.46	2.83	0.73	1.85	1.88	0.55	0.01	0.65	0.43	<0.01	0.02

DM - dry matter; LAB - lactic acid bacteria; FAMB - facultative anaerobic mesophilic bacteria; cfu - colony-forming units; SEM - standard error of the mean.
[1] L and Q - linear and quadratic effects, concerning the inclusion of crude glycerin.

Table 3 - Chemical composition of Piatã grass silages added with different doses of crude glycerin (0, 100, 200, and 300 g kg⁻¹ of DM) and cut at two ages (45 and 60 days)

Parameter	Crude glycerin (g kg⁻¹ of DM)				SEM	Age		SEM	P-value			P-value for contrasts[1]	
	0	100	200	300		45	60		Glycerin (G)	Age (A)	G × A	L	Q
DM (g kg⁻¹)	277.81	292.21	317.87	320.11	10.70	294.41	339.98	9.08	0.03	<0.01	0.45	<0.01	0.03
Ash (g kg⁻¹)	86.21	86.30	82.57	78.91	4.65	83.33	83.66	3.10	0.05	0.39	0.07	0.08	0.19
CP (g kg⁻¹ of DM)	123.44	112.39	104.20	89.10	12.25	114.01	100.55	8.70	<0.01	0.02	0.43	<0.01	0.05
EE (g kg⁻¹ of DM)	40.00	44.00	52.80	68.64	11.28	59.06	43.66	6.90	<0.01	0.02	0.21	<0.01	0.06
NDF (g kg⁻¹ of DM)	544.37	500.56	469.49	429.78	40.94	462.00	510.10	28.66	<0.01	<0.01	0.22	<0.01	0.04
ADF (g kg⁻¹ of DM)	304.33	271.49	248.79	231.59	27.12	250.23	277.87	19.20	<0.01	<0.01	0.32	<0.01	0.02
NFC (g kg⁻¹ of DM)	163.59	226.10	248.47	330.57	53.88	248.04	296.33	36.50	<0.01	0.04	0.32	<0.01	0.07
IVDMD (g kg⁻¹ of DM)	769.68	810.08	819.91	841.55	30.36	830.11	790.50	21.00	<0.01	<0.01	0.34	<0.01	0.07
TDN (g kg⁻¹ of DM)	777.46	820.28	830.70	853.64	32.18	841.52	799.53	20.50	<0.01	<0.01	0.51	<0.01	0.03

DM - dry matter; CP - crude protein; EE - ether extract; NDF - neutral detergent fiber; ADF - acid detergent fiber; NFC - non-fibrous carbohydrates; IVDMD: in vitro dry matter digestibility; TDN - total digestible nutrients; SEM - standard error of the means.
[1] L and Q - linear and quadratic effects, concerning the inclusion of crude glycerin.

of DM losses was 22.39% (from 231.03 to 144.34 g kg^{-1} DM). These values are consistent with the results reported by Carvalho et al. (2017), comparing different levels of inclusion in sugarcane silage. These authors reported reductions of 20.28% of DM losses by adding *Lactobacillus hilgardii* and 80 g kg^{-1} DM of glycerin.

The decrease of gas losses was also observed with increasing levels of crude glycerin inclusion. Gas losses decreased on average by 78% when comparing the lowest with the highest level of crude glycerin inclusion. Gas losses 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, important to drop silage pH. In contrast, heterofermentative inoculants are related to the production of alcohol, and acetic and butyric acids, associated to greater gas losses during ensiling (Viana et al., 2013).

The supposition of more efficient fermentation associated to increasing levels of crude glycerin inclusion may be addressed to the fact that *Lactobacillus* strains break down glycerol as a source of energy (Garai-Ibabe et al., 2008; Pasteris and Strasser de Saad, 2009). In the present study, the most expressive number of microorganisms was addressed to lactic acid bacteria. This type of bacteria is usually related to the decrease of DM losses due to greater acidification during ensiling (Goeser et al., 2015).

The increase in the growth rates of lactic acid bacteria were not associated to the final pH in any silage (P>0.05). The final pH of the silage is usually related to the quality of fermentation, but does not necessarily explain the speed in which pH drops, neither the type of fermentation (microorganism populations), nor the nutritional value of the silage. Still, the dry matter and gas losses observed in the present study are suggestive of rapid decline of pH during fermentation as a result of the inclusion of crude glycerin. This was also discussed by Goeser et al. (2015) and reported by Santos et al. (2014), testing the effect of the inclusion of crude glycerin and microbial additives to sugarcane silage. In addition, these authors observed an increment in the energy density of sugarcane silage.

Microorganisms such as yeast and molds use glycerol as a source of energy (Taccari et al., 2012). Thus, the inclusion of crude glycerin was expected to increase the population of these types of microorganisms in the silage. These are undesired microorganisms, related to hygienic and nutritional aspects of the silage (Bravo-Martins et al., 2006). However, the population of yeast and molds increased in a level (Table 2) considered irrelevant to configure any depreciation in the quality of the silages (Cogan et al., 2016).

All silages were considered to show good quality in terms of microbiology.

The nutritional value of the silages was in accordance with the expectations. Greater amounts of NDF (510.10 g kg^{-1} of DM) (P<0.01) were related to more mature grass (60 days at cutting), probably due to greater proportions of cell wall. The composition of crude glycerin, i.e., mainly non-fibrous carbohydrates, reduced the proportions of NDF and ADF with increasing levels of crude glycerin, which is related to the dilution effect (Table 3). Martins et al. (2014) observed reductions by 25.3 and 24.7% of NDF when 450 g kg^{-1} of DM of glycerin was added to silage produced with corn and sunflower, respectively.

Likewise, the linear reduction (P<0.01) in CP concentration, related to glycerin inclusion, may be also related to a dilution effect (Table 3). Similar results were reported by Gomes et al. (2015), who observed a reduction by 37.56% in the CP concentration of sugarcane silages added with 150 g kg^{-1} of fresh matter glycerin compared with control silages. This is often reported because additives used to promote fermentation are rich in energy and present low protein content.

The association of crude glycerin inclusion and superior amounts of EE, IVDMD, and TDN (Table 3) is probably due to the composition of the additive used for ensiling. The byproduct sampled from biodiesel production consisted of low-purity glycerin (high levels of residual lipids originated from vegetable oils). This is relevant to livestock production because high amounts of EE in the diet, usually above 70 g kg^{-1} of DM, may reduce intake as a consequence of poor microbiological degradation of fiber in the rumen (Doreau and Ferlay, 1995; Gudla et al., 2012; Buccioni et al., 2012; Manso et al., 2016). However, the EE concentration observed in the silages was considered as within suitable intervals for intake (Table 3).

A disadvantage of using silages prepared with tropical grasses is associated to low energy concentration (TDN) for animal feed. Dietary energy in grass silage may be considerably low compared with corn or sorghum silages (Carvalho et al., 2016), which may be compensated by offering greater proportions of concentrate to ensure weight gain in animal production systems. However, this may configure considerable increases in the production costs.

An average increase by 2.5 g TDN kg^{-1} of DM was observed for each inclusion of 10 g kg^{-1} of DM crude glycerin in the silage, reaching a maximum of 853.64 g of TDN kg^{-1} of DM. The increased nutritional value of the silage was probably related to the energetic value of the crude glycerin, which enabled lactic acid bacteria to grow and optimize fermentation. It is noteworthy that

energetic additives, such as molasses and pure glycerol, would probably enable superior results. However, low-cost additives seem to be the key to make the inclusion of additives and the ensiling of grass feasible. In contrast, low-price additives may present little amount of glycerol, which could result in poor fermentation.

Currently, there is little information regarding intake (acceptability) of silages containing crude glycerin. Therefore, consistent contribution towards livestock performance is incipient. However, the results reported in this study are convincing that the inclusion of crude glycerin may alleviate fermentative losses and generate grass silage with superior nutritional value. Overall, the results are potential contributions to support sustainable livestock production within tropical regions and scenarios of biodiesel production.

Conclusions

The use of crude glycerin in the ensiling of Piatã grass is recommended to increase the population of lactic acid bacteria, reduce fermentation losses, and improve the nutritional value of silage. Overall, the dose of 300 g kg^{-1} of dry matter crude glycerin (in relation to the ensiled dry matter) yields the best results.

Acknowledgments

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