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Quality characteristics of haylage from forage grasses of tropical pastures: losses, gas production, nutritional value, microbial population and organic acids

Características de qualidade de silagem pré-secada de gramíneas forrageiras de pastagens tropicais: perdas, produção de gases, valor nutricional, população microbiana e ácidos orgânicos

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Highlights

The haylage of *U. brizantha* has better chemical characteristics.
The highest acetic acid levels were in the Xaraés, Planaltina and Tupã genotypes.
No mold colonies were observed in the *in natura* material of Massai.

Abstract

Tropical pastures have been little explored for haylage, due to the lack of information on nutritional quality. This study aimed to evaluate the fermentation quality of different genotypes of forage grasses from tropical pastures in the form of haylage. Six genotypes of grasses were used to evaluate the fermentation characteristics, losses and presence of microorganisms in the in the haylage. The completely randomized design was used for all variables. Data were subjected to analysis of variance with all genotype's means compared by the Scott Knott's test at 5% probability. There was effect of

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interaction between form of material x genotype (P<0.01) on all variables of chemical composition evaluated in the *in natura* and haylage at the moment of haylage making, except for NDF and $N\text{-}NH_3$ (P>0.05). The concentrations of O_2 in the haylage after 60 days of storage were lower for the genotypes Xaraés and Tupã. The highest levels of acetic acid were observed in genotypes Xaraés, Planaltina and Tupã. The Planaltina genotype presented the highest DM loss in the process of production and storage of the haylage, averaging 8.2 ± 0.37%. The haylage of the species *B. brizantha* and *M. maximus* presented better nutritional characteristics.
Key words: Aerobic stability. Cultivation. Fermentation. Microbiology. Nutrition. Pasture.

Resumo

As pastagens tropicais têm sido pouco exploradas para silagem pré-secada, devido à falta de informações sobre a qualidade nutricional. Este estudo teve como objetivo avaliar a qualidade da fermentação de diferentes genótipos de gramíneas forrageiras de pastagem tropical na forma de haylage. Foram utilizados seis genótipos de gramíneas para avaliação das características fermentativas, perdas e presença de microrganismos na silagem pré-secada. Foi utilizado delineamento inteiramente casualizado para todas as variáveis. Os dados foram submetidos à análise de variância com todas as médias dos genótipos comparadas pelo teste de Scott Knott a 5% de probabilidade. Houve efeito da interação da forma do material x genótipo (P<0,01) sobre todas as variáveis de composição química avaliadas no material *in natura* e haylage no momento da fenação, exceto para FDN e $N\text{-}NH_3$ (P>0,05). As concentrações de O_2 no silagem pré-secada após 60 dias de armazenamento foram menores para os genótipos Xaraés e Tupã. Os maiores teores de ácido acético foram observados nos genótipos Xaraés, Planaltina e Tupã. O genótipo Planaltina apresentou a maior perda de MS no processo de produção e armazenamento, 8,2 ± 0,37%. A silagem pré-secada das espécies *B. brizantha* e *M. maximus* apresentou melhores características nutricionais.
Palavras-chave: Estabilidade aeróbia. Cultivo. Fermentação. Microbiologia. Nutricional. Pastagens.

Introduction

Even with the good-potential of tropical grasses, as it is the case of the species *Urochloa brizantha, Megathyrsus maximus,* and *Andropogon gayanus,* which stand out for their high yield, resistance to water shortages, and flexibility of use (Pequeno et at., 2017; Akbari et al., 2018; Henry et al., 2018), tropical climate regions suffer from productive seasonality arising from rainfall concentration in certain months of the year, which results in increased forage availability during the rainy period and feed shortage during the dry period (Silva et al., 2021). In addition, tropical grasses have low soluble carbohydrate content (Khiaosa-Ard et al., 2020; Klevenhusen & Zebeli, 2021), low dry matter (DM) content in the phase of better nutritional value, and high passivity to losses in sun exposure due to greater leaf blade width and morphological structure (Collins & Moore, 2017; Nascimento et al., 2022), which hinders their use in the production of conserved roughages such as silage and hay.

The possibility of using conserved tropical grasses has been of interest to cattle farmers, and the focus of several studies (Deutschmann et al., 2017; Nascimento et al., 2020; Trytsman et al., 2020; Nascimento et al., 2021a; Rufino et al., 2022; Nascimento et al., 2022). Haylage has been pointed out as an option for the conservation of forage species considering their chemical and morphological characteristics. The principle of haylage making is based on anaerobic fermentation, where microorganisms transform soluble carbohydrates into acids to promote pH drop and inhibit the proliferation of undesirable microorganisms, preserving the nutritional characteristics and aiming to keep the feed as close as possible to the original forage (Nath et al., 2018). This requires that the forage has adequate amounts of fermentable substrate, relatively low buffer capacity, and dry matter content above 300 g/kg (McDonald et al., 1991). Thus, the quality of the haylage is dependent on chemical and morphological characteristics that vary among genotypes (Soundharrajan et al., 2017).

Among the genotypes commonly used in tropical pastures it is possible to identify plants with potential for haylage making, meeting the demand of producers who do not have conditions to perform the cultivation of exclusive areas for conservation and forage (Nascimento et al., 2020). Thus, using the forage surplus of plants commonly produced on the property to meet the demands of animals and maintain stable production rates throughout the year through the production of haylage as conserved feed, provides a viable alternative to meet the demand in the period of scarcity (Walker & Vendramini, 2018).

The hypothesis of this study is that at least one of the evaluated pasture forage grass genotypes presents characteristics suitable for the production of haylage. Thus, the objective was to evaluate the fermentation quality of different tropical pasture forage grass genotypes in the form of haylage.

Material and Methods

Location and experimental design

The experiment was carried out at the Federal University of Piauí (UFPI), Bom Jesus campus, located in Piauí - Brazil at latitude 09º04’28” South, longitude 44º21’31” West and altitude of 277 meters. The climate of the region is classified as BSh, with summer rainfalls and dry winter according to the Köppen classification of 1936, described by Alvares et al. (2013).

Six pasture grass genotypes were used: Marandú grass and Xaraés grass (*Urochloa brizantha*), Massai grass and Paredão grass (*Megathyrsus maximus*), Planaltina grass and Tupã grass (*Andropogon gayanus*). For the determination of the chemical composition, total soluble carbohydrates and microbial population, the completely randomized design (CRD) was used in a 6×2 factorial scheme with three replications, where the factors corresponded to the six genotypes and two forms of material (*in natura* and haylage). CRD was also used for yield, gas content, aerobic stability, pH, ammonia nitrogen ($N\text{-}NH_3$) and organic acids content of the haylage, with six treatments (genotypes) and four replications, which corresponded to the haylage bales.

Planting and soil composition

The experimental area was sown in February 2016, which is a period that coincides with the beginning of the rains in the region. Sowing was performed in 4-cm-deep furrows, using 20 seeds per linear meter. Before planting, a soil analysis was performed, resulting in the classification of the soil as Dystrophic Yellow Latosol, with clay loam texture (clay: 257 g kg^{-1}; silt: 34 g kg^{-1}; sand: 709 g kg^{-1}) presenting the following chemical characteristics: pH in water: 5.5; phosphorus: 74.5 mg dm^{-3}; potassium: 127.0 mg dm^{-3}; calcium: 1.68 cmol c dm^{-3}; magnesium: 0.77 cmol c dm^{-3}; aluminum, & lt: 0.00 cmol c dm^{-3}; hydrogen + aluminum: 1.94 cmol c dm^{-3}; sum of basis: 2.78 cmol c dm^{-3}; cation exchange capacity at pH 7.0: 4.72 cmol c dm^{-3}; base saturation: 59%; and aluminum saturation: 0.00%.

For planting, the soil was corrected according to the soil analysis, and fertilized with 30 kg ha^{-1} of potassium (potassium chloride) and 45 kg ha^{-1} of phosphorus (single superphosphate). During the productive cycle, the soil was fertilized with nitrogen (urea), at a level of 150 kg N ha^{-1}, which was divided according to the productive cycles over the year, and was repeated throughout the years. The area was irrigated with the equivalent of 5 mm per day, using a sprinkler system.

Haylage making

The plants were cut at the pre-established residue height for each genotype, according to the recommendations made by Dias (2012). For Marandú, Xaraés and Massai, cuts were made at a residue height of 15 cm, and for Tupã, Planaltina and Paredão at 30 cm. The harvested forage was immediately exposed to the sun, and during dehydration the material was turned over every 30 minutes for uniformity of the dehydration process (Nascimento et al., 2022). The baling point was determined by the microwave method according to Souza et al. (2002). The material was baled in 3-kg bales and wrapped with conventional plastic film when the material reached 45% DM at the baling point, and stored in a ventilated shed for 60 days.

Chemical composition, total soluble carbohydrates and ammonia nitrogen

For the determination of the chemical composition of the *in natura* and haylage material, 500g of each material was harvested weighed and pre-dried in a forced ventilation oven at 60°C until reaching constant weight. The samples were ground in a Willey mill in a 1-mm mesh sieve to determine the contents of dry matter (DM) (934.01), crude protein (CP) (981.10), mineral matter (MM) (930.05) and organic matter (OM) (Association of Official Analytical Chemists [AOAC], 1990). The neutral detergent fiber (NDF) was determined according to Van Soest et al. (1991).

The total soluble carbohydrate (TSC) concentration was obtained by the concentrated sulfuric acid method, described by Dubois et al. (1956), with adaptations by Corsato et al. (2008). Briefly, after collection, the samples were frozen to minimize the action of endogenous enzymes (Cecato et al., 2001). The WSC contents were calculated in mg x 100 mL^{-1}, in 100% DM, and subsequently adjusted based on the DM.

To evaluate the ammonia nitrogen content (N-NH_3), samples of 100 g of the haylage were collected for further analysis, according to the methodology described by Mizubuti et al. (2009).

Gas production, internal temperature and losses

The O_2 and CO_2 contents were determined at the opening of the haylage through the Instruthern O_2 meter® (model MO-900), and the Testoryt® CO_2 analyzer. The analyses were performed through valves (PVC pipes) inserted in each bale and closed with polyethylene plastic films. At this time, the internal temperatures of the haylage were also determined using a skewer thermometer. The losses were calculated by subtracting the TFGM and TFDM, expressed in g kg^{-1}.

Aerobic stability and pH

After opening the haylage bales and removing the material for the previously described analyses, the material present in the bales of each treatment was homogenized and decompressed with the objective of favoring air penetration. After this procedure, haylage samples were transferred to a room with controlled temperature at 25.3 ± 0.30 ºC, and average air relative humidity of 57.56 ± 15.5%. The material was exposed to air for a period of 0 to 120 hours. The room temperature was controlled by the thermostat of the air conditioner and periodically checked by thermometers distributed in the room.

The internal and surface temperatures of the haylage were recorded every 4 hours for 48 hours and after this period, they were checked every 8 hours up to 120 hours, as proposed by Johnson et al. (2002). The temperature was determined using a digital thermometer (Incoterm®) inserted at 10 cm in the center of the haylage mass and a digital infrared thermometer with laser sighting (Benetech®). The aerobic stability was calculated as the time observed for the haylage to increase 2 °C in relation to room temperature after exposure to air, according to Taylor et al. (2002b). From the same sample, approximately 50 g were taken every 8 hours (three samples per day) to determine the pH values during aerobic exposure according to the methodology described by Mizubuti et al. (2009).

Organic acids

The content of organic acids was determined using the method described by Kung and Ranjit (2001), in samples of the haylage of each treatment from which a liquid sample was extracted. The samples were analyzed for acetic, propionic, iso-butyric, butyric, isovaleric and valeric acid contents in a commercial laboratory through high performance liquid chromatography on a high performance liquid chromatograph (HPLC), (SPD-10ª VP) coupled to an ultraviolet (UV) detector at a wavelength of 210 nm.

Microbial population

The microbiological evaluation was performed in both in natura and haylage materials according to the recommendations of González and Rodrigues (2003). Plating was performed in duplicate for each culture medium. The populations were determined by the selective technique of cultures in anaerobic medium, through the counting of lactic acid bacteria, enterobacteria, molds and yeasts. The plates considered susceptible to counting were those that presented values between 30 and 300 CFU.

Statistical analysis

For the characterization of the haylage quality in the analysis of chemical composition, gas production, ammonia nitrogen and organic acids, the means were compared at 5% probability using the following model: $Y_{ij} = \mu + EF_i + e_{ij}$, where: Y_{ij} = analyzed parameter i; μ = general constant; EF_i = fixed effect; e_{ij} = residual effect.

For aerobic stability and pH as a function of time, the means were subjected to linear regression analysis at 5% probability, and unfolding was performed when the interactions were significant, considering the model: $Y_{ijk} = \mu + EF_i + H_j + (EF_iH_j) + e_{ijk}$, where: Y_{ijk} = analyzed parameter i; j time; μ = general constant; EF_i = fixed effect of cultivar; H_j = fixed effect of hours; EF_iH_j = fixed effect of the interaction between cultivar × hours; e_{ijk} = residual effect.

The means of the tropical grass pasture genotypes were compared by the Scott Knott's adjusted test ($P<0.05$). All analyses were performed using the software SISVAR version 5.3. Data regarding the quantification of microbial groups (in logarithmic unit, $\log_{10}$) were analyzed descriptively.

Results and Discussion

Chemical composition, total soluble carbohydrates and ammonia nitrogen

There was effect of the interaction between form of material x genotype ($P<0.01$) on all variables of chemical composition evaluated in the *in natura* and haylage material during the production of the haylage, except for NDF and N-NH_3 ($P>0.05$; Table 1). The Planaltina genotype showed the highest contents of DM in both *in natura* and haylage (141.8 and 372.5± 0.62 g kg^{-1}). The haylage of all genotypes evaluated, presented values of DM higher than 300 g kg^{-1}, which are characterized as adequate values to allow fermentation and stabilization of the material (McDonald et al., 1991).

Table 1
Chemical composition of forage in natura and haylage of six genotypes of tropical pasture forage grass

Genotype	Form of Material (FM)		Mean	*P-value*			SEM
	in natura	Haylage		FM	Genotype	FM x Genotype	
Dry matter (DM; g kg^{-1})							
Massai	145.3 Ab	343.7 Ba	244.5	<0.01	<0.01	<0.01	0.62
Paredão	119.3 Bb	347.6 Ba	233.4				
Marandu	104.6 Bb	318.1 Ca	211.3				
Xaraés	110.6 Bb	332.2 Ca	221.4				
Planaltina	141.8 Ab	372.5 Aa	257.1				
Tupã	135.2 Ab	325.9 Ca	230.5				
Mean	126.8	340.3					
Crude protein (CP; g kg^{-1})							
Massai	104.8 Bb	140.0 Aa	122.4	<0.01	<0.01	<0.01	0.47
Paredão	110.2 Bb	136.0 Aa	123.1				
Marandu	127.6 Aa	127.2 Aa	127.4				
Xaraés	109.8 Bb	130.8 Aa	120.3				
Planaltina	92.6 Ba	92.1 Ba	92.3				
Tupã	99.8 Ba	86.8 Ba	93.3				
Mean	107.4	118.3					
Neutral detergent fiber (NDF; g kg^{-1})							
Massai	713.4	707.9	710.7 A	0.07	0.27	0.20	2.21
Paredão	736.8	643.7	690.3 A				
Marandu	654.7	657.3	656.0 A				
Xaraés	678.6	682.9	680.8 A				
Planaltina	706.1	666.7	686.4 A				
Tupã	682.9	681.6	682.2 A				
Mean	696.1	672.9					
Mineral matter (MM; g kg^{-1})							
Massai	108.1 Da	76.7 Bb	92.4	<0.01	<0.01	<0.01	0.26
Paredão	142.6 Ba	86.0 Ab	114.3				
Marandu	122.0 Ca	83.5 Ab	102.7				
Xaraés	152.7 Aa	75.2 Bb	113.9				
Planaltina	135.8 Ba	61.9 Cb	98.8				
Tupã	127.3 Ca	73.6 Bb	100.4				
Mean	130.5	74.2					

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Organic matter (OM; g kg^{-1})							
Massai	891.9 Ab	923.4 Ba	907.6				
Paredão	857.3 Cb	913.9 Ba	885.6				
Marandu	877.9 Bb	916.4 Ba	897.2	<0.01	<0.01	<0.01	0.39
Xaraés	847.3 Db	934.8 Aa	891.0				
Planaltina	864.1 Cb	933.1 Aa	898.6				
Tupã	872.6 Bb	926.8 Aa	899.7				
Mean	869.4	924.3					
N-NH_3 (%)							
Massai	0.30	0.60	0.45 B				
Paredão	0.43	0.51	0.47 B				
Marandu	0.53	0.82	0.68 A				
Xaraés	0.40	0.56	0.48 B	<0.01	<0.01	0.38	0.05
Planaltina	0.30	0.45	0.37 B				
Tupã	0.28	0.50	0.39 B				
Mean	0.37b	0.57a					
Total soluble carbohydrates (TSC; g kg^{-1})							
Massai	48.3 Aa	36.6 Ab	42.4				
Paredão	34.0 Ba	29.5 Ba	31.7				
Marandu	38.0 Ba	16.7 Cb	27.3				
Xaraés	49.6 Aa	35.5 Ab	42.6	<0.01	<0.01	<0.01	1.8
Planaltina	44.5 Aa	18.8 Cb	31.7				
Tupã	49.9 Aa	21.0 Cb	35.5				
Mean	44.0	26.3					

SEM: standard error of the mean; means followed by the same uppercase letter in the column and lowercase in the row do not differ statistically from each other by the Scott-Knott test at 5% probability.

The highest CP content was observed for the genotype Marandu in the *in natura* material. For the haylage material, the genotypes Planaltina and Tupã had the lowest values of CP recorded. There was an increase in the percentage of CP between the *in natura* material and the haylage of the genotypes Massai, Paredão and Xaraés in the order of 33.58, 23.41 and 19.12%, respectively. The increase in the CP content of the haylage material in comparison to the *in natura* material may be related to the dilution effect increasing in proportion due to the water loss that occurred and that this component is expressed in relation to the percentage of DM.

The highest concentration of MM was observed for genotype Xaraés in the *in natura* material, averaging 152.7 ± 0.26g kg^{-1}. The haylage from Paredão and Marandu genotypes had the highest MM contents, which were 86.0 and 83.5 ± 0.26g kg^{-1}, respectively. The opposite was observed for DM, where Xaraés showed the lowest value observed for the in natura material, and the genotypes Paredão, Marandu and Massai showed the lowest values for the haylage. The highest value of OM in the *in natura* material was observed for the genotype Massai (891.9 ± 0.39 g kg^{-1}). The reduction in the MM contents of the *in natura* material compared to the haylage of all genotypes may be an indication that full sun exposure in the field may result in mineral losses by respiration and leaching (Nascimento et al., 2020). Withering can reduce the soluble carbohydrate content due to increased respiratory activity, and consequently the production of lactic acid, which can increase the mineral content in the preserved product. With this, there may be a combined effect of leaching of minerals drained by effluent from the material during the process of haylage making (Müller et al., 2016). As for OM, the observed values were similar to those found by Costa et al. (2018) who reported 916.7 g kg^{-1} DM in Tiffton85 haylage.

For the isolated effect of form of material shape there was a higher ($P<0.01$) content of $N\text{-}NH_3$ in the haylage, the Marandu grass showed the highest content of $N\text{-}NH_3$ among the genotypes evaluated. The $N\text{-}NH_3$ concentrations observed for the haylage of the genotype Marandu are lower than results obtained in the literature, which presents values of 1.25% (Costa et al., 2018) and 1.10 % (Guimarães et al., 2019) for temperate climate grasses. $N\text{-}NH_3$ is a parameter indicative of the proteolysis process of amino acids into ammonia, CO_2 and amines. Values less than 10% indicate low nutritional losses in the preserved material (McDonald et al., 1991). This shows the potential of these pasture grass genotypes for haylage making, indicating adequate fermentation and maintenance of the nutritional value of the preserved material.

The lowest TSC concentrations in the *in natura* material were observed for the genotypes Paredão and Marandu (34.0 ± 1.8 and 38.0 ± 1.8 g kg^{-1}, respectively). The TSC values obtained in the haylage indicate losses of soluble carbohydrates in all genotypes when compared to the *in natura* material, except for the genotype Paredão. This result may indicate the utilization of carbohydrates by microorganisms during the fermentation process of the haylage. It is important to note that the TSC values observed for the pasture grasses are considered low because they are related to high contents of NDF. In grasses that have NDF values higher than 500 g kg^{-1}, the TSC content can decrease as a result mainly of lignification (Klevenhusen & Zebeli, 2021).

The mean values of NDF of the *in natura* material found in this study were 696.1 ± 2.21 g kg^{-1}. However, the TSC values obtained for the haylage in this study were lower than that found by Nath et al. (2018) in haylage of Tifton 85, which was 32.29 g kg^{-1} DM, thus, it can be said that even with possible degradation and reduction in TSC contents, the haylage of tropical pasture grasses can be equivalent to that of temperate grasses that are already consolidated in forage conservation for their nutritional quality and acceptance by animals.

The excess or lack of TSC can result in inadequacies during the production process and directly influence the aerobic stability of the haylage mass, given that with exposure there is an increase in the respiration rate and in the consumption of TSC. The TSC values obtained in the haylage indicate losses of soluble carbohydrates in all genotypes when compared to the *in natura* material, except for the genotype Paredão. This result may indicate the utilization of carbohydrates by microorganisms during the fermentation process of the haylage. It is important to note that the TSC values observed for the pasture grasses are considered low because they are related to high contents of NDF. In grasses that have NDF values higher than 500 g kg^{-1}, the TSC content can decrease as a result mainly of lignification (Klevenhusen & Zabeli, 2021).

Gas production, internal temperature and losses

There was effect (P<0.01) on gas production (O_2 and CO_2), internal temperature and DM losses of the haylage of the six genotypes of tropical pasture forage grass (Table 2). Oxygen concentrations in the haylage after 60 days of storage were lower for the genotypes Xaraés and Tupã, while for CO_2, the concentrations were higher for the genotypes Massai, Marandu, Planaltina and Tupã, which obtained 23.7, 20.7, 23.1 and 20.2 ± 1.30 %, respectively. The presence of O_2 after 60 days of haylage baling indicates that the medium was not completely anaerobic during preservation. However, the presence of O_2 observed in the present study is lower than that indicated by Coblentz et al. (2016), which defines values of 6.93 for adequate fermentation. Thus, even with the presence of O_2, the values observed are within acceptable levels for adequate fermentation and stabilization of the material. In the fermentation process of forage, the microorganisms degrade soluble carbohydrates and release CO_2 and acids, besides generating heat (McDonald et al., 1991). In the present study, the genotypes with the greatest reductions in TSC in the *in natura* and haylage material were those with the highest CO_2 values. This indicates that higher concentrations of TSC may have increased the presence of undesirable microorganisms in the haylage resulting in higher production of CO_2, acetic, propionic and isovaleric acid, as observed for the Andropogon genotypes.

The highest internal temperatures of the haylage were observed for the genotypes Marandu, Xaraés, Planaltina and Tupã (30.7, 30.6, 30.7 31.0 ± 0.17 °C, respectively). The Planaltina genotype presented the highest DM loss in the process of production and storage of the haylage, which was 8.2 ± 0.37%, associated with the balance between the heat rate produced by microbial activity and heat losses, which are directly related to the oxidation of DM, causing losses in the form of CO_2 (Hill & Leaver, 2002). Corroborating this, the Planaltina genotype presented the highest DM loss observed in the present study. An adequate level of TSC is one of the main concerns in forage conservation (Nath et al., 2018), as shown by these results.

Table 2
Determination of O_2 concentration, CO_2, internal temperature, and losses in haylage of six genotypes of tropical pasture forage grass

Genotypes	O_2	CO_2	Internal temperature (ºC)	DM losses (g kg^{-1})
Massai	2.77 A	23.7 A	29.8 B	61.1 B
Paredão	3.57 A	18.2 B	29.2 C	57.0 B
Marandu	2.55 A	20.7 A	30.7 A	68.0 B
Xaraés	1.82 B	14.0 C	30.6 A	62.3 B
Planaltina	3.15 A	23.1 A	30.7 A	82.4 A
Tupã	1.42 B	20.2 A	31.0 A	66.5 B
P-value	<0.01	<0.01	<0.01	<0.01
SEM	0.37	1.30	0.17	0.37

SEM: standard error of the mean; means followed by the same uppercase letter in the column do not differ statistically by the Scott-Knott test at 5% probability.

Aerobic stability and pH

There was a significant effect of interaction ($P<0.01$) between the genotypes and time of air exposition of the haylage on surface and internal temperature (Table 3). As for the haylage pH, there was only effect of the genotype ($P<0.01$). Regarding the surface temperature there was effect among the genotypes only at hours 0, 72 and 96. At hour 0, the genotype Xaraés presented the highest surface temperature (22.0 ± 0.22 °C), while the genotypes Massai and Paredão presented the lowest temperatures (19.0 and 18.8 ± 0.22 °C, respectively). At hour 72, the highest temperatures were observed for the Massai, Planaltina, and Tupã genotypes (24.0, 24.3, and 24.5 ± 0.22 °C, respectively). At hour 96, the highest surface temperatures were observed for the genotypes Xaraés, Tupã and Planaltina. The internal temperature of the haylage had effect only at 24 and 96 hours of air exposure, where the highest averages were observed for the genotypes Marandu, Planaltina and Tupã at 24 hours and for and Xaraés, Planaltina and Tupã at 96 hours.

There was an increasing linear effect ($P<0.01$) for all genotypes of the hours of air exposure on the surface and internal temperature of haylage, except for the internal temperature of Marandu grass. The Massai and Marandu genotypes obtained the highest pH values observed during the hours of air exposure (6.8 and 7.1 ± 0.32, respectively). No aerobic stability break was observed over the 120 hours of exposure to air of the haylage, which may be associated with the presence of acetic acid and for its bactericidal effect inhibiting the development of microorganisms and the consequent heat production. The break of aerobic stability is obtained when the material, after exposure to air, presents an increase of 2 °C in relation to the room temperature, (Taylor & Kung, 2002a). The results obtained indicate greater stability of the pasture grass haylage when subjected to animal consumption in natural environment, avoiding degradation of the material.

Table 3
Evaluation of surface temperature (°C), internal temperature (°C), and pH of the haylage of six genotypes of tropical pasture forage grass

Genotype (Gen.)	Hour						Mean	P-value Linear
	0	24	48	72	96	120		
			Room temperature (°C)					
	25.4	25.6	25.2	25.3	25.2	25,3		
			Air relative humidity (%)					
	62.0	50.0	65.5	56.6	60.0	51,3		
			Surface temperature (°C)					
Massai	19ªOC	19.9A	24.4A	24.0A	24.5B	25,5A	22,9	<0,01
Paredão	18.8C	20.3A	24.5A	23.7B	24.3B	25,1A	22,8	<0,01
Marandu	20.1B	20.0A	23.9A	23.2B	24.6B	25,4A	22,9	<0,01
Xaraés	22.0A	20.1A	24.5A	23.7B	25.8A	25,0A	23,5	<0,01
Planaltina	20.5B	20.0A	24.5A	24.3A	26.3A	24,5A	23,3	<0,01
Tupã	20.5B	20.2A	24.1A	24.5A	26.2A	25,0A	23,4	<0,01
Mean	20.1	20.1	24.3	23.9	25.3	25,1		
			Internal temperature (°C)					
Massai	21.7A	20.6B	23.7A	23.7A	23.0B	24,2A	22,8	<0,01
Paredão	22.7A	20.5B	23.5A	23.2A	22.7B	25,2A	23,0	<0,01
Marandu	24.0A	22.7A	23.5A	24.2A	22.0B	24,5A	23,6	0,42
Xaraés	22.8A	20.8B	23.8A	24.1A	23.8A	25,1A	23,4	<0,01
Planaltina	22.2A	22.0A	23.2A	23.5A	25.0A	25,2A	23,5	<0,01
Tupã	23.0A	22.0A	23.5A	23.5A	23.7A	25,5A	23,5	<0,01
Mean	22.7A	21.4	23.5	23.7	23.5	24,9		
			pH					
Massai	7.1	6.7	6.9	6.8	6.5	6,9	6,8 A	
Paredão	6.3	6.2	6.2	6.6	6.3	6,4	6,3 B	
Marandu	7.5	7.4	7.0	7.2	6.6	6,6	7,1 A	
Xaraés	6.6	6.3	6.3	6.4	6.4	6,6	6,4 B	
Planaltina	5.9	5.9	6.4	6.1	6.3	6,4	6,2 B	
Tupã	5.8	6.0	5.6	6.0	6.1	6,2	6,0 B	
Mean	6.5	6.4	6.4	6.5	6.4	6,5		0,88
Analysis of variance				*P*-value			SEM[b]	
			Gen.	Hours	Gen. × Hours			
Surface temperature			0.01	<0.01	<0.01		0.22	
Internal temperature			0.02	<0.01	<0.01		0.49	
pH			0.01	0.90	0.91		0.32	

SEM: standard error of the mean; means followed by the same uppercase letter in the column do not differ statistically by the Scott-Knott test at 5% probability.

Organic acids

For the content of organic acids in the haylage of genotypes of tropical forage grasses, there was effect (P<0.01) on acetic, propionic and isovaleric acid (Table 4). The highest levels of acetic acid were observed in genotypes Xaraés, Planaltina and Tupã. For all genotypes, the presence of acetic acid was higher than what was recommended by Almeida et al. (2013), who define values of 20 g kg^{-1} for good-quality materials. The increase in the values of acetic acid in the haylage of the Planaltina and Tupã genotypes is due to the peak development of enterobacteria that occurs in these genotypes, since the end product of these microorganisms from the consumption of glucose is a molecule of lactate and a molecule of acetate or ethanol (McDonald et al., 1991). According to Luis and Ramirez (1988), enterobacteria generally multiply until about the seventh day of fermentation, when they are replaced by lactic groups. At the beginning of fermentation, enterobacteria compete with LAB for available soluble carbohydrates, and the greater competitive and antagonistic capacity from bacteriocins produced by LAB is crucial, which is an effect that was not observed in the present experiment possibly due to resistance in decreasing pH, which hindered the development of lactic bacteria.

Table 4
Evaluation of organic acids in the haylage of six genotypes of tropical pasture forage grass

	Organic Acids (g kg^{-1} DM)					
Genotypes	Acetic	Propionic	Iso-butyric	Butyric	Isovaleric	Valeric
Paredão	302.6B	26.6B	13.7	93.3	15.1 B	10.6
Massai	400.1B	39.1B	24.7	159.4	21.4 B	13.6
Marandu	505.1B	26.2B	47.1	102.3	23.6 B	11.3
Xaraés	593.0A	69.9A	47.7	150.8	22.6 B	12.7
Planaltina	557.1A	50.7A	42.5	125.3	25.4 B	13.4
Tupã	586.0A	34.9B	23.7	119.8	60.3 A	13.1
P-value	<0.01	0.05	0.83	0.18	<0.01	0.30
SEM	30.6	5.8	22.9	16.8	5.1	0.9

SEM: standard error of the mean; means followed by the same uppercase letter in the column do not differ statistically by the Scott-Knott test at 5% probability.

The values of organic acids observed in this study are higher than those observed by Ribeiro et al. (2014), who evaluated *Andropogon gayanus* silage, and found averages of 17.5, 0.68 and 10.6 g kg^{-1} for acetic, propionic and butyric acids, respectively; and also, to those observed by Musco et al. (2016) regarding iso-butyric, isovaleric, valeric acids for *Brachiaria ruziziensis* and *Panicum maximum* that presented values of 6.53, 6.81, 6.30 and 7.28 g kg^{-1}, respectively. The highest levels of propionic acid were observed in the genotypes Xaraés and Planaltina. The genotype Tupã showed the highest isovaleric acid content, which was 6.03 ± 0.51 g kg^{-1}.

Microbial population

The microbial population of the *in natura* and haylage material from the six genotypes of tropical pasture forage grass are shown in Figure 1. There was no change in the presence of lactic acid bacteria (LAB) in the *in natura* and haylage material, with a mean value for the form of material and genotypes of 6.98 log CFU g^{-1}.

For enterobacteria and yeasts in the *in natura* material, it was observed higher populations for the genotype Tupã (4.98 and 7.48 log CFU g^{-1}, respectively). The Andropogon genotypes showed the highest presence of mold, yeast and enterobacteria. In addition to indicating greater passivity to DM losses by gas and effluent production, high enterobacteria values may influence acceptance and consumption by the animals (Schenck & Müller, 2013). According to Pahlow et al. (2003), yeasts are usually the initiators of aerobic spoilage, consuming sugars and fermentation acids and increasing temperature and pH. Thus, the presence of yeast can be associated with high pH values (Nath et al., 2018), as observed in this study. It is important to highlight that the pH of the haylage should not be compared to that of other high moisture silages, considering that the high concentration of DM may limit the bacterial fermentation capacity (Costa et al., 2018). In addition, *L. buchneri* can consume lactate during the silage fermentation process and transform it into acetate, raising the pH (Driehuis et al., 2001).

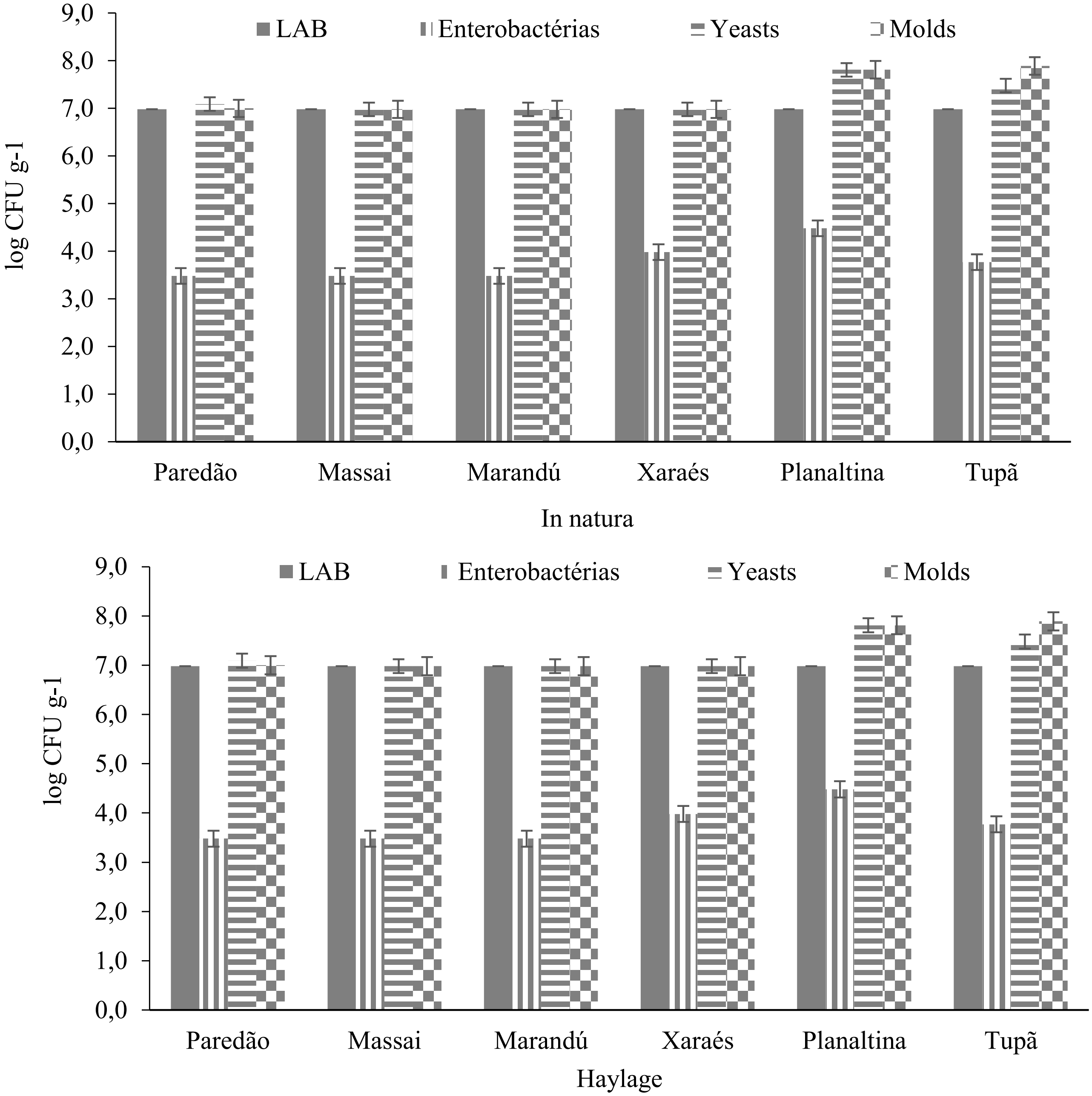

Figure 1. Microbial population of *in natura* and haylage material from six genotypes of tropical pasture forage grass. LAB: Lactic acid bacteria.

No mold colonies were observed in the *in natura* material of the Massai and Tupã genotypes. In the haylage the highest populations of enterobacteria, yeasts and molds were observed for the genotypes Planaltina and Tupã. Among the explanations for the increased populations of undesirable microorganisms, the DM content of the material at the moment of haylage making has been described as the factor with the greatest impact due to the major influence on improper fermentations and proliferation of undesirable microorganisms (McDonald et al., 1991). However, in the present study, the genotypes of the Andropogon species showed DM contents adequate for conservation, with values similar to genotypes that showed adequate fermentation and little presence of spoilage microorganisms. This may indicate that the high presence of these microorganisms in the *in natura* material was the main reason for the high presence of molds and yeasts in the haylage.

Different from the *in natura* forage, the presence of mold was observed in the haylage of all genotypes, with a higher concentration for the Planaltina genotype. The presence of mold indicates the degradation of residual sugars and lactic acid produced in the anaerobic phase (McDonald et al., 1991). The LAB values obtained in the present study were higher than those found by Nath et al. (2018), who obtained values of 5.83 log CFU g^{-1}. However, the absence of numerical difference in the LAB population between the *in natura* and haylage material can be explained by the lack of effective action of microorganisms in the fermentation of these haylages, indicating less production of strong acids to lower the pH and control the fermentation of undesirable microorganisms (Nascimento et al., 2021), as confirmed by the results for pH.

Conclusions

The results obtained in this study show that the haylage of *U. brizantha* and *M. maximus* has better characteristics regarding fermentation pattern, chemical and bromatological composition, and aerobic stability, making these genotypes the most suitable.

However, it is important to emphasize that planning based on the productive history of these genotypes, harvest season and climatic conditions are indispensable for the success in the production of haylage. It is noteworthy that there is a need for new studies evaluating this type of preserved material in the diet of ruminant animals.

Declaration of Competing Interest

The authors declare no conflict of interest.

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