PASTURE AND FORAGE UTILIZATION

Effect of using inoculant on elephant grass silage with additives

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ABSTRACT. This study aimed to evaluate the use of inoculant on silage of elephant grass, wet brewery waste and buriti meal at different silo opening times. The experimental silage tested was composed of 50% elephant grass, 30% wet brewery waste, and 20% buriti meal. The experimental design was randomized block in a factorial arrangement (2 x 4), where the treatments consisted of the use or not of inoculant during silage processing, and four silo opening times (7, 14, 21, and 28 days). Data collected were firstly subjected to ANOVA and subsequent Tukey's test. Results were considered significant at $p \leq 0.05$. The use of inoculant in silage production provided more acidic pH ($p < 0.05$) and higher ($p < 0.05$) effluent losses. Longer silo opening times resulted in more alkaline pH ($p < 0.05$) and higher ($p < 0.05$) effluent losses. The use of inoculant in silage production and longer silo opening times provided higher ($p < 0.05$) content of ash, crude protein, and fats, and lower ($p < 0.05$) content of dry matter and fiber (neutral and acid detergent). However, there was no interaction between the factors evaluated in this study. It was concluded that the inoculant may be used in the production of elephant grass silages, wet brewery waste, and buriti meal, providing a more acidic pH, higher effluent losses, lower contents of dry matter and fiber, and higher nutritional content. As the opening time was extended up to 28 days, there was more alkaline pH, higher effluent losses, lower dry matter and fiber content, and higher nutritional content.

Keywords: dry matter; effluent losses; ensiling; fiber, forage.

Introduction

Silage production from tropical grasses either in an area designed for direct production or as a way of using produced surplus in pasture or forage areas, during the favorable period of time to the forage growth, is an interesting strategy for the livestock roughage supplementation (Faria et al., 2010). Because *Pennisetum* grasses has a high green mass production per hectare during the rainy season, silage is one of the main preservation methods to improve the use of the nutritional potential of this grass (Ferrari Junior, Paulino, Possenti, & Lucenas, 2009). Elephant grass (*Pennisetum purpureum* Schum.) is a forage with excellent dry matter potential that may be used as an alternative to annual crops frequently used to silage production. For this purpose, cuts of this forage have been recommended when it is young aiming a better nutritional value and eliminate the excess of moisture (Monteiro, Abreu, Cabral, Ribeiro, & Reis, 2011).

A high content of moisture at the time of cutting, low soluble carbohydrate content, and high buffering capacity of grasses inhibit the fermentation process, impairing the production of good quality silages. Furthermore, grasses with high moisture produce large amounts of effluents with high content of organic and mineral compounds that are lost (Ferrari Junior et al., 2009). 60 days is the ideal cutting time for the production of elephant grass silage (Muck, 2010; Santos et al., 2010). However, the dry matter content at this age is very low, between 15 and 20%, and it is not recommended for ensiling mainly because it negatively influenced both the fermentation process, and also promotes some increase in nutrients loss due to effluent production (Monteiro et al., 2011; Cardoso et al., 2016). Thus, it is recommended the use of products to improve the dry matter content during the silage production (Pires et al., 2009; Monteiro et al.,

2011) either through the improvement of fermentation characteristics or by the reduction of losses inherent to the process (Ferrari Junior et al., 2009).

For decades, producers have had a wide variety of silage additives available to assist in forage preservation. Silage additives are generally classified in four categories based on their effects on silage preservation: (1) fermentation stimulants, (2) fermentation inhibitors, (3) aerobic deterioration inhibitors, and (4) nutrients and absorbents. Silage additives can have more than one mode of action based on the 4 categories above. In addition, the above classification focuses on effects largely within the silo; however, the effects of these additives on livestock are often more important to the producer to choose their use (Muck et al., 2018).

Previous studies pointed that the use of nutrient-rich additives (usually higher than 80%) can enable the absorption of moisture, increasing the dry matter content of the ensiled material, and even promote improvements in the chemical composition of the produced silage (Carrera et al., 2012; Souza, Goes, Silva, Yoshihara, & Prado, 2015; Cardoso et al., 2016). In this sense, wet brewery waste is an alternative food source widely used for ruminants, which is generated in large amounts during the production of beer, being obtained throughout the year at a low cost, presenting an excellent nutritional content (protein, fiber, and soluble carbohydrates), especially after the dehydration process. However, this feedstuff presents a low dry matter content, which hinders the transport, storage and preservation of this residue (Souza et al., 2012; Muck et al., 2018). In turn, buriti meal is a by-product from the processing of buriti fruit that is constituted by seed (40%), peel (30%), cellulose wrap (20%) and pulp (10%), presenting an interesting nutritional potential, especially its content of dry matter, fibers, and fats (Cavalcante, Garcez, Moreira Filho, & Alves, 2014; Rufino, Cruz, Tanaka, Melo & Feijó, 2017), and is highly available in the Amazon region (Calbo & Moraes, 1997).

Furthermore, inoculant additives contain in their formulation a combination of lactic acid bacteria and enzymes derived from microbial by-products. Microorganisms such as *Bacillus* and *Aspergillus* produce cellulases, hemicellulases, amylases, glycoamylases, and proteases that may promote digestion of structural and nonstructural carbohydrates such as starch, producing soluble sugars used as a substrate for lactic fermentation during the ensiling process (Ferrari Junior et al., 2009; Pires et al., 2009; Zopollatto et al., 2009). In silage processing, these basically aim to influence the fermentation process favoring preservation and improving the nutritional value (Zopollatto et al., 2009).

Thus, the objective of this study was to evaluate the effect of the use of inoculant on silage made up of elephant grass, wet brewery waste and buriti meal at different silo opening times.

Material and methods

The study was conducted in Manaus, AM (2° 38' 43.8" S 60° 02' 27.4" W). The soil of the experimental area used to plant the grass is classified as clayey yellow latosol (Latossolo Amarelo argiloso) (Santos et al., 2013) and the climate is characterized as hot and humid tropical, being limited to winter (rainy season), from December to June, and summer (dry season) from July to November.

The experimental silage tested was composed of 50% elephant grass, 30% wet brewery waste, and 20% buriti meal. The experimental design was randomized block in a factorial arrangement (2 x 4), where the treatments consisted of the use or not of inoculant during silage processing, and four silo opening times (7, 14, 21, and 28 days). Experimental silos (50 cm height per 10 cm diameter) with wooden lids were used, being five silos per treatment where each silo was considered a replicate. The silos were stored and sealed in a place protected from the influence of environmental conditions.

Elephant grass (*Pennisetum purpureum* cv Napier) for silage production was cut at 60 days of age (vegetative stage) at a height of 0.20 cm from the ground. The particle size used was 3 -5 cm aiming to facilitate the compaction of forage in the silo and control the effluent production. The wet brewery waste was obtained from Batuta brewery© (Manaus, AM). The buriti meal was obtained in the Association of Residents of the Santo Antonio do Abonari Community, located at Km 200 of BR-174 highway.

The proportions of each material were individually calculated based on their natural matter contents, and distributed in the silos. Samples of each material were collected for individual analysis of chemical composition. Each silo was individually loaded, compacted, and sealed, presenting an average weight of 4 kg. The commercial inoculant used contained the biological agents *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Lactobacillus rhamnosus*, *Lactobacillus lactis*, *Bacillus subtilis*, and

dextrose. This was applied according to the manufacturer's indications in proportion to the amount of ensiled material.

At each seven-day period (7, 14, 21, and 28 days), the silos were opened in order to calculate the effluent losses (Schmidt et al., 2011), and measure pH (Wilson & Wilkins, 1972). At the opening of the silos, the upper layer (approximately 10 cm) was discarded due to the possible presence of fungi and molds. Samples of 350g silage were collected in each period to evaluate the dry matter (%), organic matter (%), ash (%), crude protein (%), fats (%), neutral detergent fiber (%), and acid detergent fiber (%) according to methods described by Van Soest, Roberton & Lewis (1991), and Schmidt et al. (2011).

All data collected in this study were analyzed using the GLM procedure of SAS (Statistical Analysis System, 2008) and estimates of treatments were firstly subjected to ANOVA and subsequent Tukey's test. Results were considered significant at $p \leqslant 0.05$.

Results and discussion

The feedstuffs used to produce the silage presented an individual chemical composition similar to reported by previous studies (Ferrari Junior et al., 2009; Santos et al., 2010; Zanine et al., 2010), where elephant grass and wet brewery waste show a low dry matter content. However, all feedstuffs presented high organic matter content (Table 1). These studies also reported a high moisture of the elephant grass at growth stage, which may difficult to obtain a silage with good quality (Cavali et al., 2010). Consequently, the low dry matter content of elephant grass results in a low osmotic pressure, permitting the development of *Clostridium* sp. that break down sugars, lactic acid, proteins, and amino acids into butyric acid, acetic acid, ammonia, carbonic gas, and starches, which result insignificant losses and a good environment to silage production (Zanine et al., 2010).

Table 1. Chemical composition of feedstuffs used to produce the silage.

Chemical composition	Elephant grass	Wet brewery waste	Buriti meal
Dry matter, %	17.45	19.56	61.38
Organic matter, %	93.21	96.05	96.10
Ash, %	6.79	3.95	3.99
Crude protein, %	6.89	39.52	22.36
Fats, %	2.25	5.88	42.79
Neutral Detergent Fiber, %	68.10	48.60	62.48
Acid Detergent Fiber, %	44.50	18.83	50.12

In this sense, the use of wet brewery waste and buriti meal may cause an effective increase in the nutrient content of the ensiled forage, especially the buriti meal that provided an increase on dry matter. In all grasses, the main limiting factor for the production of good quality silage is the low dry matter content at the appropriate time for cutting. This fact leads to the occurrence of secondary fermentations caused mainly by bacteria of the genus *Clostridium,* generating nutrient losses with effluent production. Moisture-absorbing additives are an important tool to minimize the effects of low dry matter of tropical grasses at the time of ensiling (Andrade et al., 2010; itavo, itavo, Morais, Coelho, & Dias, 2010; Negrão et al., 2016; Dias, Cândido, Furtado, Pompeu, & Silva, 2019).

Wet brewery waste and buriti meal presented high crude protein content (Table 1). Previous studies reported that these feedstuffs are good sources of protein (Souza et al., 2012; Chanie & Fievez, 2017). In the wet brewery waste, protein is mainly located in the germ portion of the spent grain and is digested to a partial amount in the rumen and to a greater amount in the small intestinal tract (Chanie & Fievez, 2017). This variable indicates the nitrogen that is bound to the cell wall of the silage, which renders it unavailable throughout the digestive tract of the animal. However, the observed values do not compromise the quality of the forage (Santos et al., 2013).

There was no significant ($p > 0.05$) interaction between the use of inoculant and silo opening times on these variables. However, the use of inoculant in silage production provided more acidic pH ($p < 0.05$) and higher ($p < 0.05$) effluent losses (Table 2). These results may indicate a greater activity of lactic acid bacteria and higher lactic acid production. But this drop in pH was not sufficient to reduce losses of effluents. Previous studies reported that lactic acid is not a good inhibitor of fungi and yeast activity (Silva, Pedreira, Figueiredo, Bernadinho, & Farias, 2010). Unfortunately, lactic acid bacteria fermentation rarely sufficiently

lower pH and produce enough acetic acid to prevent yeasts and molds from growing in silage (Muck, 2010). According to Schmidt et al. (2011), under anaerobic conditions, fungi and yeast may develop at a lower pH when have soluble carbohydrates available in the medium. In the present study, the pH was low in all silages evaluated, which is an indicator of good fermentation quality in the silages.

Table 2. pH and effluent losses of the silage of elephant grass, wet brewery waste and buriti meal using or not inoculant at different silo opening times (SOP).

Factors[1]	pH	Effluent losses, %
Inoculant inclusion		
No	4.07[a]	3.75[b]
Yes	4.02[b]	3.77[a]
SOP		
7 days	4.03[b]	2.85[c]
14 days	4.03[b]	2.86[c]
21 days	4.05[ab]	4.18[b]
28 days	4.09[a]	5.17[a]
Effect	p-value	
Inoculant	0.01[*]	0.05[**]
SOP	0.01[*]	0.01[*]
Inoc. x SOP	0.12[ns]	0.15[ns]
CV[2], %	1.70	6.33

[1]Mean values followed by different letters are significantly different by Tukey's test at 5%; [2] CV – Coefficient of variation; * Significant effect ($p < 0.01$); ** Significant effect ($p < 0.05$); ns – non-significant.

In relation to the environmental aspect, it is important to mention that the successful use of inoculants in silage production depends on the growth of the inoculated bacteria, presence of adequate substrate, and population of bacteria in proportion to the forage mass (Zopollatto et al., 2009). Furthermore, bacterial inoculants have more advantages than chemical additives due to their easy capacity and safe application, do not pollute the environment, and are regarded as natural products (Jalč, Lauková, Simonová, Váradyová, & Homolka, 2009).

The results also indicated that longer silo opening times resulted in more alkaline pH ($p < 0.05$) and higher ($p < 0.05$) effluent losses. In general, elephant grass does not result in a good silage due to its high moisture content at the harvest time and low levels of soluble carbohydrates, required for the development of lactic acid bacteria during the fermentation process. In the long-term, there may be a loss of soluble nutrients in the form of wastewater due to the excess moisture in the forage used, and also a loss of nutrients in the form of gases generated by a secondary fermentation by spoilage microorganisms that grow in high humidity environments and produce ammonia nitrogen and butyric acid (Guerra et al., 2016). In order to reduce effluent losses, techniques such as wilting and the application of moisture absorbing additives can be used (Zanine et al., 2010).

There was no significant ($p > 0.05$) interaction between the use of inoculant and silo opening times on these variables. However, the use of inoculant during silage production resulted in higher ($p < 0.05$) contents of ash, crude protein, and fats. (Table 3). In the current market, there are inoculants containing different species and strains of bacteria, whose effects have been variable. Most studies pointed that this inclusion has provided positive effects on chemical and microbiological composition of the silage, and animal performance (Contreras-Gouveia, Muckb, Mertens, & Weimer, 2010). However, for an inoculant to be effective, the plant and the selected microorganism must be compatible (Assis et al., 2014), which naturally provide a better content of available nutrients, such as those observed in the results of the present study. Normally, the nutritional availability of good quality silage is close to that of the feedstuffs and is also highly palatable (Vu et al., 2019).

On the other hand, the use of inoculant during silage production provided lower ($p < 0.05$) contents of dry matter and fiber (neutral and acid detergent) (Table 3). It is important to mention that losses of dry matter occur due to the respiration of microorganisms that convert soluble carbohydrates and organic acids into heat, water, and CO_2 (Schmidt et al., 2011).

Longer silo opening times resulted in higher ($p < 0.05$) content of organic matter, ash, crude protein, and fat (Table 3). In this case, the same effect that caused the alkaline pH in the long-run in silage material favored the development of *Clostridium* and other microorganisms with similar characteristics because they have their maximum efficiency in environments with high water activity, high pH, and high temperature (Zopollatto et al., 2009; Muck, 2010).

However, this treatment also resulted in lower ($p < 0.05$) contents of dry matter and fiber (neutral and acid detergent) (Table 3). The measurement of dry matter losses during the ensiling process is a characteristic of great importance for the evaluation of the fermentation quality. Dry matter losses in general are greater at the beginning of the fermentation process, when the activity of microorganisms is more intense (Assis et al., 2014). In this study, the occurrence of a greater loss was observed until the 28th day of fermentation.

Table 3. Dry matter (DMT), organic matter (OMT), ash (ASH), crude protein (CPT), fats (FTS), neutral detergent fiber (NDF), and acid detergent fiber (ADF) of silage of elephant grass, wet brewery waste and buriti meal using or not inoculant at different silo opening times (SOP).

Factors[1]	DMT, %	OMT, %	ASH, %	CPT, %	FTS, %	NDF, %	ADF, %
Inoculant inclusion							
No	92.30[a]	95.65	4.34[b]	23.70[b]	25.26[b]	70.25[a]	41.28[a]
Yes	91.33[b]	95.62	4.37[a]	24.34[a]	27.07[a]	69.67[b]	40.33[b]
SOP							
7 days	92.28[a]	95.58[b]	4.25[b]	23.03[b]	24.21[c]	70.36[a]	41.59[a]
14 days	92.23[a]	95.60[b]	4.39[a]	23.82[b]	25.70[b]	70.29[a]	41.08[a]
21 days	92.18[a]	95.60[b]	4.39[a]	24.19[ab]	26.82[ab]	69.87[b]	40.52[b]
28 days	90.58[b]	95.74[a]	4.41[a]	25.03[a]	27.94[a]	69.32[b]	40.03[b]
Effect				p-value			
Inoculant	0.03[**]	0.14[ns]	0.05[**]	0.05[**]	0.03[**]	0.05[**]	0.02[**]
SOP	0.05[**]	0.02[**]	0.01[*]	0.01[*]	0.01[*]	0.02[**]	0.05[**]
Inoc. x SOP	0.10[ns]	0.16[ns]	0.15[ns]	0.17[ns]	0.14[ns]	0.10[ns]	0.13[ns]
CV[2], %	2.59	0.13	2.91	13.77	10.78	1.76	5.30

[1] Mean values followed by different letters are significantly different by Tukey's test at 5%; [2] CV – Coefficient of variation; * Significant effect ($p < 0.01$); ** Significant effect ($p<0.05$); ns – non-significant.

And regardless of the use or not of inoculants, ensiling process provides more favorable fermentation conditions to fiber degradation, especially by stimulus to the activity of cellulolytic bacteria (Tavares et al., 2009; Gomes et al., 2017). It is expected in good quality silage that the homofermentative lactic bacteria become dominant as quickly as possible and promote - because of low pH and reduced oxi-reduction potential - inhibition of enterobacteria and bacteria of the genera *Listeria*, *Bacillus*, *Clostridium* and the heterofermentative lactic bacteria. An ideal fermentation profile is the one where the maximum lactic acid is produced because lactic acid fermentation does not result in losses from gas formation and secondary metabolites (Ferreira et al., 2014).

Conclusion

It was concluded that the inoculant may be used in the production of silage of elephant grass, wet brewery waste, and buriti meal, providing a more acidic pH, higher effluent losses, lower dry matter and fiber content, and higher nutritional content. As the opening time was extended up to 28 days, there was more alkaline pH, higher effluent losses, lower dry matter and fiber content, and higher nutritional content.

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