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The quality of crude glycerine influences the fermentation and nutritive value of Piatã grass silage

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ABSTRACT - We aimed to evaluate the effects of crude glycerine purity and levels of glycerol on the fermentative profile, microorganisms counting, and nutritional value of Piatã grass silage. The experiment was carried out in a 3×3+1 completely randomized factorial design using three different types of crude glycerine [low purity (40% of glycerol), medium purity (60% glycerol), and high purity (80% glycerol)], three doses of glycerol (20, 40, and 60 g/kg DM), and a control (no crude glycerine added) as an additional treatment, which were stored for 80 days. Statistical differences were not observed on fermentative losses of the treatments tested. The increase of glycerol doses resulted in higher levels of non-fibrous carbohydrates and lower levels of fibre components. The addition of low-purity crude glycerine increased the levels of fat and *in vitro* digestibility of silages. The highest levels of lactic acid and acetic acid occurred in the treatments with 60 g/kg of glycerol when using crude glycerine with a low purity. The lowest lactic acid content was observed in the control treatment and in the lowest dose of glycerol. The highest glycerol dose (60 g/kg), associated with the lowest degree of crude glycerine purity (40%) resulted in the greatest nutritional value and provided the best silage conservation.

Keywords: byproduct of biodiesel, fermentation profile, forage conservation, ruminant feed

1. Introduction

In tropical regions, most grass production is concentrated in the rainy season, generating a surplus production during this period and a deficit during the dry season. Therefore, silage production is an alternative method to manage the forage supply by retaining much of its nutritional value and allowing storage for long periods (Epifanio et al., 2014). *Brachiaria brizantha* grass cv. Piatã has a vigorous forage production and high nutritional value, having the potential to improve animal performance (Orrico Junior et al., 2013; Silveira et al., 2018).

Tropical grasses, including Piatã grass, are characterized by a low DM and soluble carbohydrate content at harvesting, which, associated with high buffer capacity (Khota et al., 2016), hinders the silage process and can result in higher dry matter loss and silage with low acceptability by ruminants. During silage fermentation, high levels of soluble sugars are essential for the proliferation of lactic acid and acetic acid bacteria (Vendramini et al., 2016). The use of energetic additives may compensate for the low

soluble carbohydrate content of tropical grasses, as well as reduce DM losses and increase organic acid production, consequently improving silage quality.

The addition of crude glycerine (a byproduct of biodiesel production) has presented improvement on the fermentative profile and nutritive value in different grass silages (Dias et al., 2014; Orrico Junior et al., 2017). According to Hong et al. (2010), some bacteria species may use glycerol as an energetic substrate for the production of organic acids, among them, lactic acid. In a recent research, Cunha et al. (2020) also observed improvements in the production of lactic acid and the nutritional value of Tifton 85 silages, even when the forage has high levels of dry matter, which is a condition that hampers the fermentation process.

The above-mentioned studies have investigated the effect of different doses of crude glycerine during the ensilage; however, in none of them has the effect of crude glycerine purity been evaluated in combination with predefined levels of glycerol. The production of each 100 kg of biodiesel generates approximately 10 kg of crude glycerine as a byproduct, which contains variable amounts of glycerol and other contaminants, including methanol, residual fats, and catalyst salts (Santibáñez et al., 2011). The efficiency of the transesterification process determines the level of glycerol in crude glycerine. High levels of glycerol indicate better utilization of fatty acids during processing, while low levels of triglycerides are left intact. On the other hand, low levels of glycerol may signal process inefficiency, which results in a greater concentration of residual products, mainly fat (Zavarize et al., 2014). These residual products may interfere in the type of microorganism population, fermentative profile, nutritional value of the silage, and, consequently, animal performance (Chanjula, 2017), mainly when low-purity crude glycerine is used (increasing the levels of fat in silage).

Little is known about the effects of the different types of crude glycerine (low, medium, and high purity) on the fermentation pattern, lactic acid bacteria, mould and yeast counting, fermentative losses, and nutritional value of Piatã grass silage. Thus, the hypothesis of this work is that high levels of residual products of low-purity crude glycerine (40% glycerol) at the dose up to 60 g/kg DM do not affect negatively the chemical and microbiological parameters of Piatã grass silage. Therefore, this study as carried out to evaluate the quality effect of three types of crude glycerine [low (40% glycerol), medium (60% glycerol), and high (80% glycerol) purity] and three levels of glycerol addition (20, 40, and 60 g/kg DM) on the fermentation pattern, microorganism population, fermentative losses, and nutritional value of Piatã grass silage.

2. Material and Methods

The research was carried out in the city of Dourados, Mato Grosso do Sul, Brazil (22°11'55" S, 54°56'7" W, and 452 m altitude). According to the Köppen classification, the climate in the region is humid mesothermal – Cwa, with average temperature and rainfall between 20 and 24 °C and 1250 and 1500 mm, respectively.

The experiment was carried out in completely randomized design in a factorial scheme 3×3+1, using three different types of crude glycerine [low purity (L; 40% of glycerol), medium purity (M; 60% glycerol), and high purity (H; 80% glycerol)], three doses of glycerol (20, 40, and 60 g/kg DM), and a control (no crude glycerine added) as an additional treatment, with three repetitions per treatment. To facilitate the discussion of results, the treatments mentioned will be represented by the following acronyms: L20, L40, L60, M20, M40, M60, H20, H40, and H60 (the letter corresponds to the type of glycerine and the number correspond to the glycerol dose).

Approximately, 960 g/kg of total solids, 142 g/kg of glycerol, 61 g/kg of methanol, and 747 g/kg of lipids were used as the basis to prepare the crude glycerine for the treatments (commercial product, Biocar). Following this, a mix with different volume of pure glycerol (99.5% of glycerol, Sigma-Aldrich®) was added to obtain crude glycerine with 40, 60, and 80% glycerol (Table 1). After having obtained the crude glycerine with three degrees of purity, a calculation was carried out based on the DM content of the forage (338.49 g/kg of DM) to achieve the values of 20, 40, and 60 g of glycerol/kg of DM forage (Table 1).

The Piatã grass was collected from an area of 2000 m^2 which was treated with regular limestone and fertilizer applications as a function of the chemical analysis of the soil. Before harvesting of forage (60 days), we performed fertilization with 80 kg/ha of N, 200 kg/ha of P_2O_5, and 200 kg/ha of K_2O, using the commercial urea and chemical fertilizer at 0-20-20 as nutrient sources. A standardization mowing was carried out at 10 cm from the soil in April, 2016. After 60 days, the grass was manually cut 10 cm from the soil in the morning. The harvested forage was then taken to a covered shed, and as a consequence, the rate of dehydration was decelerated. After that, the forage was chopped in a stationary electric grinder (TRF 300, TRAPP®), the average particle size was standardized in 2 cm.

The chopped forage was homogenized and partitioned into small samples (3.5 kg) to form each of the experimental plots; they were let scattered over a plastic coating awaiting the distribution of the treatments before being ensiled. The crude glycerine was sprinkled over the forage according to the proposed doses using a manual sprinkler (Volder®1.5 l). After the application of the treatments, all material was re-mixed, and a sample (approximately 300 g) was collected to perform the chemical determinations in each of the treatments to be ensiled. The forage mass was stored in laboratory silos built with PVC pipes (10 cm in diameter and 50 cm in height) with a useful volume of 3.8 L. The material was manually compacted with wooden rods to obtain the mean density of 365 kg DM/m^3 among the treatments. At the bottom of each silo, a layer of approximately 2.5 cm of sand was added for effluent drainage. A thin plastic canvas and cotton cloth were used to avoid contact between the forage and the sand. The experimental silos were sealed with airtight lids containing Bunsen valves for gas release and stored in the laboratory at room temperature.

All the components of the experimental mini-silos, as well as the wrapped forage, were weighed before ensilage, and after 80 days of storage, the silos were opened for the determination of gas loss (g/kg of DM ensiled), effluent production (g/kg of forage ensiled), and DM loss (g/kg of DM ensiled) (Li et al., 2017).

After the silos were opened, the internal material was homogenized. Forage samples from each treatment were collected and dried at 55 °C for 72 h. The samples were ground in a Wiley mill with a 1.0 mm mesh, the DM was obtained by placing the ground samples in a dry oven at 105 °C for 2 h (AOAC, 2005). The crude protein (CP; method 976.06), ether extract (EE; method 2003.05), and ash (method 942.05) contents of each sample were determined according to AOAC (2005). The measurement of neutral detergent fibre (NDF) was carried out with α-amylase and sodium sulfite, and acid detergent

Table 1 - Composition of three types of crude glycerine and volume used in each treatment

Parameter	Crude glycerine purity		
	Low (40% of glycerol)	Medium (60% of glycerol)	High (80% glycerol)
	Composition of mixture of crude glycerine and pure glycerol		
Crude glycerine (mL/L)	698.25	464.99	232.19
Glycerol (mL/L)	301.75	535.01	767.81
	Crude glycerine composition		
Glycerol (g/kg)	400.0	600.0	800.0
Total solids (g/kg)	972.1	981.4	990.7
Methanol (g/kg)	42.6	28.4	14.2
Lipids (g/kg)	521.6	347.4	173.5
	Crude glycerine volume (mL/kg of DM)		
Control	0.0	0.0	0.0
20 g glycerol/kg of DM	50.0	33.3	25.0
40 g glycerol/kg of DM	100.0	66.7	50.0
60 g glycerol/kg of DM	150.0	100.0	75.0

fibre (ADF) was expressed with ash content (Mertens, 2002). The NDF digestibility was determined by incubating the silage (1mm particle size) for 48 h in a TE-150 incubator (Tecnal, Piracicaba, SP, Brasil) using rumen-fluid collected from rumen-fistulated Nellore steers receiving grass silage during three weeks (Holden, 1999). Non-fibrous carbohydrates (NFC) and total carbohydrates (TC) were obtained using the following equations (Sniffen et al., 1992):

$$NFC = 100 - (NDF + CP + EE + ash) \quad (1)$$

$$TC = 100 - (CP + EE + ash) \quad (2)$$

To obtain the aqueous extract, a 25-g sample of silage was blended in 225 mL of sterile water and homogenized in a shaker (Thermo Scientific™ MaxQ™ 4000 Benchtop Orbital Shakers) for 1 min. The pH of each sample was then determined using a pH meter (DIGIMED® DM 20 Potentiometer, Digicrom Instruments, SP, Brazil) according to the methodology described by Playne and McDonald (1966). A portion of this extract was filtered and centrifuged for 15 min at 10,000 × *g*. The supernatant was frozen at −20 °C for further analysis of the organic acids. Organic acids were determined in a gas chromatograph with a mass detector (GC-MS; GCMS QP 2010 plus, Shimadzu, Kyoto, Japan) using a capillary column (Stabilwax, Restek, Bellefonte, USA, 60 m, 0.25 mm Ø, 0.25 µm cross bond carbowax polyethylene glycol) and the analytical parameters recommended by the manufacturer. The concentration of lactic acid was determined by the colorimetric method proposed by Pryce (1969).

The microorganisms counting was also performed with an aqueous extract. To obtain it, a 25 g sample of silage was blended in 225 mL of peptone water (0.1 %) and homogenized in a shaker for 10 min, followed by a sequential 10-fold dilution (from 10^{-1} to 10^{-8}). For the lactic acid bacteria (LAB) count, Man Rogosa Sharpe – Difco (MRS Micro Med, Isofar) agar plus 0.4 % nystatin (to prevent fungal growth) was used, and the plates were incubated at 35 °C for 72 h. For the mesophilic bacteria count, the plates were incubated at 35 °C for 72 h using nutrient agar medium (KASVI). Dichloran-Glycerol (DG18 Acumedia Neogen) medium was used for the filamentous fungi count, and the plates were incubated at 28 °C from five to seven days. Yeasts were counted in yeast extract peptone-dextrose medium (YEPD; HiMedia) with chloramphenicol added (to prevent bacterial growth); the plates were incubated at 35 °C for 48 h. The colonies were counted on plates containing a minimum of 30 and a maximum of 300 colony-forming units (Bravo-Martins et al., 2006).

Data were statistically analysed using the GLM procedure of the SAS software (Statistical Analysis System, version 9.0). The statistical model used in this study was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha^*\beta_{ij} + \varepsilon_{ijk}, \quad (3)$$

in which Y_{ijk} = dependent variable, μ = general mean, α_i = type of crude glycerine effect (fixed effect; i = 40, 60, and 80), β_j = level of glycerol effect (fixed effect; j = 20, 40, and 60), $\alpha^*\beta_{ij}$ = interaction of type of crude glycerine and level of glycerol effect plus control, and ε_{ijk} = random error associated with each observation.

Treatments were evaluated by the least significance difference (LSD) test using 0.05 significance level. Means were compared by Tukey's test when significant effects were identified in the variance analyses. The SigmaPlot® 11.0 was used to make the surface plot.

3. Results

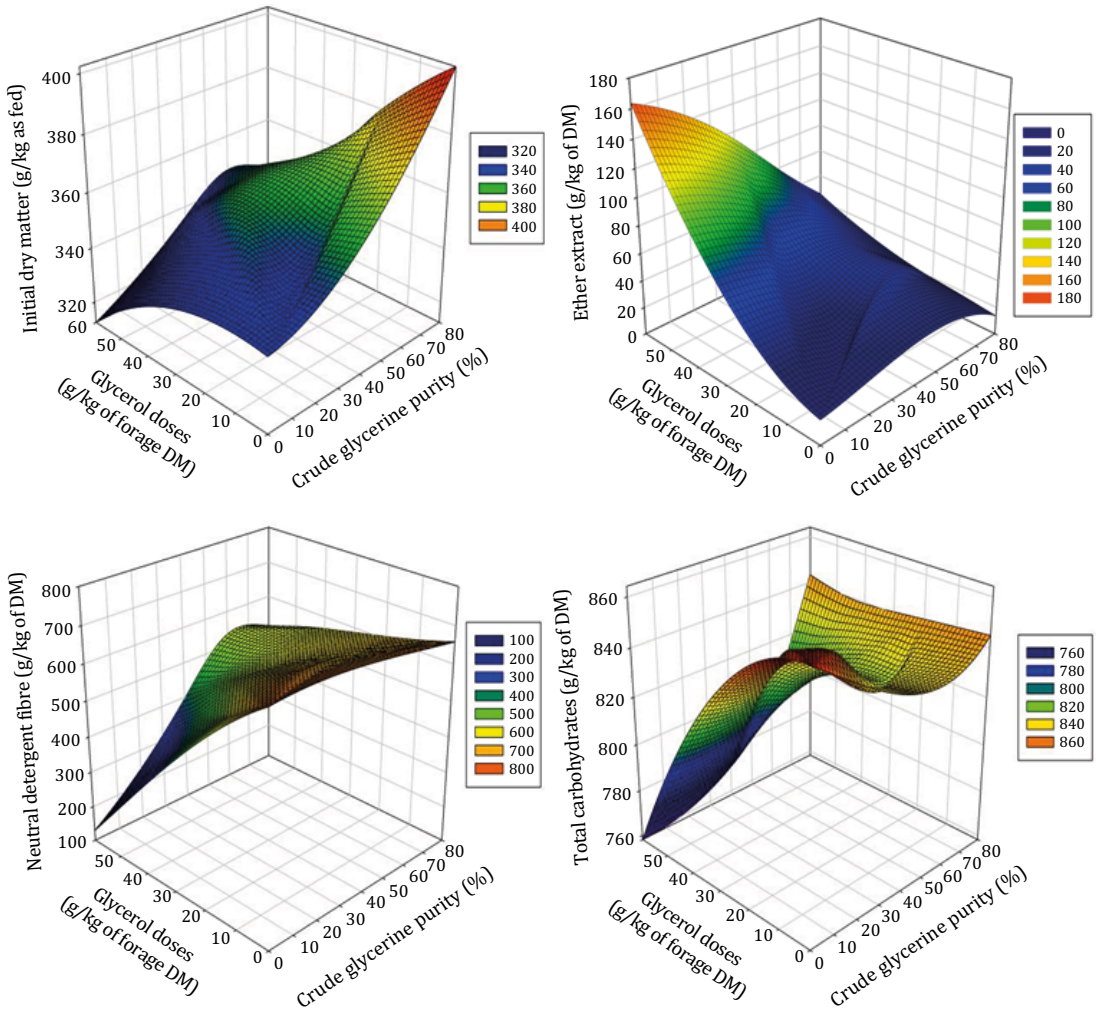
The DM content was reduced by 48.3 g/kg over time before ensiling, the forage presented 352.0 g DM/kg forage, and this level was reduced to 303.7 g DM/kg forage after 80 days of storage (Figure 1 and Table 2). There was an interaction between the degree of crude glycerine purity and the doses of glycerol for the initial DM, EE, NDF, and TC contents (Figure 1). The initial DM (Figure 1) was greater with high-purity crude glycerine and low glycerol doses, and decreased with the reduction of crude glycerine purity. However, when the glycerol doses were increased, there was no discrepancy in the initial DM with the different degrees of crude glycerine purity. The EE content was greater for the L60

treatment. There was no difference (P>0.05) in EE between the control treatment and the doses of glycerol with a high-purity crude glycerine (H20, H40, and H60).

The NDF content was greater (P<0.01) in the control, H20, H40, M20, and M40 treatments (Figure 1). The lowest (P<0.01) NDF values were obtained in the L60 treatment. The TC content was higher (P<0.01) in the control, L20, M20, and H20 treatments.

The degree of crude glycerine purity influenced the levels of final DM, CP, hemicellulose, and NFC (Table 2). The final DM and CP contents were greater (P<0.01) with the highest purity levels of crude glycerine (60 and 80%). The hemicellulose contents reduced when crude glycerine was used in Piatã grass silage, whereas NFC increased with the use of crude glycerine.

Greater CP levels (P<0.01) were observed for the glycerol doses of 20 and 40 g/kg (Table 2), whereas a greater NFC content was found with glycerol doses of 40 and 60 g/kg. The hemicellulose and ADF contents gradually reduced as the glycerol doses increased. Increasing glycerol doses (20, 40, and 60 g/kg) also resulted in the increase (P<0.01) of *in vitro* NDF digestibility by 15.5, 19.8, and 26.5%, respectively. There were no changes in total DM losses, effluent losses, or gas losses.



SEM - standard error of the means.
Effects of interaction between crude glycerine purity and glycerol doses: DMi: P = 0.02, SEM: 8.463; EE: P<0.01, SEM: 12.815; NDF: P = 0.03, SEM: 40.613; TC: P = 0.01, SEM: 15.924.

Figure 1 - Initial dry matter (DMi), ether extract (EE), neutral detergent fibre (NDF), and total carbohydrates (TC) of Piatã grass ensiled with three types of crude glycerine [low purity (40% of glycerol), medium purity (60% glycerol), and high purity (80% glycerol)], three doses of glycerol (20, 40, and 60 g/kg of forage DM), and control (no crude glycerine added).

The initial pH was higher when the low-purity crude glycerine was used, mainly in the greatest doses of glycerol (Table 3). However, the addition of crude glycerine resulted in lower final pH values, with no changes in relation to the different doses of glycerol. There was no difference (P>0.05) in the contents of isobutyric, valeric, and isovaleric acids, but these acids were only detected in the control treatment, at 4.0, 0.9, and 1.2 g/kg of DM, respectively.

For lactic, acetic, propionic, and butyric acids (Figure 2), there was an interaction between the degree of crude glycerine purity and the doses of glycerol. The highest levels of lactic and acetic acids occurred in the L60 treatment. The lowest (P<0.01) lactic acid content was observed in the control and H20 treatment. The concentration of acetic acid was diminished (P<0.01) with the lowest glycerol doses, mainly with the crude glycerine at the highest degree of purity.

For propionic acid, the greatest values (P<0.01) were obtained in the control and M20 treatments. For butyric acid, the three higher values (P<0.01) were obtained in the control, H20, and M60 treatments. The lowest butyric acid content was observed for the L40 and L60 treatments.

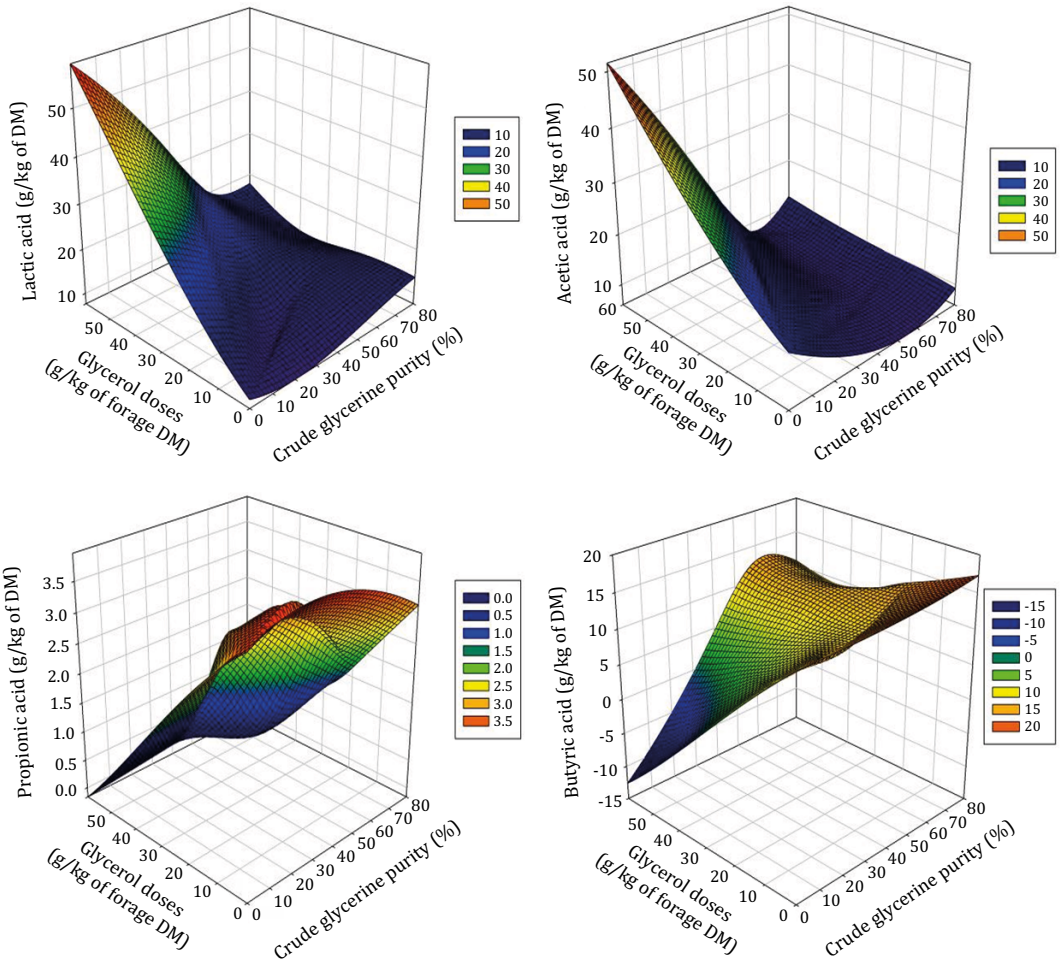
The population of LAB increased when crude glycerine was added, regardless of the crude glycerine purity (Table 3), and also with the highest glycerol doses. Greater (P<0.01) mould counts were observed in the M20 silage.

For mesophilic bacteria and yeasts, there was an interaction between crude glycerine purity and glycerol doses (Figure 3). The mesophilic bacteria count increased as there was an increase of the glycerol doses and the crude glycerine purity. The smallest mesophilic bacteria counts were observed in the control and L20 treatment. The greatest yeast counts were obtained in the treatments using 20 g/kg glycerol with medium-purity crude glycerine, and the lowest yeast counts were obtained in the control treatment, and with high glycerol doses with low-purity crude glycerine.

Table 2 - Levels of dry matter (DM) chemical composition (g/kg of DM), DM losses (g/kg of DM), effluent loss (g/kg of forage), and gas loss (g/kg of DM) of Piatã grass ensiled with three types of crude glycerine [low purity (40% of glycerol), medium purity (60% glycerol), and high purity (80% glycerol)], three doses of glycerol (20, 40, and 60 g/kg of forage DM), and control (no crude glycerine added)

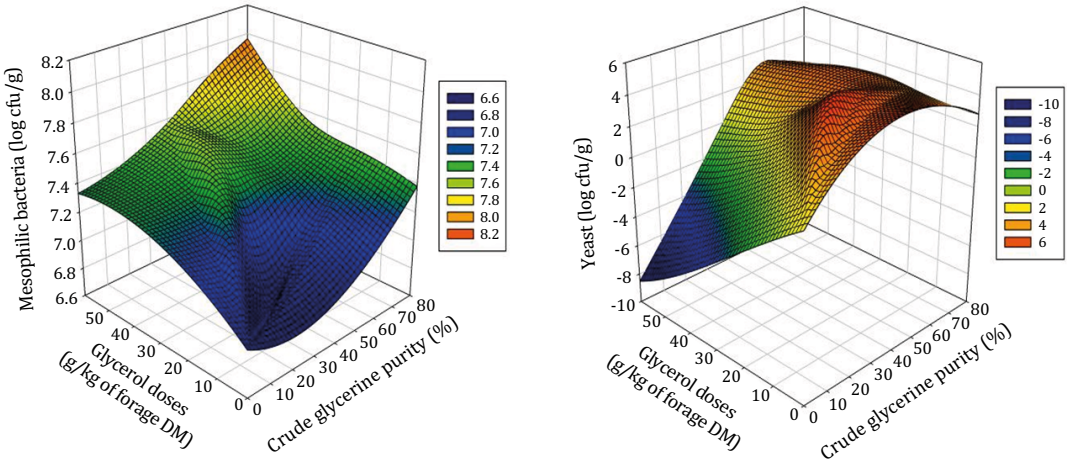
	Control	Types of crude glycerine (CG)			Glycerol (GL) doses (g/kg)			SEM	P-value		
		Low	Medium	High	20	40	60		CG	GL	CG*GL
Initial DM[1]	338.49cB	346.44bc	355.70ab	358.39a	363.82A	355.39A	341.33B	8.463	0.02	<0.01	0.02
Final DM[1]	283.71c	297.43bc	310.09ab	315.45a	312.55	308.61	301.71	12.26	0.02	0.19	0.25
Crude protein	38.75bB	39.74b	45.68a	46.82a	48.42A	44.08AB	39.75B	3.35	<0.01	<0.01	0.19
Ether extract	17.52bC	67.65a	57.62a	28.05b	31.70BC	45.33B	76.29A	12.815	<0.01	<0.01	<0.01
Neutral detergent fibre	709.11aA	515.80b	566.47b	561.97b	613.81B	549.96C	480.48D	40.613	0.03	<0.01	0.03
Acid detergent fibre	411.50A	294.16	314.82	303.09	344.91B	304.85B	262.31C	29.61	0.35	<0.01	0.05
Hemicellulose	297.61aA	225.60c	251.66b	258.88b	268.90A	245.11B	222.14C	14.71	<0.01	<0.01	0.19
Non-fibre carbohydrate	154.87bC	295.79a	245.33a	279.56a	221.88BC	276.47AB	322.34A	39.65	0.04	<0.01	0.17
Total carbohydrates	863.98aA	811.59b	811.80b	841.53a	835.68AB	826.43B	828.10C	15.924	<0.01	<0.01	0.01
NDF digestibility	691C	835	829	836	798B	828B	874A	34.3	0.92	<0.01	0.43
DM loss	160.16	176.01	140.3	134.08	134.42	167.65	148.32	48.66	0.17	0.37	0.71
Effluent loss	6.32	4.88	3.75	4.24	3.71	4.22	4.97	1.89	0.46	0.38	0.93
Gas loss	58.37	42.96	42.82	42.29	45.15	41.65	41.27	12.78	0.99	0.78	0.99

NDF - neutral detergent fibre; SEM - standard error of the mean.
[1] g/kg of forage.
Means with different lowercase letters differ at P≤0.05 by Tukey's test for types of crude glycerine. Means with different uppercase letters differ at P≤0.05 by Tukey's test for levels of glycerol. Significant interaction effects were presented in the figures.



SEM - standard error of the means.
Effects of interaction between crude glycerine purity and glycerol doses: lactic acid: P<0.01; SEM: 3.725; acetic acid: P<0.01, SEM: 2.057; propionic acid: P = 0.01, SEM: 0.642; butyric acid: P = 0.01; SEM: 1.548.

Figure 2 - Lactic, acetic, propionic, and butyric acids of Piatã grass ensiled with three types of crude glycerine [low purity (40% of glycerol), medium purity (60% glycerol), and high purity (80% glycerol)], three doses of glycerol (20, 40, and 60 g/kg of forage DM), and control (no crude glycerine added).



SEM - standard error of the means.
Effects of interaction between crude glycerine purity and glycerol doses: mesophilic bacteria: P = 0.01, SEM: 0.111; yeasts: P<0.01; SEM: 0.693.

Figure 3 - Mesophilic bacteria and yeasts of Piatã grass ensiled with three types of crude glycerine [low purity (40% of glycerol), medium purity (60% glycerol), and high purity (80% glycerol)], three doses of glycerol (20, 40, and 60 g/kg of forage DM), and control (no crude glycerine added).

Table 3 - Measurements of pH, volatile fatty acids (g/kg DM) and microorganisms counting (log cfu/g) of Piatã grass ensiled with three types of crude glycerine [low purity (40% of glycerol), medium purity (60% glycerol), and high purity (80% glycerol)], three doses of glycerol (20, 40, and 60 g/kg of forage DM), and control (no crude glycerine added)

	Control	Types of crude glycerine (CG)			Glycerol (GL) doses (g/kg)			SEM	P-value		
		Low	Medium	High	20	40	60		CG	GL	CG*GL
pH (initial)	5.97cC	6.31a	6.22ab	6.08bc	6.10BC	6.21AB	6.29A	0.113	0.01	0.01	0.25
pH (final)	4.78a	4.24b	4.43b	4.36b	4.41	4.31	4.30	0.145	0.03	0.22	0.92
Lactic acid	9.47cC	22.17a	16.92b	14.24bc	12.91BC	17.00A	13.42B	3.725	<0.01	<0.01	<0.01
Acetic acid	16.43aA	15.17a	7.58c	10.86b	9.03B	10.54B	14.02A	2.057	<0.01	<0.01	<0.01
Butyric acid	15.33aA	6.90c	11.64b	11.08b	11.21B	9.17C	9.24BC	1.548	0.01	0.02	0.01
Propionic acid	3.63A	1.51	1.07	1.39	2.39B	0.91C	0.92C	0.642	0.34	<0.01	0.01
Lactic acid bacteria	7.22bC	7.45a	7.44ab	7.60a	7.40BC	7.52AB	7.58A	0.133	0.01	<0.01	0.97
Mesophilic bacteria	6.92cD	7.35b	7.48b	7.67a	7.22C	7.50B	7.77A	0.111	<0.01	<0.01	0.01
Yeast	0.83cC	2.07bc	3.67a	2.16b	3.94A	2.68B	1.27C	0.693	<0.01	<0.01	<0.01
Mould	0.76abAB	0.92ab	1.26a	0.26b	1.51A	0.92AB	0.00B	0.798	0.04	<0.01	0.07

SEM - standard error of the means.
Means with different lowercase letters differ at P≤0.05 by Tukey's test for crude glycerine. Means with different uppercase letters differ at P≤0.05 by Tukey's test for glycerol. Significant interaction effects are present in the figures.

4. Discussion

The changes in DM content observed between fresh forage and the silage stored for 80 days (48.3 g/kg) were probably a result of the evaluation method that was used (oven drying), which removes volatile components (Woolford, 1984). For silage DM evaluation, the toluene distillation method is more indicated because the volume of distillate is corrected for total acids (by titration), ammonia, and ethanol (Brahmakshatriya and Donker, 1971). Therefore, the possible loss of volatile silage compounds may have influenced the final DM levels and, consequently, the calculation of DM losses.

The variations in DM components, such as CP, NDF, ADF, hemicellulose, and TC, occurred in response to the addition of crude glycerine. Since crude glycerine does not have these components (only glycerol, methanol, and lipids), its addition results in the dilution of these components on a DM basis. A similar result was observed by Orrico Junior et al. (2017) and Cunha et al. (2020) with the addition of different doses of crude glycerine to tropical grass silage. The effect of the interaction between the degree of crude glycerine purity and glycerol content on the NDF and TC contents can be explained by the change in the volume of crude glycerine that was needed to achieve the target percentages of glycerol (Table 1); the opposite occurred for the EE content, which is present at high levels in crude glycerine with low degrees of purity (Zavarize et al., 2014). Adding low-purity crude glycerine resulted in an increased concentration of EE, whereas high-purity crude glycerine led to little variation in EE content.

The use of diets with levels of up to 70 g/kg DM of EE can improve the production of milk, mainly to high-production cows; however, higher EE values may lead to a reduced intake and the digestibility of the diet (Palmquist and Jenkins, 1980). Therefore, care must be taken when high doses of low-purity glycerine are used during ensilage, which results in silages with higher EE values (114.6 g EE/kg DM). However, considering that a regular diet of high-producing dairy cows has around 50% of silage inclusion, the EE content of the diet could be adjusted with the inclusion of concentrate with low EE content.

The increase of NDF digestibility in function of the level of glycerol observed in this work is in accordance with the results presented by Orrico Junior et al. (2017). According to Chanjula (2017), the increase in NDF digestibility is probably due to the amount of energy provided by glycerol that contributed with the growth of ruminal microorganisms.

The addition of crude glycerine, as well as the increase in glycerol dosage before ensilage, increased initial pH values. However, after 80 days of storage, the addition of crude glycerine resulted in a low pH, regardless of the glycerol doses. The pH remained above the levels considered adequate for good fermentation (between 3.8 and 4.2). These pH values restrict the proteolytic enzymes of the plant as well as the growth of non-desirable bacteria (Borreani et al., 2018).

However, the levels of butyric acid observed were above the recommended (10 g/kg DM) by Kung Jr. et al. (2018) in the control treatment and in the treatment containing 20 g/kg of glycerol with high-purity crude glycerine. The high levels of butyric acid in the control treatment can be explained by the low concentration of soluble carbohydrates in the forage (50 g/kg DM) (Epifanio et al., 2014), which may have resulted in a less effective fermentation and with probable growth of *Clostridium spp*. On the other hand, the high levels of butyric acid in the H20 treatment may be linked to the difficulty of distributing the dose of 25 mL/kg of DM. This difficulty may have led to the growth of *Clostridium spp* at specific points into the silo, interfering in the final result. The use of organic fertilizers in the forage area, six months before this experiment, may have helped to guarantee the presence of *Clostridium spp* spores in the ensiled material (Müller et al., 2014).

Nevertheless, the addition of crude glycerine resulted in a larger population of LAB, which also increased with higher glycerol doses. *Lactobacillus spp*. can use glycerol as a source of energy (Rivaldi et al., 2013), which may result in a greater amount of lactic and acetic acids in the treatments with the highest doses of glycerol (60 g/kg), mainly with low-purity crude glycerine. The treatment with low-purity crude glycerine and highest dose of glycerol had the lowest propionic and butyric acid contents and the lowest mould and yeast counts. These changes can be justified by the lowest degree of crude glycerine purity, which requires a greater crude glycerine volume to reach the target level of glycerol. The greatest doses of crude glycerine (mL/kg DM) result in better homogenization of crude glycerine with forage, ensuring a greater uniformity of fermentation inside the silo. A good fermentation results in a quick reduction of pH, which inhibits the growth of yeast, mould, and *Clostridium spp* (Todorova and Kozhuharova, 2010; Borreani et al., 2018; Kung Jr. et al., 2018; Lopes et al., 2018).

For the parameters evaluated in this research, low-purity crude glycerine presented a similar or superior performance when compared with the other types of crude glycerine tested. In addition, its low market prices can make it an important additive to improve the fermentation process of tropical grasses. However, special attention should be given to the high levels of fat which may be present in silages that received high doses of this type of glycerine. In these cases, the mixture of this forage with low-fat concentrates is necessary to maintain the levels of dietary fat within the limits recommended by literature (Palmquist and Jenkins, 1980).

5. Conclusions

The use of low-purity crude glycerine (40% glycerol) at the dose of up to 60 g/kg DM does not affect negatively the fermentative profile and the lactic acid bacteria count in comparison with other types of crude glycerine. In addition, this combination results in greater nutritional value by increasing the ether extract contents, associated with a reduction in the levels of fibrous components.

The addition of glycerol (independent of crude glycerine types) is important to improve the levels of lactic acid and the nutritive value of Piatã grass silage.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: A.W. Schwingel, M.A.P. Orrico Junior and J. Lucas Junior. Data curation: A.W. Schwingel. Formal analysis: A.W. Schwingel, M.A.P. Orrico Junior and A.C.A. Orrico. Funding acquisition: A.W. Schwingel and R.A. Reis. Investigation: A.W. Schwingel and T. Fernandes. Methodology: A.W. Schwingel and T. Fernandes. Project administration: A.W. Schwingel and J. Lucas Junior. Visualization: J. Lucas Junior. Writing-review & editing: R.O. Souza.

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