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Nutritional and fermentation parameters of Xaraés grass silage produced with bacterial additive

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ABSTRACT. The use of bacterial additives in forage silages with low content of dry matter prevents undesirable fermentation and reduces losses by gases (PG) and effluents (PE) during the ensiling process. This study aimed to evaluate the fermentation parameters, chemical composition and *in vitro* digestibility of silage of *Urochloa brizantha* cv. Xaraés produced with bacterial additive. The inoculant contained the following strains: *Propionibacterium acidipropionici* + *Lactobacillus plantarum; Lactobacillus buchnari; Propionibacterium acipropionici* + commercial enzymes and *Lactobacillus plantarum* and *Pediococcus pentosaccus*, at 0, 25, 50, 75, 100, and 125% of the recommended level for sugarcane (2 g ton^{-1}). The experiment was a completely randomized design with four replications, and six levels of inoculant (0, 25, 50, 75, 100, and 125%). There was a quadratic relationship between the inoculant addition and the levels of pH, PE, DMIVD, NDF, ADF and LIG of the silage. PG and MM increased linearly with the addition of inoculant. The N-NH_3, DM, CP, CEL, HEM and EE were not affected by the inoculant. Bacterial additive at 50% provided increased DMIVD. Appropriate values were found for pH and NH_3.

Keywords: ensiling, forage, inoculant.

Parâmetros fermentativos e nutricionais de silagem de capim Xaraés produzidas com aditivo bacteriano

RESUMO. O uso de aditivos bacterianos em silagens de forrageiras com baixo teor de matéria seca inibe fermentações indesejáveis e reduz perdas por gases (PG) e efluentes (PE) durante o processo da ensilagem. Objetivou-se avaliar as características fermentativas, a composição química e a digestibilidade "in vitro" de silagens de *Urochloa brizantha* cv. Xaraés produzidas com aditivo bacteriano. Foi utilizado inoculante contendo as seguintes cepas: *Propionibacterium acidipropionici* + *Lactobacillus plantarum; Lactobacillus buchnari; Propionibacterium acipropionici* + enzimas comerciais e *Lactobacillus plantarum* + *Pediococcus pentosaccus*, nas doses de 0, 25, 50, 75, 100 e 125% do nível recomendado para cana (2 g t^{-1}). O delineamento utilizado foi o inteiramente casualizado com quatro repetições, sendo utilizado inoculante comercial e seis doses de adição (0, 25, 50, 75, 100 e 125%). Observou-se relação quadrática entre a inclusão do inoculante e os teores de pH, PE, DIVMS, FDN, FDA e LIG da silagem. PG e MM aumentaram linearmente à medida que foi adicionando o inoculante bacteriano. Os teores de N-NH_3, MS, PB, CEL, HEM e EE não foram influenciados pelos níveis de inclusão do inoculante. O aditivo bacteriano no nível de 50% proporcionou aumento na DIVMS. Foram encontrados valores adequados para pH e N- NH_3.

Palavras-chave: ensilagem, forrageiras, inoculante.

Introduction

The Brazilian cattle production mostly adopts the pasture feed, however, animals raised on pasture, usually require supplementation to maintain their growth curve and hence their performance, especially during the dry season.

The Xaraés grass represents an option for grassland diversification, and the genus *Brachiaria* is among the most important tropical forages in Brazil. The Xaraés grass has several advantages over other cultivars, such as higher germination rate and higher production, ensuring higher stocking rate and productivity per area (FLORES et al., 2008) and beyond grazing it can also be used for silage production.

Silage is the forage storing in an anaerobic environment, where the forage is chopped, compressed and sealed for its preservation by fermentation, to provide a feed with a nutritional value close to the original, with minimum loss during the process.

It is necessary the predominance of lactic acid fermentation to conserve forage as silage. Several factors can affect the fermentation quality, changing its final product, such as the presence of homo and heterofermentative bacteria, soluble carbohydrates, moisture and dry matter content, compaction and rapid closing of the silo (PATRIZI et al., 2004).

Grass silages usually have high moisture, which favors butyric fermentation and ammonia release, and may adversely affect the intake of animals. The use of additives in silage grass improves the quality of the fermentation process, also improving nutritional quality, or decreasing losses during the conservation process (AVILA et al., 2009). Improvement in the silage fermentation pattern may therefore increase the intake of these silages by animals.

Given the limited information about *Urochloa brizantha* cv. Xaraés ensiling process, this study aimed to evaluate fermentation parameters, chemical composition and *in vitro* dry matter digestibility of Xaraés grass silages produced with bacterial additive.

Material and methods

The experiment was carried out at the Farm Monte Alegre Pindaibas, located in the municipality of Rio Verde, Goiás State (20º 25' S, 54° 51' W and altitude of 530 m). The Xaraés grass (*Urochloa brizantha* cv. Xaraés) was planted on February 15, 2012, and then managed under sprinkler irrigation, maintaining a rainfall of 15 mm per day. At 45 days, after planting, a standardization cut was performed at 15 cm from the ground and the residual material was removed from the experimental field.

The soil of the experimental area is dystrophic red oxisol, and the chemical analysis showed the following results: pH 5.1 in $CaCl_2$; 43.1% of base saturation; 1.9% of aluminum saturation; 42 g kg^{-1} organic matter; 2.5 mg dm^{-3} P (Mehlich 1); and 54.7 mg dm^{-3} K (Mehlich 1). On April 19, 2012, maintenance fertilization was performed with 50 kg ha^{-1} of 20-05-20 of NPK. 100 kg ha^{-1} of N were applied as urea on two dates: April 24, 2012 and June 7, 2012.

The planted area was used only for producing silage. The harvest was performed on July 1, 2012, the material was collected using a steel blade brush cutter (STIHL®), at 15 cm from the ground and then crushed in stationary chopper with seven knives (Nogueira®) to particles about 1cm. The chopped material was manually mixed with the inoculant.

It was used an inoculant containing the following strains: *Propionibacterium acidipropionici* + *Lactobacillus plantarum*; *Lactobacillus buchnari*; *Propionibacterium acipropionici* + commercial enzymes and *Lactobacillus plantarum* and *Pediococcus pentosaccus*, at levels of 0, 25, 50, 75, 100, and 125% of the recommended level for sugarcane (2 g ton^{-1}). The dilutions were performed in water, with the 100% of dilution equivalent to 0.5 g of inoculum/liter of water/ton of silage. The design was completely randomized with four replications and six levels of inoculant addition (0, 25, 50, 75, 100, and 125%).

Silages were made in PVC experimental silos, measuring 10 cm in diameter and 40 cm long. The silage was manually compacted, covered with canvas and sealed with adhesive tape. At the bottom of the silos was placed 1 kg of dry sand for draining effluents, as well as a thin plastic screen and TNT to prevent contact of the material with sand. The silos had a mean specific mass of 965.06 kg of GM m^{-3}.

We weighed the set silo + canvas + dry sand + screen, before ensiling, and the filled and capped silos, for quantitative determination of gases and effluent losses, based on gravity differences.

After 60 days, silos were opened and samples processed in a blender, filtered through cheesecloth to extract the fluid, immediately used to determine the pH value using a digital potentiometer and the content of ammonia nitrogen ($N\text{-}NH^3$) according to the methodology described by Silva and Queiroz (2006).

Loss of silage, in the forms of gases and effluents, and dry matter recovery were quantified by weight difference, using equations adapted from (SANTOS et al., 2008) as follows.

Gas loss was obtained by the equation: GL (% DM) = [(WfsST - WfsOT) / (GMEF × DMEF)] × 100, where: GL – Gas loss; WfsST - weight of the filled silo at the sealing time (kg); WfsOT - weight of the filled silo at the opening time (kg); GMEF- green matter of ensiled forage (kg); DMEF - dry matter of ensiled forage (%).

The effluent production was based on the weight difference of the sand on the silo bottom, at sealing and opening times: EL (kg ton^{-1} of GM) = [(WES - Ts) - (WEYS - Ts)] / FMi × 100, where: EL - effluent losses; WES - weight of the empty silo + weight of sand at the opening time (kg); Ts –tare weight of the silo; WEYS – weight of the empty silo + weight of sand at the sealing time (kg); FMi - forage mass at the sealing time (kg).

DM, CP and EE were determined according to the methodology described by Silva and Queiroz (2002) and NDF, ADF and lignin were evaluated by the sequential method as described by Van Soest et al. (1991). The *in vitro* dry matter digestibility was determined using a Daisy Incubator (Ankon

Technology Corporation, Fairport, USA), with incubation for 48h in ruminal fluid plus 24 hours in HCL and pepsin.

Data of fermentation and nutritional characteristics were subjected to regression analysis using the SAEG (2009) program.

Results and discussion

There was a quadratic relationship between the addition of bacterial inoculant and the values of pH and effluent loss (EL) of the silage. According to the regression equation, the inflection points for pH and EL were found with the addition of 70 and 85.89% of the inoculant, respectively. The $N\text{-}NH_3$ was not affected by the levels of inoculant.

The pH showed a positive quadratic relationship, increasing up to the level of 70%, decreasing thereafter. As the pH is related to lactic acid production during fermentation, levels above 70% were probably more effective in the production of this acid.

The effluent loss had a negative quadratic relationship, decreasing until the level of 85.89%. Therefore, levels below the inflection point were more effective in controlling losses of effluents.

For gas loss, there was a linear increase with the addition of the inoculant; this indicates a greater proliferation of enterobacteria, heterofermentative and proteolytic bacteria, responsible for the greatest losses (Table 1).

The pH is a good indicator of fermentation quality in silages with low DM content (CHERNEY; CHERNEY, 2003). In the present study, pH values (4.25 to 4.47) were lower than that observed by Azevedo et al. (2012) for Xaraés grass (4.58) and similar to that of Piatã grass (4.20).

With decreasing pH, there are lower gas losses. The lowest GL was observed with the addition of 25% inoculant. GL results (0.26 to 0.56%) may be considered satisfactory and are lower than the results verified by Zanine et al. (2007), who found GL for elephant grass silages at 1.51%.

Pinho et al. (2008) pointed out that the grass ensiling without additive cause effluent loss, which contains large amounts of organic compounds, including sugars, organic acids and proteins, confirming the importance of adding inoculants, especially those with high dry matter content to control effluent losses.

However, this was not observed in this study, once the addition of the additive to Xaraés grass silage did not reduce effluent losses. This is because the bacterial additive used in this experiment does not have this characteristic. The lowest loss was registered in the control treatment with 16.6% effluent loss, and the highest loss was with the addition of 75% inoculant, with 31.5% EL. There was a negative correlation (-0.46974) between DM content and effluent loss.

There was no significant effect on $N\text{-}NH_3$, demonstrating that the used inoculant did not affect this process. The $N\text{-}NH_3$ is a good indication of the silage quality because it is directly correlated with the proteolysis process in silages. This result was different from that observed by Santos et al. (2008) who, however, evaluated the Tanzania grass (*Panicum maximum*).

There was a quadratic relationship between the addition of bacterial inoculant and the levels of IVDMD, NDF and ADF of the silage. According to the regression equation, the inflection points for levels of IVDMD, NDF, ADF were 20.36, 66.40, and 50.07 of the inoculant recommended dose, respectively. The MM increased linearly with the inoculant addition. DM, CP, LIG, CEL and HEM were not affected by inoculant addition (Table 2).

The addition of 75% inoculant decreased the IVDMD. The control silage showed 74.54% IVDMD, which is higher than that observed by Coan et al. (2005) and Bergamaschine et al. (2006), which was 54.5 and 60.23%, respectively, for tropical forages silages.

The inoculant addition did not alter the DM of silage. We found an average of 22.41% for DM, similar to the value recorded by Penteado et al. (2007), who evaluated Mombaça grass silage with bacterial inoculants, 22.9%. According to the authors, DM preservation in inoculated silages occurs by the activity of lactic acid bacteria present in the material, which when in favorable environment produce homolactic fermentation that results in minimum DM loss.

Table 1. Mean values and regression equations for pH, gas loss (GL), effluent loss (EL), ammonia nitrogen ($N\text{-}NH_3$) according to the addition of bacterial inoculant★.

Variable	Bacterial Inoculant (%)								
		25	50	75	100	125	Equation	P	R^2
pH (%)	4.25	4.27	4.37	4.47	4.37	4.27	$Y = 4.21 + 0.0056x - 0.00004x^2$	0.0204	0.74
GL (%)	0.28	0.26	0.27	0.56	0.52	0.57	$Y = 0.25 + 0.0023x$	0.0380	0.61
EL (%)	16.6	26.9	30.1	31.5	29.8	31.1	$Y = 17.90 + 0.3264x - 0.0019x^2$	0.0209	0.92
$N\text{-}NH_3$(%)	4.37	3.65	4.07	4.02	4.10	3.27	NS[1]	0.2579	-

★*Propionibacterium acidipropionici* + *Lactobacillus plantarum*; *Lactobacillus buchnari*; *Propionibacterium acidipropionici* + commercial enzymes and *Lactobacillus plantarum* + *Pediococcus pentosaccus*. [1]NS = non-significant.

Table 2. Mean values and regression equations for *in vitro* dry matter digestibility (IVDMD), dry matter (DM), mineral matter (MM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (LIG), cellulose (CEL), hemicellulose (HEM) and ether extract (EE), according to the addition of bacterial inoculant*.

Variable	Bacterial Inoculant (%)								
	0	25	50	75	100	125	Equation	P	R^2
IVDMD(%)	74.54	74.42	75.54	68.41	73.45	68.51	$Y = 74.65 + 0.0285x - 0.0007x^2$	0.0144	0.86
DM (%)	22.95	23.71	22.61	21.04	21.58	22.62	NS[1]	0.2689	-
MM (%)	8.83	9.05	10.12	10.41	10.02	10.48	$Y = 9.0005 + 0.0131x$	0.0027	0.75
CP (%)	10.97	11.35	10.72	12.22	12,02	11.32	NS	0.4832	-
NDF (%)	81.68	79.36	79.12	75.21	73.07	83.20	$Y = 82.94 - 0.2125x + 0.0016x^2$	0.0327	0.53
ADF (%)	41.83	41.29	41.98	40.42	40.19	45.02	$Y = 42.375 - 0.0701x + 0.0007x^2$	0.0048	0.54
LIG (%)	11.18	10.30	14.85	13.28	12.46	14.33	NS	0.2805	-
CEL (%)	26.5	31.00	27.1	27.11	27.75	30.67	NS	0.1809	-
HEM (%)	39.85	38.07	37.14	34.79	32.87	38.18	NS	0.0976	-
EE (%)	3.98	3.96	3.95	3.94	3.94	3.94	NS	0.1028	-

**Propionibacterium acidipropionici* + *Lactobacillus plantarum*; *Lactobacillus buchnari*; *Propionibacterium acidipropionici* + commercial enzymes and *Lactobacillus plantarum* + *Pediococcus pentosaccus*. [1]NS = non-significant.

MM increased linearly with the inoculum addition, due to the reduction of organic matter in the silage. The highest result was observed with 125% of the bacterial additive with 10.48% for MM. Loures et al. (2005) found similar values of MM, in Tanzania grass silages with biological additive.

The inoculant did not affect CP of silages with a mean value of 11.43. Paziani et al. (2006) investigated the chemical composition of Tanzania grass silage with bacterial inoculant and observed that the inoculant increased the CP content. This was not observed in our study, in which the inoculant addition was not significant.

Regarding the NDF, with quadratic effect, the lowest value was observed for the treatment with 100% inoculant (73.07% NDF) and the highest value was verified with 125% inoculant (83.20% NDF). These values were higher than those found by Monteiro et al. (2011) with 65.96% NDF in inoculated elephant grass silage. This demonstrates that the high NDF and the low ADF increased the IVDMD.

The ADF content also showed a quadratic effect. The lowest value was obtained with the addition of 100% inoculant (40.19% ADF), and the highest value, with inoculant addition at 125% (45.02% ADF). A study developed by Rezende et al. (2008) obtained higher values for elephant grass silage, 48.23% of FDA, indicating that Xaraés grass silage has a lower content of fiber unavailable to the animal, probably due to the morphology of Xaraés grass when compared to that of elephant grass.

For CEL, the inoculant addition increased its content; the best result was found for the addition at 50%. Nevertheless, the CEL values were lower than that found by Azevedo et al. (2012) for Xaraés grass (35.25%) and piatã grass (35.30%). The reduction in our levels may be related to the cellulose content (28.45%) of Xaraés grass at the ensiling time. Ferrari Junior et al. (2009) studied additives in elephant grass silages and observed that the addition of enzymatic bacterial inoculant promoted a decrease in CEL values (38.21% to 36.19%) of silages.

Furthermore, considering values of LIG (Table 2), we observed that they were not influenced by the inoculant. Values of LIG were higher than those found by Azevedo et al. (2012) for Xaraés grass (4.83%) and Piatã grass (4.66%). In turn, Rodrigues et al. (2003) evaluated the addition of microbial inoculants on the fermentation characteristics and chemical composition of elephant grass silage and observed no effect of inoculants on the LIG content.

Values of HEM were not influenced by the inoculant (Table 2). They were higher than those reported by Azevedo et al. (2012) for Xaraés grass (28.84%) and Piatã grass (28.52%).

Likewise, there were no effects of the use of inoculant on EE, whose values were higher than those reported by Ferrari Junior et al. (2009) for elephant grass silages with bacterial additive, 2.57%.

Conclusion

In summary, the bacterial additive applied to Xaraés grass silages changes the pH, losses of gases and effluents, as well as the *in vitro* dry matter digestibility, mineral matter and fibrous portion (NDF and ADF) of silages.

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