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Fermentative and bromatological value of Piatã palisadegrass ensiled with different additives

Bolson, D.C.[1@]; Pereira, D.H[1]; Pina, D.S.[1]; Pedreira, B.C.[2]; Mombach, M.A.[2] and Xavier, I.M.[1]

[1]Institute of Agricultural and Environmental Sciences. Federal University of Mato Grosso. Mato Grosso. Brazil.
[2]Embrapa Agrosylvopastoral. Mato Grosso. Brazil.

Additional keywords

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Summary

This study assessed the fermentation profile, losses and the chemical composition of Piatã palisadegrass (*Brachiaria brizantha* cv. Piatã) ensiled with different additives. The experimental design was completely randomized, with four replicates per treatment, and five treatments with different additives, as follows: T1 control without additives; T2 - microbial inoculant SiloMax Centurium (Matsuda); T3 - enzyme-bacterial inoculant Sil All C4 (Alltech do Brasil); T4 - corn meal (100 g kg^{-1} in the fresh matter); and T5 - crude glycerin (100 g kg^{-1} in the fresh matter), totaling 20 experimental silos. Addition of cornmeal caused an increase in the dry matter (DM) and non-fiber carbohydrates (NFC) contents and a decrease in the neutral detergent neutral (NDF) content of the silage. The highest loss of total DM (124.7 g kg^{-1}) was observed for the silage without additives, whereas addition of cornmeal provided the lowest loss of total DM (32.2 g kg^{-1}). Lower pH values and higher titratable acidity values were observed in the silages containing cornmeal and crude glycerin, respectively, as well as greater production of lactic acid. It is recommended to include corn meal at 100 g kg^{-1} in the fresh matter, as it provides a better fermentative profile and nutritive value, in addition to lower losses of DM. In addition, the inclusion of glycerin at 100 g kg^{-1} of the fresh matter suggested it as a potential additive.

Valor bromatológico e fermentativo da silagem de capim piatã

Palavras chave-adicionais

Glicerina bruta.
Capim tropical.
Ensilagem.

Resumo

Objetivou-se avaliar o perfil fermentativo, as perdas e a composição química da silagem de *Brachiaria brizantha* cv. Piatã com diferentes aditivos. O delineamento experimental foi inteiramente casualizado, com quatro repetições por tratamento e cinco tratamentos com diferentes aditivos, como segue: T1- controle sem aditivos; T2 - inoculante microbiano SiloMax Centurium (Matsuda); T3 - inoculante ênzimo microbiano Sil All C4 (Alltech do Brasil); T4 - fubá de milho (100 g kg^{-1} na matéria natural) e T5 - glicerina bruta (100 g kg^{-1} na matéria natural), totalizando 20 silos experimentais. A adição de fubá de milho elevou os teores de matéria seca (MS) e carboidratos não-fibrosos (CNF) com diminuição na fibra em detergente neutro (FDN). A maior perda de MS total (124,7 g kg^{-1}) foi observado para a silagem sem aditivos, enquanto que a adição de fubá de milho reduziu as perdas de MS total (32,2 g kg^{-1}). Os menores valores de pH foram observados nas silagens com fubá de milho e acidez titulável e os mais elevados nas silagens com glicerina bruta, bem como uma maior produção de ácido láctico. Recomenda-se a inclusão de fubá de milho em 100 g kg^{-1} de matéria natural, uma vez que fornece um melhor perfil fermentativo e composição química, além de reduzir as perdas de MS. Além disso, a inclusão de glicerina em 100 g kg^{-1} de matéria fresca mostrou-se como um aditivo com potencial de utilização.

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dheymecristina@hotmail.com

INTRODUCTION

Forage production is dependent on seasonal climatic factors and thus varies between high in the rainy season, and low in the dry season under tropical conditions. In this sense, forage conservation is an essential management practice to ensure good-quality food in the periods of forage scarcity to meet the animal requirements throughout the entire year (Azevedo *et al.*, 2014) and increase the use efficiency the pasture on the period of greater production.

The most commonly adopted technique is preserving the surplus forage production during the rainy season as silage. Nevertheless, according to Evangelista *et al.* (2004), grasses have a low dry matter content, high buffering capacity, and low concentration of soluble carbohydrates. Those characteristics impair the preservation process through silage because of the possibility of secondary fermentation, which cause large losses of dry matter. Conversely, when ensiled at young stage, they present a greater nutritional value (Silva *et al.*, 2011).

Given these difficulties, the grass silage requires additives to provide good fermentation conditions. The microbiological and enzymatic additives and by-products should be evaluated for their potential of use.

By-products such as meals, citrus pulp, and currently crude glycerin, have been employed as a complement in animal feeding. Additionally, according to Santos *et al*. (2010) these products have high concentrations of soluble carbohydrates, which improves the fermentation profile and nutritional value and consequently reduces the losses during the ensiling process.

The main objectives of using additives in the ensiling process are to improve the quality of fermentation in the silo. The dry matter, soluble carbohydrates and/or decreasing the pH of the ensiled material can be changed, inhibiting the growth of undesirable microorganisms such as enteric bacteria, clostridia, yeasts, *Listeria*, bacilli, etc. (Zopollatto *et al*. 2009). The adding of beneficial microorganisms to dominate the fermentation generate end products that will not inhibit the intake and production of animals, besides contributing to increasing the recovery of dry matter from the preserved material (Kung Jr. *et al*., 2003).

In this way, this study assessed the fermentative and chemical characteristics of Piatã palisadegrass (*Brachiaria brizantha* cv. Piatã) ensiled with different additives and hence know its potential for silage.

MATERIAL AND METHODS

Sample preparation

The experiment was conducted in the Animal Nutrition Laboratory and Forage Crops and Institute of Agricultural and Environmental Sciences, at Federal University of Mato Grosso, located in the municipality of Sinop/MT, Brazil, between at 2012 and 2013. Silages were prepared by using 20 PVC silos with four replicates per treatment and volume of 2.75 liters, provided with Bunsen valves and the average specific mass in fresh matter was 636.93 ± 11 kg/m^3. Five additives were evaluated in the ensiling process, composing in the following treatments: T1 control without additives; T2 - microbial inoculant SiloMax Centurium (Matsuda); T3 - enzyme-microbial inoculant Sil All C4 (Alltech do Brasil); T4 - cornmeal (100 g kg^{-1} in the fresh matter) and T5 - crude glycerin (100 g kg^{-1} in the fresh matter).

The enzyme-microbial inoculant Sil All C4 consisted of homofermentative bacteria (*Lactobacillus plantarum*, *Pediococcus acidilactici* and *Lactobacillus salivarius*) and heterofermentative bacteria (*Enterococcus faecium*), in addition to enzymes (amylase, cellulase, xylanase, and hemicellulolytic enzymes), with inoculation rates of 1.89×10^{10} cfu/g for total lactic bacteria and 2.10×10^9 cfu/g for heterofermentative bacteria. The microbial inoculant SiloMax Centurium was composed of *Lactobacillus plantarum*, *Pediococcus pentosaceus* and sucrose, with an inoculation rate of 2.5×10^{10} cfu/g. The inoculation dose was applied as recommended by the manufacturer. The composition of crude glycerin was 820 g kg^{-1} of glycerol, 5.2 g kg^{-1} of methanol, 70.34 g kg^{-1} of mineral and pH of 6.00.

Chemical analyses

The analysis of dry matter (DM), ash, crude protein (CP) and ether extract (EE) were determined by the procedures of AOAC (1990). Acid detergent fiber (ADF) was determined by method of Van Soest and Robertson (1985). Neutral detergent fiber (NDF) was determined with heat-stable alpha-amylase without the use of sodium sulfite (Mertens, 2002). The chemical composition before of ensilage is shown in **table I**.

For the calculation of non-fiber carbohydrate (NFC) levels the method proposed by Hall (2000) was used, as follows: NFC = 100 - [CP + NDF + EE + Ash]. Ammoniacal nitrogen ($N\text{-}NH_3$) was evaluated by the method proposed by Chaney and Marbach (1962) in a sample of silage diluted in water and trichloroacetic acid (10%), using a spectrophotometer (Bioespectro SP-220) with 625 nm wavelength reading.

Soluble carbohydrates (SC) were determined by spectrophotometry utilizing a spectrophotometer (Bioespectro SP-220) with 490 nm reading, according to the technique described by Johnson *et al*. (1966).

The pH and titratable acidity were determined according to the technique described by Cherney and Cherney (2003), following the method proposed by Playne and McDonald (1966), utilizing a pH meter. Based on the DM content, buffering capacity, and soluble carbohydrate content of the samples of fresh

Table I. Chemical composition of Piatã palisadegrass before ensilage (Composição química do capim Piatã antes da ensilagem).

	Control	SiloMax[3]	Sil All[4]	Cornmeal[5]	CG[6]
DM[1]	254.9	242.7	230.3	278.0	273.6
Ash[2]	78.4	78.8	80.7	70.7	83.1
CP[2]	126.4	123.4	130.9	129.2	102.9
EE[2]	16.0	30.8	26.8	16.8	34.6
NDF[2]	539.6	669.5	613.5	518.2	488.2
ADF[2]	270.3	261.4	270.6	239.9	204.2
pH	5.48	5.06	5.21	4.65	4.74
SC[2]	20.9	31.5	25.1	38.3	20.5

[1]g kg^{-1}; [2] g $kg^{-1}$of DM; [3]microbial inoculant SiloMax Centurium (Matsuda); [4]enzyme-microbial inoculant Sil All C4 (Alltech do Brasil); [5]100 g kg^{-1} in the fresh matter; [6]crude glycerin (100 g kg^{-1} in the fresh matter). DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber: SC: soluble carbohydrates.

forage, the coefficient of fermentation was calculated according to the equation proposed by Weissbach and Honig (1996), cited by Oude Elferink *et al*. (2000).

A High-Performance Liquid Chromatograph (HPLC) with reversed-phase C18 column coupled to an UV detector with 210 wave length was used to determine the acetic, propionic, lactic, and butyric organic acids (Oliveira *et al*., 2010).

The losses of effluent, gas and dry matter were quantified by the method Jobim *et al*. (2007) as the difference in the weight of the set before and after ensiling, compared with the fresh mass of the ensiled sample.

Statistical Analysis

The experiment was conducted in a completely randomized design with four replicates per treatment, and the treatments' means were compared using Tukey's test at 5% probability for Type I error.

RESULTS AND DISCUSSION

Chemical composition

The addition of 100 g kg^{-1} of cornmeal and glycerin in the fresh matter elevated DM contents of 277.4 g kg^{-1} and 256.5 g kg^{-1}, respectively **(table II)** with significance in relation with others treatments ($p<0.05$). This response was due to the high levels of DM of the utilized additives, despite the fluid form of crude glycerin.

These values were higher than 250 g kg^{-1} DM suggested by Haigh (1999) for minimum formation of effluent, and close to the 300 g kg^{-1} recommended by Rotz and Muck (1994) to limit the growth of bacteria of the genus *Clostridium*. In this regard, the control silages and the silages with microbial additive and microbial-enzyme inoculant can to have been subjected to undesirable fermentations due to increase N-NH_3 values **(table III)** and losses of total dry matter **(table V)**.

The use of additives that contribute to the increase in dry matter content in grass silage is commonly used because of the low DM content in the development phase with better nutritional value (Azevedo *et al*., 2014; Epifanio *et al*., 2014; Silva *et al*., 2011). Therefore, the use of cornmeal is always the best option as an additive as well as other available meal.

Regarding Ash **(table II)**, the treatment with glycerin displayed the highest concentrations of this nutrient compared with other treatments, possibly due to the original composition of this compound, which did not interfere with the composition of the end product.

The EE values were higher in the treatments with cornmeal and crude glycerin, 33.1 g kg^{-1} and 32.4 g kg^{-1}, respectively, compared with the control treatment, microbial and enzyme-microbial inoculants **(table II)**. Probably because of the EE content of the cornmeal and glycerin, which increased the values of this nutrient in the silage.

The inclusion of microbial and enzyme-microbial inoculants, and cornmeal provided higher CP contents, given that inoculant provide bacteria that contribute to improving fermentation, which can decrease the occurrence of proteolysis and deamination resulting from the control of undesirable microorganisms (McDonald *et al*., 1991). The presence of cornmeal contributes to a rapid pH decline, which promotes the lactic fermentation and contributes to reducing proteolysis, in addition to decreasing the forage moisture content.

Evaluating the use of moisture-absorbing additive, Costa *et al*. (2011) included 150 g kg^{-1} of millet meal in Piatã grass silage and found CP contents of 139 g kg^{-1} with the use of millet and 97.8 g kg^{-1} without additive. It should be stressed that the millet usually has higher CP contents than corn.

The CP levels using crude glycerin were similar to the control treatment, because it has low concentration of nitrogen compounds in the total composition of the additive, providing a dilution effect on the silage, or

Table II. Mean contents of DM, Ash, CP, EE, NDF, ADF, NFC and hemicellulose of Piatã palisadegrass silage with different additives (Médias dos teores de MS, cinzas, PB, EE, FDN, FDA, CNF e hemicelulose da silagem de capim Piatã com diferentes aditivos).

	Treatment					SEM[6]	P-value
	Control	SiloMax[2]	Sil All[3]	Cornmeal[4]	CG[5]		
DM (g kg^{-1})	238.8[c]	233.9[c]	232.4[c]	277.4[a]	256.5[b]	1.75	0.0001
Ash[1]	72.2[b]	81.5[b]	83.8[b]	70.6[b]	94.5[a]	1.66	0.0001
CP[1]	100.7[b]	114.0[a]	115.6[a]	117.5[a]	99.1[b]	1.34	0.0001
EE[1]	19.7[b]	15.3[b]	20.5[b]	33.1[a]	32.4[a]	1.47	0.0012
NDF[1]	624.8[a]	628.9[a]	633.6[a]	449.0[b]	601.1[a]	4.58	0.0001
ADF[1]	220.2[bc]	233.7[bc]	248.6[b]	205.2[c]	290.7[a]	2.97	0.0001
NFC[1]	182.6[b]	160.4[b]	146.6[b]	329.8[a]	172.9[b]	4.47	0.0001
Hemicellulose[1]	404.6[a]	395.2[a]	385.0[a]	243.8[b]	310.4[ab]	4.72	0.0005

[1] g kg^{-1} of DM; [2]microbial inoculant SiloMax Centurium (Matsuda); [3]enzyme-microbial inoculant Sil All C4 (Alltech do Brasil); [4]100 g kg^{-1} in the fresh matter; [5]crude glycerin (100 g kg^{-1} in the fresh matter); [6] standard error of the means. Means followed by the same letters in the row do not differ by Tukey's test (α = 0.05). DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: non-fiber carbohydrates.

even from the carrying of the compounds towards the effluent, due their liquid form.

Except for the addition of cornmeal, the inclusion of the other additives did not cause changes in the NDF contents of the silages as compared with control **(table II)**. For the treatment with cornmeal, the NDF values were reduced, since this additive is poor in fiber. Reduction in the fiber values were found by Azevedo *et al*. (2014), in silage of Piatã grass, which showed 691.7 g kg^{-1} and 661 g kg^{-1} of NDF in the treatments with enzyme-microbial inoculant and with inclusion of millet meal, respectively, as compared with the control treatment (727.5 g kg^{-1}).

For the treatment with enzyme-microbial inoculant, the ADF values were similar **(table II)** to those in the treatment with microbial inoculant and the control treatment. This fact can be explained by the lack of activity of the enzymes present in the enzyme-microbial inoculant causing solubilization of the cell-wall components and increasing the availability of soluble carbohydrates for fermentation by lactic-acid bacteria. Similar results were observed in silages of Tanzania and Mombaça grasses containing enzyme-microbial inoculant, with 473 g kg^{-1} and 454 g kg^{-1} ADF, respectively, as compared with the control treatment, which had 461 g kg^{-1} of ADF (Coan *et al*., 2005).

The inclusion of cornmeal reduced the NDF and Hemicellulose values **(table II)** and presented to higher the NCF values. Probably due to the dilution effect on the fiber because of the lower NDF in chemical composition (NRC, 2016). This effect can be observed in studies reported by Coan *et al*. (2005) and Azevedo *et al*. (2014). The other treatments were similar of the fiber content.

Crude glycerin increased the ADF value **(table II)** but this is not in agreement with the literature that attributed reduction in the ADF of silage with additives. The increase in the levels of ADF occurs mainly due to the utilization of soluble carbohydrates by microorganisms during ensiling and consequent increase of the cellular wall (Balieiro Neto *et al*., 2007).

There was no significant effect on the treatment with enzyme-microbial inoculant in relation to the hemicellulose content, though it contained hemicellulolytic enzymes in its composition; therefore, it can be inferred that there was lack of enzymatic activity.

Conservation efficiency

The pH values **(table III)** with inclusion of microbial and enzyme-microbial inoculant did not differ in relation to the control treatment. Thus, these additives did not meet the expected objective, yielding higher final pH values. These values are above the 3.8 to 4.2 considered ideal in the literature to obtain good-quality silages (Van Soest 1994).

Divergent results were found by Azevedo *et al*. (2014), with final pH values considered ideal, of 4.00 and 4.25. In Tanzania grass silage, Santos *et al*. (2008) evaluated the addition of enzyme-microbial inoculant and reported a lower pH value in the treatment with inoculant than control: 4.32 and 4.74, respectively.

The pH values in the treatments with cornmeal and glycerin were considered acceptable, characterizing them as having good fermentation quality in relation to this parameter. A fast pH decline is essential for the final quality of the ensiled mass, ensuring reduction in the proteolytic activity and reducing the growth of undesirable microorganisms, particularly enteric bacteria and clostridia.

However, according to Woolford (1984), the pH alone cannot be considered a safe criterion to evaluate the fermentations, because its inhibiting effect on the bacteria depends on the rate of decline of the ionic concentration and on the moisture degree of the medium. This demonstrates the importance of evaluating the titratable acidity, which, according to Silva and Queiroz (2002), is based on the fact that the pH does not have a perfect correlation with the lactic acid content of the silage, which should contribute to reducing it.

In the treatments with cornmeal and crude glycerin, there was a significant decrease in pH as compared with the other treatments **(table III)** and a considerable increase in the titratable acidity values, which is possibly explained by the greater production of lactic acid. For the other treatments, in turn, it is possible to infer that the fermentation took longer to stabilize. This is explained not only by the high pH, but also by

Table III. Mean of pH, TACIDITY, N-NH_3, SC and CF of Piatã palisadegrass silage with different additives (Médias de pH, acidez tituilável (TACIDITY), nitrogênio amoniacal (N-NH_3), carboidratos solúveis (SC) e coeficiente de fermentação (CF) da silagem de capim Piatã com diferentes aditivos).

	Treatment					SEM[9]	p-value
	Control	SiloMax[5]	Sil All C4[6]	Cornmeal[7]	CG[8]		
pH	4.55[a]	4.63[a]	4.63[a]	4.22[b]	4.29[b]	0.17	0.0001
TACIDITY[1]	34.92[b]	35.09[b]	34.09[b]	53.00[a]	52.81[a]	0.88	0.0001
N-NH_3[2]	157.1[a]	167.8[a]	135.0[b]	100.2[c]	156.2[a]	2.20	0.0001
SC[3]	15.1[c]	28.9[a]	22.6[b]	20.8[b]	15.1[c]	1.00	0.0001
CF[4]	25.95	24.97	23.61	28.64	27.77	-	-

[1]expressed as mL of NaOH 0.1N until reaching pH 7.0; [2]g kg^{-1} of nitrogen total; [3]g kg^{-1} of DM; [4]%; [5]Microbial inoculant SiloMax Centurium (Matsuda); [6]Enzyme-microbial inoculant Sil All C4 (Alltech do Brasil); [7]100 g kg^{-1} in the fresh matter; [8]Crude glycerin (100 g kg^{-1} in the fresh matter); [9]standard error of the means. Means followed by the same letter in the row do not differ by Tukey's test (α= 0.05). TACIDITY: titratable acidity; N-NH_3: ammoniacal nitrogen; SC: soluble carbohydrates; CF: coefficient of fermentation.

Table IV. Mean contents of AC_{AC}, $PROP_{AC}$, BUT_{AC} and LAC_{AC} of Piatã palisadegrass silage with different additives (Médias dos teores de ácido acético, propiônico, butírico e lático da silagem de capim Piatã com diferentes aditivos).

	Treatments					SEM[6]	p-value
	Control	SiloMax[2]	Sil All [3]	Cornmeal[4]	CG[5]		
AC_{AC}[1]	20.6[a]	25.2[a]	23.8[a]	23.1[a]	22.3[a]	1.32	0.4458
$PROP_{AC}$[1]	0.64[a]	0.59[a]	0.64[a]	0.57[a]	0.47[a]	0.18	0.4182
BUT_{AC}[1]	0.05[a]	0.08[a]	0.07[a]	0.06[a]	0.07[a]	0.07	0.0074
LAC_{AC}[1]	19.7[ab]	17.5[ab]	15.7[b]	22.7[a]	21.7[ab]	1.25	0.0435

[1]g kg^{-1} of DM; [2]microbial inoculant SiloMax Centurium (Matsuda); [3]enzyme-microbial inoculant Sil All C4 (Alltech do Brasil); [4]100 g kg^{-1} in the fresh matter; [5]crude glycerin (100 g kg^{-1} in the fresh matter); [6]standard error of the means. Means followed by the same letter in the row do not differ by Tukey's test (α= 0.05). AC_{AC}: acetic acid; $PROP_{AC}$: propionic acid; BUT_{AC}: butyric acid; LAC_{AC}: lactic acid.

the low titratable acidity, which shows that there was no significant production of lactic acid or other acids.

The treatment with cornmeal showed acceptable $N\text{-}NH_3$ values, which, according to Van Soest (1994), indicates that there was no excessive protein breakdown into ammonia and amino acids compose the largest part of the non-protein nitrogen. On the other hand, the control treatment, SiloMax and glycerin presented undesirable values - above 150 g kg^{-1} - which shows that there was a considerable protein breakdown and such silages are consumed less by animals, whereas treatment Sil-All showed an intermediate value of 135 g kg^{-1} of nitrogen total **(table III)**.

With regard to the SC levels after ensiling **(table III)**, treatment SiloMax showed better results (28.9 g kg^{-1}) followed by treatments Sil-All and cornmeal, with 22.6 and 20.8 g kg^{-1}, respectively. The lowest proportions of soluble carbohydrates were observed in the control treatment and in the treatment with glycerin, both with 15.1 g kg^{-1} of DM.

Alterations in the SC contents are related to the use of these carbohydrates by the bacteria from the fermentation process as substrate for their growth, leading to the synthesis of latic acid (Muck 2010). Another factor that contributed to the reduction in the SC contents is the losses by effluent, which carry highly digestible substances such as these carbohydrates out of the silo.

Regarding the CF, values were lower than those indicated by Weissbach and Honig (1996), cited by Oude Elferink *et al*. (2000), because materials ensiled with a low DM content or insufficient concentrations of soluble carbohydrates present a low CF (<35), which may result in inappropriate fermentations and the production of silage with low nutritive value.

The amounts of acetic (AC_{AC}), propionic ($PROP_{AC}$), butyric (BUT_{AC}) acids produced during the fermentation **(table IV)** did not differ among the treatments. However, Perim *et al*. (2014) observed lower acetic acid values in silage of Piatã grass treated with energetic meals.

The values observed for propionic acid can be accepted, as it showed low results (below 1 g kg^{-1}), indicating that there was no degradation of lactic acid by butyric bacteria during the fermentation process. This corroborates the result for butyric acid, which was satisfactory for the fermentation process since values were relatively low and the content of this acid is the main negative indicator of quality in the silage's fermentation process.

Production of lactic acid was highest **(table IV)** in the treatment with cornmeal (only numerically, but without significative differences in relation with the control, Silo Max and glycerine treatments), consequently, it improved the quality of the fermentation process with a rapid pH decline and inhibition of the growth of spoiling microorganisms. However, for the treatment with enzyme-microbial inoculant, the lactic acid levels were low as compared with the others, thus limiting the quality of the end product. For the other treatments, the lactic acid contents were not significant in relation to control.

Losses in the conservation

Regarding the losses during the fermentation process **(table V)**, for the treatment with microbial and enzyme-microbial additives, the losses by effluent (EFL_L)

Table V. Mean contents of EFF_L, GAS_L and TDM_L of Piatã palisadegrass with different additives (Média dos teores de perdas por efluente (EFF_L), gas (GAS_L) e matéria seca total (TDM_L) da silagem de capim Piatã com diferentes aditivos).

	Treatment					SEM[6]	p-value
	Control	SiloMax[2]	Sil-All C4[3]	Cornmeal[4]	CG[5]		
EFF_L[1]	35.6[b]	34.3[b]	39.3[b]	16.7[c]	54.2[a]	1.60	0.0001
GAS_L[1]	96.2[a]	46.5[b]	14.2[b]	15.4[b]	51.0[b]	3.04	0.0014
TDM_L[1]	127.0[ab]	79.6[bc]	42.1[cd]	32.2[d]	104.1[b]	3.17	0.0001

[1]g kg^{-1} of DM; [2]microbial inoculant SiloMax Centurium (Matsuda); [3]enzyme-microbial inoculant Sil All C4 (Alltech do Brasil); [4]100 g kg^{-1} in the fresh matter; [5]crude glycerin (100 g kg^{-1} in the fresh matter); [6]standard error of the means. Means followed by the same letter in the row do not differ by Tukey's test (α= 0.05). EFF_L: effluent losses; GAS_L: gas losses; TDM_L: total dry matter losses.

did not differ as compared with the control treatment. However, addition of glycerin to the ensilage increased the EFL_L (54.2 g kg^{-1}), possibly due to its being in liquid form.

The lowest EFL_L values were found with addition of cornmeal (16.7 g kg^{-1}), as this additive's function is to absorb moisture. In this regard, McDonald *et al*. (1991) report the effect of the lower production of effluent with inclusion of moisture-absorbing additives, which also provided an increased DM content in the ensiled mass.

The dry matter content is also relevant for EFL_L, since the greater the DM content of the ensiled mass, the lower the moisture level in it, and thus the lower the effluent losses. These losses are not favorable during ensiling, because the generated fluid contains highly digestible compounds such as soluble carbohydrates, organic acids, minerals, and soluble nitrogen compounds, which will decrease the silage's nutritive value (McDonald *et al*., 1991).

The gas losses (GAS_L) in the treatments with cornmeal and enzyme-microbial inoculant **(table V)** were within the range considered acceptable for silages (10 to 20 g kg^{-1} of the total DM losses), since this type of loss is considered unavoidable during the ensiling process (McDonald *et al*., 1991).

Decreased gas losses were observed by Penteado *et al*. (2007) when evaluating silage of Mombaça grass at different regrowth ages with addition of an enzyme-microbial inoculant, resulting in average gas losses of 13.0 g kg^{-1} in the DM.

Higher production of gas is associated with enteric bacteria. Butyric fermentation, caused by bacteria of the genus *Clostridium* sp., is also noteworthy. The highest gas loss values being in the control (96.2 g kg^{-1}) and glycerin (51.0 g kg^{-1}) treatments may be a result of the high moisture content and the greater proteolysis observed in these treatments, respectively. Greater gas losses were also reported by Ribeiro *et al*. (2009), who evaluated Marandu grass silages treated with inocula and moisture absorbers, with 85.0 g kg^{-1} of losses in the control treatment.

The total DM losses depend on the losses of effluent and gas; thus, these losses will have a direct influence on the loss of total DM. The treatments with enzyme-microbial additive and cornmeal reduced the total losses of DM due to the improvement in the fermentation profile, with reduction in CO_2 production. Inclusion of glycerin and microbial inoculant did not differ from control in relation to DM losses, and gas production was considered above the ideal. However, the DM contents of the silage treated with glycerin did not affect the fermentation profile, with no direct consequences to undesirable fermentations.

CONCLUSION

It is recommended to include 100 g kg^{-1} cornmeal in the fresh matter in the ensiling of *Brachiaria brizantha* cv. Piatã, because it provides a better fermentation profile and nutritive value, besides lower dry matter losses. In addition, inclusion of crude glycerin in the fresh matter of the ensilage don't is recommended because it promotes higher losses during the fermentation process, with difficulties of being used as an additive in grass silage.

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