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Nutritional value, fermentation losses and aerobic stability of elephant grass (*Pennisetum purpureum* Schum.) silage treated with exogenous fibrolytic enzymes

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ABSTRACT. The aim of this study was to evaluate nutritional value, fermentation losses, and aerobic stability of elephant grass silage (*Pennisetum purpureum* Schum.) treated with exogenous fibrolytic enzymes. The experiment was conducted in a completely randomized design with four replicates (experimental silos) and five levels of fibrolytic enzymes (0, 1.5, 3.0, 4.5 and 6.0%). For this, the elephant grass was ensiled at 70 days of age in plastic buckets with 20L capacity. Silos were opened 60 days after sealing. Analyses were made for chemical composition, *in vitro* dry matter digestibility (IVDMD), effluent losses (EL), gas losses (GL) and dry matter recovery (DMR), as well as the aerobic stability of the silage. Data were analyzed with PROC REG of SAS® University, at 5% probability. There was an increase in IVDMD content ($p < 0.0001$) and reduction in NDF and ADF contents ($p < 0.0001$) according to enzyme levels. These results were related to the increase in the degradation of fiber fractions. There were higher EL ($p = 0.0062$) as a function of enzyme levels and aerobic deterioration after silo opening, at all levels tested. Thus, it can be concluded that the exogenous fibrolytic enzymes change the chemical composition of elephant grass silage, and increase its digestibility and nutritional value. Moreover, when used alone as an additive, fibrolytic enzymes are not able to recover all dry matter of this silage (with effluent and gas losses), and are not able to maintain aerobic stability in the first hours after opening the silos.

Keywords: aerobic deterioration; digestibility; effluent losses; dry matter recovery.

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Introduction

The uneven distribution of rainfall in Brazil causes many rural producers to adopt food conservation practices, so that they can maintain the performance of their herds during periods of low forage production, usually in the dry season (Rassini, 2004). In this sense, silage is one of the alternatives for the conservation of forage through anaerobic fermentation (Veriato, Tolentino, Alves, Jayme, & Moura, 2018).

Among the potential crops for ensiling, corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) have high contents of dry matter and soluble carbohydrates, as well as high digestibility and nutritional value in general (Stella, Peripolli, Prates, & Barcellos, 2016). However, in recent years, there has been growing interest in the use of forage grasses for silage production. The ensiling of these grasses has some advantages: high forage production, better use of the cultivated areas of the property, with a reduction in the areas destined to crops; in addition to lower forage losses in the dry period of the year (Bonfá et al., 2015).

In the case of tropical grasses that can be ensiled, elephant grass (*Pennisetum purpureum* Schum.) is the most used for this purpose, as it presents high dry matter production, vigor, persistence and quality as forage. In addition, it is quite suitable for silage production due to the high levels of soluble carbohydrates, when compared to other grasses (Pereira, Ledo, & Machado, 2017).

However, compared to traditional silage crops such as corn and sorghum, elephant grass has lower dry matter content. On the other hand, when harvested late or managed inappropriately, it has high content of NDF, especially lignin, a plant component that has very reduced digestibility. For these reasons, it is necessary to use additives that modulate anaerobic fermentation and increase the nutritional value of elephant grass silage (Furtado, Carneiro, Coutinho, Cândido, & Silva, 2019).

In this scenario, the use of exogenous fibrolytic enzymes has gained prominence in Brazilian scientific research. Exogenous fibrolytic enzymes are biotechnological products, mostly composed of a mixture of cellulases and hemicellulases that can complement the digestion of cellulose and hemicellulose of endogenous enzymes. Therefore, the inclusion of this type of additive can increase dry matter digestibility and reduce NDF levels. Consequently, its use can also improve the fermentation kinetics of lactic acid-producing bacteria, in addition to reducing fermentation losses and aerobic deterioration of silages (Muck et al., 2018).

However, the application method and the levels of inclusion of this additive can be decisive for the action of the enzymes and, consequently, for the increase in the silage quality (Santos et al., 2010). In addition, there are few results in the literature on the exclusive use of these enzymes, without their combined use with microbiological, moisture-absorbing or nutritional additives. Based on the above, the goal of the study was to evaluate the nutritional value, fermentation losses and aerobic stability of elephant grass silage treated with levels of exogenous fibrolytic enzymes.

Material and methods

The experiment was conducted at the Laboratory of the Center for the Study and Research in Animal Production, State University of Bahia, Campus - IX, Barreiras, State of Bahia, 12º09'10" S and 44º59'24" W, from January to May 2017. The experimental design was completely randomized, with 5 treatments and 4 repetitions. Elephant grass (*Pennisetum purpureum* Schum. Cv. Roxo Botucatu) was harvested at 70 regrowth days (Table 1), with the cut made 10 cm from the ground, in a grass field already established on campus since 2012.

Table 1. Dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose and crude protein (CP) of elephant grass (*Pennisetum purpureum* Schum.) before ensiling.

DM (g kg^{-1})	272.0
NDF[1] (g kg^{-1})	728.0
ADF[1] (g kg^{-1})	448.0
Hemicellulose	301.0
CP[1] (g kg^{-1})	22.1

[1]Results on a dry matter basis.

The material was ground to 2.5 cm particles, homogenized and subsequently, the exogenous fibrolytic enzymes were applied at the levels of 0, 1.5, 3.0, 4.5 and 6.0% dry matter kilogram (DM) elephant grass, with the aid of a manual sprayer. The set of enzymes was composed of the products Novozymes Celluclast® (cellulase) and Novozymes Viscozyme® (xylanase), at a proportion of 75 and 25%, respectively. The products were diluted in water and the solution (water + enzymes) was applied at the proportion of 100 mL kg^{-1} DM elephant grass.

The material was ensiled in plastic buckets with a capacity of 20L, the experimental silos. The silos lids were equipped with Bunsen valves to allow the escape of gases from fermentation and to enable the quantification of DM losses from the fermentation process. Before ensiling, the experimental silos were previously filled with dry sand at the bottom (4 kg). The forage was separated from the sand by a layer of non-woven fabric. In this sense, the sets composed of silos, lids, dry sand and non-woven fabric were weighed before ensiling. Then, the silos were filled and sealed, with a density of 520 kg m^{-3} natural matter. Gases and effluent losses were determined, in addition to dry matter recovery, quantified by weight difference according to the equations described by Jobim, Nussio, Reis and Schimidt (2007).

Effluent losses were calculated based on the difference in weight of the sand placed at the bottom of the silo when closing and opening the silos, as follows:

$$\mathrm{PE}(\mathrm{kg.t^{-1}}) = \frac{(\mathrm{PVf} \times \mathrm{Ts}) \times (\mathrm{PVi} \times \mathrm{Ts})}{\mathrm{MFi}} \times 100$$

where: PE = effluent losses; PVf = weight of the empty silo + weight of the sand at the opening (kg); Ts = tare of the silo; PVi = weight of the empty silo + weight of the sand at closing (kg); and MFi = forage weight at closing (kg).

Gas losses were obtained by the following equation:

$$PG(\%\ MS) = \frac{(PsChf - PsCha)}{(MVFE \times MSFE)} \times 100$$

where: PG = gas losses; PsChf = weight of the filled silo at closing (kg); PsCha = weight of the filled silo at the opening (kg); MVFE = ensiled forage green matter (kg); and MSFE = ensiled forage dry matter (%). The dry matter recovery was determined by the equation:

$$RMS(\%\%) = \frac{(MFf \times MSf)}{(MFi \times MSi)} \times 100$$

where: RMS = dry matter recovery rate; MFf = forage mass at the opening (kg); MSf = forage dry matter content at the opening; MFi = forage mass at closing (kg) and MSi = forage dry matter content at closing.

The elephant grass remained ensiled for 60 days; after opening, the material was homogenized and separated into two subsamples of each repetition for analysis of pH and aerobic stability, according to Andrade et al. (2012). The silage temperature was measured at 1, 6, 9, 12, 36, 48, 72, 96 and 120 hours after opening. The ambient temperatures for each reading time were: 1h = 25.4ºC; 6h = 24.8ºC; 9h = 24.9 ºC; 12h = 25ºC; 36h = 25ºC; 48h = 25.1°C; 72h = 24.6°C; 96h = 24.9°C; and 120h = 25°C.

In addition, another aliquot (2 kg) of each repetition was dried in a forced air oven, at 60ºC for 72 hours, and subsequently ground in a Wiley mill with a 1 mm sieve. The samples were analyzed for the contents of dry matter (DM), mineral matter (MM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose, according to Detmann et al. (2012). The in vitro dry matter digestibility (IVDMD) was performed according to Tilley and Terry (1963).

Data were tested by analysis of variance and compared by regression analysis with PROC REG of SAS®, University version (Statistical Analysis System [SAS], 2015), at 5% probability. Graphics were constructed with PROC SGPLOT and SAS®, University version (SAS, 2015).

Results and discussion

In Table 2, the results indicated an effect of the levels of fibrolytic enzyme on effluent losses (p = 0.0062), gas losses (p = 0.0113) and dry matter recovery (p < 0.0001). The levels of fibrolytic enzymes increased linearly effluent losses, as already highlighted above. These results are similar to those of Nolan, Doyle and O'Kiely (2018), who observed an increase in effluent production in silages of annual ryegrass (*Lolium multiflorium* L.) treated with fibrolytic enzymes (163 and 320 g kg^{-1} for control and fibrolytic enzyme, respectively). The authors reported that fibrolytic enzymes were not able to promote total recovery of dry matter, also observed in the present study and which corroborates Andrade et al. (2012), who suggest that tropical grass silages without absorbent additives are subjected to significant effluent losses.

Table 2. Effluent losses (PE), gas losses (PG), dry matter recovery (RMS) and pH in silages of elephant grass (*Pennisetum purpureum* Schum.) treated with levels of fibrolytic enzymes.

Variables	Levels of fibrolytic enzymes					CV (%)[3]	Regression equation	R^2
	0	1.5	3	4.5	6			
PE (Kg t^{-1})[1]	5.5	6.0	6.7	7.6	8.8	25.1	$\hat{Y}$= 5.251 + 0.554x	0.35
PG (g kg^{-1})[2]	1.0	0.8	0.7	0.7	0.9	18.3	$\hat{Y}$= 1.008 – 0.191x + 0.028x^2	0.41
RMS (g kg^{-1})[2]	738.7	825.9	877.2	892.5	872.0	1.9	$\hat{Y}$= 738.676 + 70.093x – 7.978x^2	0.93
pH	4.1	4.0	3.9	3.9	4.0	1.9	$\hat{Y}$= 4.052 – 0.079x + 0.011x^2	0.33

[1]Based on natural matter. [2]Based on dry matter. [3] Coefficient of variation. [3]NS = Non-significant at 5% probability according to PROC REG of SAS® University version. R^2 = Coefficient of determination.

The greatest gas losses occurred in the control treatment, while the lowest was found in the levels of 3.0 and 4.5% fibrolytic enzymes (Table 2). This is probably because in the control treatment there were more favorable conditions for gas-producing bacteria, such as enterobacteria and the genus *Clostridium* (Furtado, Carneiro, Coutinho, Cândido & Silva, 2019). However, these results differ from those reported by Del Valle et al. (2018), who observed no significant effect of fibrolytic enzymes on gas losses in silages of sugarcane (*Saccharum officinarum* L. RB02-5799).

The dry matter recovery was lower in the control treatment and higher for the level of 4.5% fibrolytic enzymes. These results corroborate Desta et al. (2016), who reported lower dry matter losses for Napier grass silage treated with fibrolytic enzymes than the control treatment (62.8 versus 40.5 g kg^{-1}). The authors associated these results with the inhibition of secondary fermentation microorganisms (clostridia and enterobacteria) that metabolized silage nutrients, which may also have occurred in the present study. In addition, the recovery of dry matter was reduced from the level 4.5 to 6.0% (Table 2), probably due to the losses by gases and effluents, which were considerable.

The pH was lower for the control and higher for levels 3 and 4.5%. These results are in line with those obtained by Dehgani et al. (2012), who observed a lower pH value for corn residue silage treated with xylanase, when compared to the control treatment (4.0 to 3.7). Desta et al. (2016) also reported a drop in pH, from 5.1 to 4.3 when applied fibrolytic enzymes to Napier elephant grass silages. These results may indicate that fibrolytic enzymes provided better conditions for homo- and heterofermentative bacteria to produce lactate and rapidly reduce the pH, an important event to prevent loss of nutrients and growth of undesirable microorganisms (Ellis et al., 2016). However, it is worth pointing out that all the pH values observed indicated good fermentation. According to Bernardes and Chizzoti (2012), values between 3.8 and 4.2 are considered adequate for good fermentation kinetics.

From the results in Table 3, it is observed the effect of the levels of fibrolytic enzymes on DM ($p < 0.0001$), MM ($p < 0.0001$), NDF ($p < 0.0001$), ADF ($p < 0.0001$) and IVDMD ($p < 0.0001$).

Table 3. Dry matter (DM), mineral matter (MM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose (HC) in silages of elephant grass (*Pennisetum purpureum* Schum.) treated with levels of fibrolytic enzymes.

Variables ($g\ kg^{-1}$)	Levels of fibrolytic enzymes (%)					CV (%)[2]	Regression equation	R^2
	0	1.5	3	4.5	6			
DM	280.2	305.1	318.8	321.2	312.6	2.0	$\hat{Y}= 280.186 + 20.352x - 2.492x^2$	0.87
MM	78.1	81.8	85.6	89.3	93.0	2.8	$\hat{Y}= 78.150 + 2.483x$	0.84
NDF[1]	812.8	776.3	749.1	731.2	722.7	2.7	$\hat{Y}= 812.821 - 27.445x$	0.75
ADF[1]	532.4	487.8	460.4	449.9	456.6	4.4	$\hat{Y}= 532.421 - 35.395x + 3.793x^2$	0.71
HC[1]	288.1	284.5	281.0	277.4	273.4	7.7	NS[3]	0.06
CP[1]	27.5	25.0	23.0	20.8	18.9	7.8	NS[3]	0.05
IVDMD	586.6	597.9	609.2	620.5	631.8	0.8	$\hat{Y}= 586.650 + 7.533x$	0.91

[1]Results expressed on a dry matter basis. [2]Coefficient of variation. [3]NS = Non-significant at 5% probability according to PROC REG of SAS® University version. R^2 = Coefficient of determination.

The dry matter (DM) contents increased in a quadratic manner, where the level of 4.1% fibrolytic enzymes resulted in the highest observed value, reaching 321.3 g kg^{-1} DM. In terms of mineral matter (MM), a positive linear effect was observed, with an increase of 2.5 percentage units for each 1% inoculated enzymes. According to Ferrari Júnior and Lavezzo (2001), the action of fibrolytic enzymes disrupts the plant cell wall and release the cell content, which can reduce the moisture content of the silage and, consequently, increase the levels of DM and MM (Table 3). This fact occurred in the present study, since there was a positive linear effect ($p = 0.0062$) of the enzyme levels on the losses by effluents (Table 1). However, it is worth noting that all treatments resulted in DM levels suitable for good quality silage, according to recommendations by Bernardes and Chizzoti (2012).

Khota et al. (2016) verified no increase in the DM content of the ensiled Napier grass, with increasing levels of cellulase (from 0.01% to 0.1% ensiled mass). Nevertheless, it is worth pointing out that the grass remained ensiled for only 30 days, half the period used in the present study.

Contents of NDF and IVDMD (Table 3) had a linear effect, with the NDF linearly reduced by 27.4 percentage units and the IVDMD increased by 7.5 percentage units as the levels of fibrolytic enzymes increased by 1%. For the levels of ADF, a quadratic effect was found, where the level of 4.5% fibrolytic enzymes resulted in the lowest value observed, reaching 449.9 g.kg^{-1} DM. These results are in line with Antonio et al. (2018), who observed increased in situ NDF degradation of Mombasa grass silage (*Panicum maximum* Jacq. Cv. Mombaça) treated with a blend of fibrolytic enzymes (xylanase, cellulase and glucanase). These results are in line with Antonio et al. (2018), who observed an increase in in situ NDF degradation of the Mombasa grass silage (*Panicum maximum* Jacq. Cv. Mombaça) treated with a blend of fibrolytic enzymes (xylanase, cellulase and glucanase), when compared to untreated silage (540 against 509 g kg^{-1}). The same response pattern was registered for in situ degradation of ADF and DM. The authors highlighted the relative ease with which enzymes degrade fiber fractions, such as cellulose and hemicellulose. However, it is worth noting that xylanases used alone increase the digestibility of hemicellulose, while the combined use of exogenous fibrolytic enzymes (xylanases and cellulases) can reduce both NDF and ADF levels (Soltan et al., 2013), similar results were found in this study.

There was no significant effect on CP content, which on average were lower than the critical level of the basal ruminant diet (Silva et al., 2019). Importantly, these results reflect the CP levels of elephant grass before ensiling (Table 1).

Regarding the aerobic stability, there was an effect of time (hours) on the temperature of the silage ($p < 0.0001$) for all levels of fibrolytic enzyme tested (Figure 1). For levels 3.0, 4.5 and 6.0% fibrolytic enzymes, during the first 36 hours, temperatures were considerably above the respective ambient temperatures, characterizing aerobic deterioration of the silage. In addition, the lowest temperature observed when opening the silo was that of the control treatment (27.6°C) and the highest was found for 6.0% fibrolytic enzymes (30.0°C).

On the other hand, in the 48-hour reading, for most treatments, the temperatures were close to those established as ambient temperature. The only exception was for the level 6.0% fibrolytic enzymes, whose temperature recorded at that time was 26.7°C, a value close to an aerobic deterioration condition.

According to Muck et al. (2018), although fibrolytic enzymes are able to considerably increase the silage digestibility, the excessive release of soluble carbohydrates can reduce aerobic stability. Thus, soluble sugars can be quickly used by undesirable microorganisms, such as fungi and yeasts after opening the silos.

Furthermore, tropical grass silages are characterized by high moisture and are therefore subjected to deterioration by aerobic bacteria (Bernardes, Reis, & Moreira, 2005). These factors may have contributed significantly to the results found, which differ from those reported by Andrade et al. (2012), where the break of aerobic stability of elephant grass silage occurred only after the first 48 hours of observation. In this sense, the authors justified that the moisture-absorbing additives (soy hulls and cornmeal) were able to maintain aerobic stability, a fact that did not occur in the present study.

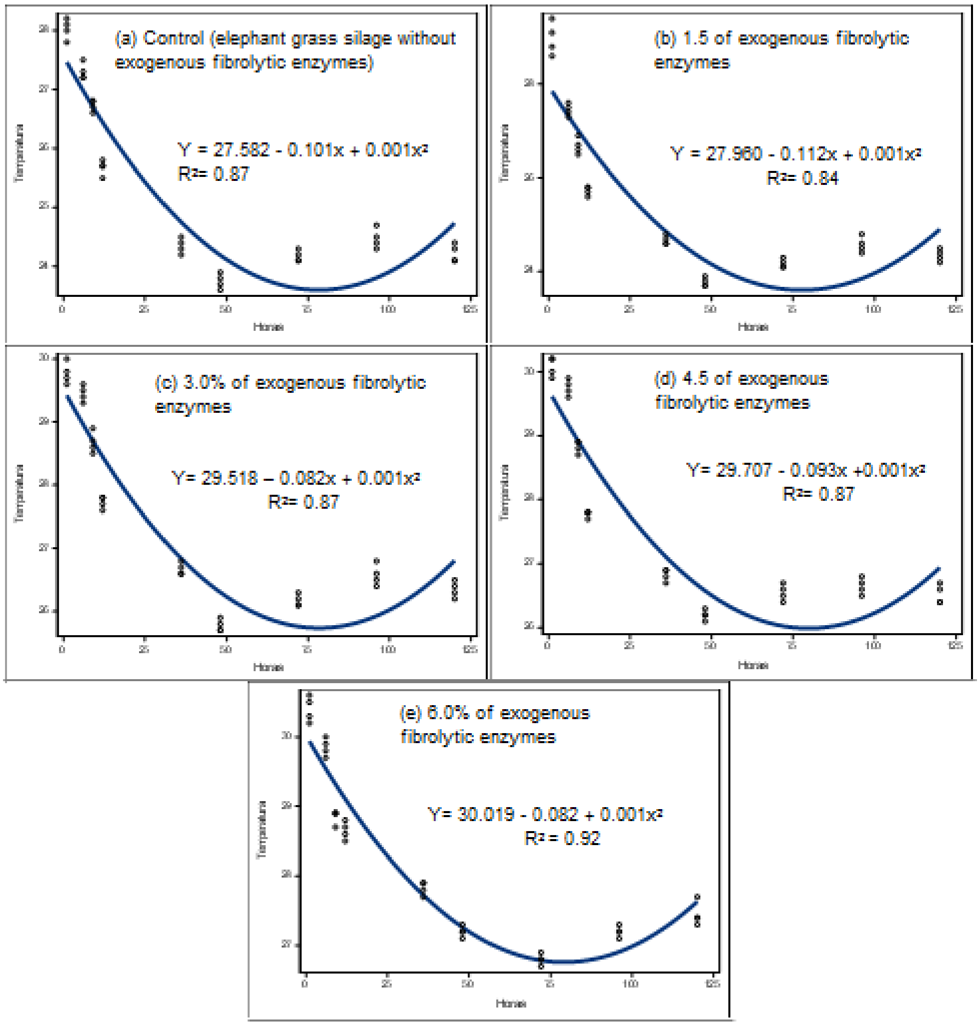

Figure 1. Aerobic stability of silages of elephant grass (*Pennisetum purpureum* Schum. Cv. Roxo Botucatu) treated with levels of fibrolytic enzymes. Elephant grass without exogenous enzymes (a); elephant grass with 1.5; 3.0; 4.5; 6.0% exogenous fibrolytic enzymes (b; c; d; e, respectively). Y = Temperature (°C); x = Hours. R^2 = coefficient of determination.

Conclusion

For the conditions of the present study, it is inferred that exogenous fibrolytic enzymes: change the chemical composition of elephant grass silage, as well as increase its digestibility and nutritional value; when used alone as an additive, they are not able to recover all dry matter from elephant grass silage (with losses by gases and effluents), and neither are able to maintain aerobic stability in the first hours after opening the silos.

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