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Quality of haylage of *Brachiaria brizantha* with different contents of dry matter in the storage

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Abstract

The purpose of this study was to evaluate the quality of Marandu grass (*Brachiaria brizantha*) haylage according to different dry matter (DM) contents in storage. The design adopted was completely randomized with four treatments and five replications. The treatments were DM contents of the plant at the moment of storage (*in natura*, 30–40, 40–50 and 50–60% DM). The analyses to assess the quality of the haylage were performed after 90 days of storage. The chemical composition, microbiological population, gas quantification, pH, N-NH_3, volatile fatty acids, soluble carbohydrates (CHO) and the aerobic stability were evaluated. The means were compared through the Tukey's test and linear regression. The treatment with 50–60% DM presented the highest DM and CHO contents which were 563.8 and 42.0 g/kg, respectively. There was a higher presence of oxygen in the haylage of *in natura* material, which was 4.8%. There was no difference between treatments for the population of lactic acid bacteria; however, the treatment with 50–60% DM had the highest concentration of enterobacteria. The haylage with 30–40% DM and 50–60% DM presented high concentrations of acetic acid. There was no break in aerobic stability for any treatment within 120 h after opening the bales. There was a smaller amount of N-NH_3 in treatments with 40–50% DM and 50–60% DM. The Marandu grass with a DM content of 50–60% for haylage making demonstrated better quality characterization of conserved forage.

Introduction

Tropical climatic conditions favour the development of forage grasses utilizing C4 metabolism, and such species have great potential for forage biomass accumulation. Species of the genus *Brachiaria* are particularly well adapted to this environment (Euclides *et al*., 2019). Grasses of the genus *Brachiaria* have great adaptation to different types of soil and climate, in addition to presenting high production of forage with good nutritional value (Ribeiro Júnior *et al*., 2015). Marandu grass (*Brachiaria brizantha*) is widely used in tropical pastures, as it adapts to the edaphoclimatic conditions of these regions, such as Africa, presenting a good response to fertilization and high-forage production (Medica *et al*., 2017).

In these regions, there is seasonal variation in the production of forage biomass, with a surplus of forage production in the rainy season and a deficit in the dry season. Therefore, it is essential to use conservation techniques that preserve the feed to be used in periods of scarcity (Gurgel *et al*., 2017; Wilkinson and Muck, 2019). Feed conservation techniques such as ensiling, haymaking and haylage making, guarantee the storage of good-quality feed for livestock. Thus, it is essential to find the right method for the conservation of forage species cultivated in the pasture (Nascimento *et al*., 2020), whether ensiling, haymaking or haylage making.

In the haymaking process, the conservation occurs through the dehydration of the forage, while conservation in the ensiling process occurs through anaerobic fermentation. For the haylage conservation technique, partial dehydration associated with anaerobic fermentation is necessary (Gayer *et al*., 2019). In order to obtain good-quality silage it is necessary that the plant has adequate chemical characteristics at harvest for adequate fermentation to occur, and in order to produce good-quality hay, it is necessary that the plant has morphological characteristics that provide fast dehydration in the field. The production of silage from tropical grasses has some disadvantages, such as the high moisture content of the plants. Thus, the suitability of the crop for silage involves characteristics such as dry matter (DM) content, soluble carbohydrates (CHO), nitrate and buffering capacity (Gurgel *et al*., 2019).

Haylage stands out for preserving the forage with good nutritional value, and for being a simple and accessible technique for farmers, as it is a hybrid method between haymaking and ensiling. The DM content of the plant at the moment of storage of the haylage influences

the fermentation and therefore, the quality of the conserved forage. The DM content after plant dehydration for haylage making can vary from 500 to 750 g/kg (Müller, 2005; Costa *et al.*, 2018*a*).

Evaluating the production of haylage with different DM contents from tropical forage grasses at the moment of storage is important to observe its influence on the quality of the feed preserved in the form of haylage, as there is little information about this under tropical conditions. It was hypothesized that the higher DM content of the forage plant at the moment of storage will produce a better quality haylage of Marandu grass. Therefore, the purpose of this study was to evaluate the influence of the DM content of Marandu grass at the moment of storage on the quality of the haylage produced, through aspects of chemical composition, gas quantification, pH, N-NH_3, volatile fatty acids (VFAs), microbiological population and aerobic stability.

Materials and methods

Location and experimental design

The experiment was carried out in Alvorada do Gurguéia, Piauí, Brazil, which is located at latitude 08°25′28″ south, longitude 43° 46′38″ west and altitude of 281 m. The climate of the region is classified as Aw according to the classification of Köppen 1931, presenting dry winter and rainy summer (Alvares *et al.*, 2013).

A pasture area of 0.5 ha established in 2013 was used from January 2017 to January 2018. A maintenance fertilization programme was carried out to correct the levels of potassium, phosphorus and nitrogen, through the application of 30 kg K_2O/ha/year, potassium chloride (60% K_2O), 45 kg P_2O_5/ha/year, single superphosphate (18% P_2O_5 and 20% Ca^{2+}) and 150 kg N/ha/year urea (45% N), respectively, as recommended by Martha *et al.* (2007) according to soil analysis and recommendation for Marandu grass.

For the analysis of chemical composition and VFAs of the haylage of Marandu grass (*B. brizantha*), a completely randomized design with four treatments and five replications was adopted. The treatments were four levels of DM in the plant, for the production of the haylage, as follows: one treatment with the *in natura* plant (non-dehydrated), and three treatments in which the forage mass was dehydrated in the field under full sun, obtaining DM contents between 30 and 40, 40 and 50 and 50 and 60%.

For the gas analysis of the haylage, a completely randomized design in a 4 × 6 factorial scheme was adopted, with five replications. The factors were four DM contents of the forage mass for the production of haylage and six gas evaluation times: 0, 7, 15, 30, 45 and 60 days, after baling.

For the analysis of aerobic stability of the haylage, a completely randomized design in a 4 × 6 factorial scheme with five replications was adopted. The factors were four DM contents of the forage mass for the production of haylage and six evaluation times: 0, 24, 48, 72, 96 and 120 h after opening the bales.

Haylage making

The forage plant was harvested in the phenological stage before flowering with approximately 50 cm of height, according to the pre-established residue height for the species of 15 cm from the ground, according to the recommendations made by Fonseca and Martuscello (2010), and the grass was harvested using a professional manual mower, that had a 62 cm^3 two-stroke engine. The harvest was carried out in the morning, aiming to allow greater efficiency in the treatments that needed dehydration.

The *in natura* material was immediately baled, and the remaining forage mass was dehydrated until reaching DM contents between 30 and 40, 40 and 50 and 50 and 60%, depending on the treatment, with variation from 2:30 to 3:00 h, then the material was collected and baled in manual balers. The baler was used for compacting the grass to make bales of ±5 kg. The equipment was made of wood and had the following dimensions: 40 cm × 40 cm × 30 cm, with a 1.5-m wooden handle for manual pressing. The dehydration of the material was performed under full sun and the forage mass was turned over to standardize the dehydration. The determination of the DM content was measured by the microwave method according to Souza *et al.* (2002). The bales were wrapped with a conventional plastic film with in a specific manual wrapper for this function. The wrapper was a wooden support (with 1.5 m of height) for the plastic film rolls (50 cm of length), and the wrapping process was performed manually. The haylage bales weighed 3 kg and were stored in a covered shed after wrapping for 90 days.

Chemical composition of the plant and haylage

Chemical analysis of the *in natura* plant (Table 1) and haylage was performed after 90 days of storage. The analyses were conducted at the Animal Nutrition Laboratory and CPCE/UFPI Laboratory, in Bom Jesus, Piauí, Brazil. The contents of DM (method INCT-CA G-003/1), crude protein (CP) (method INCT-CA N-001/1) and mineral matter (MM) (method INCT-CA N-001/1) were determined. The fractions of neutral detergent fibre (NDF) and acid detergent fibre (method INCT-CA F-002/1) were determined according to Detmann *et al.* (2012) following the procedures of the AOAC (1990) method. The concentration of total CHO was obtained through the concentrated sulphuric acid method, described by Dubois *et al.* (1956), with adaptations by Corsato *et al.* (2008). The organic compounds were extracted in ethanol solution. The concentrations of CHO were measured by reading absorbance at 490 nm using D-glucose as standard (Dubois *et al.*, 1956). The proportion of CHO, in g/100 ml, was calculated based on the solution and later adjusted based on the DM of each sample used. All analyses were performed at the Animal Nutrition Laboratory of the Federal University of Piauí in Bom Jesus, Piauí, Brazil.

Table 1. Chemical composition of Marandu grass according to the DM content in the storage

Variables	DM content			
	In natura	30–40% DM	40–50% DM	50–60% DM
DM (g/kg)	269.8	358.1	467.0	566.8
CP (g/kg DM)	88.4	90.5	81.5	101.6
NDF (g/kg DM)	689.1	667.7	668.6	714.5
MM (g/kg DM)	55.6	58.0	56.5	54.8
OM (g/kg DM)	944.3	941.9	943.4	952.3
CHO (g/kg DM)	119.1	76.9	44.4	43.5

NDF, neutral detergent fibre; MM, mineral matter; OM, organic matter; CHO, soluble carbohydrates.

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Gas analysis of the haylage

Measurements of the oxygen (O_2) and carbon dioxide (CO_2) contents were performed at the wrapping and opening of the haylage bales, according to the respective treatment. The readings were taken at 0, 7, 15, 30, 45 and 90 days, inside the bale, through two valves (polyvinyl chloride pipes) that were inserted in each bale. The analysis was performed with a gas analyser to directly determine the percentage of gases (O_2 and CO_2) inside the haylage bale. For the O_2 evaluation, an INSTRUTHERN O_2 meter (model MO-900) was used, while the CO_2 was measured by using the TESTORYT CO_2 analyser.

Evaluation of pH, N-NH_3 and aerobic stability of the haylage

At the opening of the bales, the forage mass was exposed to air at a controlled room temperature (25 °C), similar to the evaluations carried out by Johnson *et al.* (2002). The room temperature was controlled by using an INCOTERM® thermometer, while the internal temperature of the haylage was measured by using a digital INCOTERM® skewer thermometer and the surface temperature by using a digital BENETECH® infrared thermometer with Mira Laser (−50 to 420 °C). After opening the bales, the temperature was measured at 0, 24, 48, 72, 96 and 120 h. The break in aerobic stability was considered when the haylage temperature increased by 2 °C above room temperature after opening the bales (Moran *et al.*, 1996).

During the evaluation times, samples of each haylage were collected (approximately 100 g) at times 0, 24, 48, 72, 96 and 120 h and the pH was measured according to the methodology described by Mizubuti *et al.* (2009). To evaluate the ammoniacal nitrogen (N-NH_3) content, samples of 100 g were collected from the samples of the evaluated times (0, 24, 48, 72, 96 and 120 h), and the readings of N-NH_3 values were performed, according to the methodology described by Mizubuti *et al.* (2009).

Microbiological population analysis of the plant and haylage

The evaluation of the microbiological population was carried out according to the recommendations of González and Rodríguez (2003), by collecting 25 g of fresh sample of the *in natura* plant and haylage after 90 days of storage. Distilled water (225 ml) was added to the haylage and the mixture was processed in a blender for approximately 1 min. Then, 1 ml of the mixture was removed and pipetted with the appropriate dilution (10-1 to 10-9). Plating was performed in duplicate for each culture medium. The populations were determined by the selective technique of cultures in anaerobic medium, where the following were used: agar Rogosa medium for lactobacilli counting (after incubation for 48 h in an oven at 37 °C); potato dextrose agar medium acidified with 1% tartaric acid for yeasts and moulds counting (after 5 days of incubation at room temperature) and brilliant green bile agar medium for enterobacteria counting (after 24 h of incubation at 35 °C). The plates considered susceptible for counting were those that presented values between 30 and 300 colony forming unit (CFU) in a Petri dish. The means of the plates of the selected dilution were considered. The differentiation between yeasts and moulds was performed through physical structure of the colonies, which was visually perceptible, as yeasts are unicellular and moulds are multicellular.

Determination of VFAs of the haylage

In order to quantify the content of VFAs, a portion of each collected haylage sample was used, in which the acetic, propionic, isobutyric, butyric, isovaleric and valeric acids contents in the haylage after storage of 90 days were determined, using the method proposed by Kung and Ranjit (2001), where a juice was extracted by a manual press. The samples were centrifuged and, later, the VFAs were analysed through high-resolution liquid chromatography detector model SPD-10ᵃ VP coupled to an ultraviolet detector using a wavelength of 210 nm. Decimal alcohol degree of boiling was determined through an ebulliometer as recommended by Maia and Campelo (2006). The analyses were carried out at the Laboratory of the Luiz de Queiroz College of Agriculture (ESALQ), São Paulo, Brazil.

Statistical analysis

The data were subjected to analysis of variance, with mean comparison through the Tukey's test for the treatments (DM contents) and linear regression for the evaluation times, and all analyses were performed adopting a significance level of $P < 0.05$. The data were analysed by SISVAR software version 5.0 (Ferreira, 2011). In the analysis of the chemical composition data, VFAs and gases, the Tukey's test was used. While, in the evaluation of the aerobic stability data, the Tukey's test was used for the DM content of the plant and linear regression analysis for the evaluation times. The data referring to the quantification of microbiological populations (in logarithmic unit, $\log_{10}$) were analysed descriptively.

Results

Chemical composition of the haylage

The chemical composition of the haylage of Marandu grass (*B. brizantha*) according to different DM contents in the storage presented differences ($P < 0.05$) only for DM and CHO (Table 2), whereas for the other variables of chemical composition there was no effect of the treatments ($P < 0.05$).

The treatment with 50–60% DM provided the highest DM content in the haylage, that was 563.8 ± 6.9 g/kg DM, which represents 103% more DM when compared to the treatments *in natura* and 30–40% DM, that presented an average of 310.7 g/kg DM. There were losses in the CHO contents in the treatments *in natura*, 30–40% DM, 40–50% DM and 50–60% DM, which were 432, 177, 48 and 4%, respectively, in comparison with the carbohydrate content found before storage, which were 119.1, 76.9, 44.4 and 43.5 g/kg, respectively (Table 1).

After opening the haylage bales, the average CP was 94.6 ± 3.58 g/kg (Table 2), which represents an increase of 7% when compared to what was observed in the grass before storage, which was 88.4 g/kg. NDF content after opening the bales was an average of 729.5 g/kg (Table 2), representing an increase of 2% compared to the grass before storage, which was 712.7 g/kg (Table 1). The average content of MM was 59.3 ± 3.71 g/kg (Table 2), while for organic matter (OM) the average was 940.1 ± 56.5 g/kg after opening the haylage bales, compared with an average of 944.3 g/kg in the grass before storage.

Effects on O_2, CO_2 and internal temperature of the haylage

There was significant effect ($P = 0.02$) of the different DM contents of the haylage on the quantification of O_2 and CO_2, as

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Table 2. Chemical composition of the haylage of Marandu grass according to different contents of DM in the storage

Variables	*In natura*	30–40% DM	40–50% DM	50–60% DM	Mean	S.E.M.	*P*-value
	g/kg						
DM	276.8c	344.6c	453.9b	563.8a	397.3	6.90	<0.01*
	g/kg DM						
CP	96.9	87.5	94.8	99.4	94.6	3.58	0.18^{ns}
NDF	712.7	722.5	722.6	760.0	729.5	38.0	0.82^{ns}
MM	63.1	58.7	58.1	57.1	59.3	3.71	0.68^{ns}
OM	936.8	941.2	941.8	940.8	940.1	56.5	0.09^{ns}
CHO	22.4b	27.8ab	30.1ab	42.0a	30.6	3.70	0.02*

DM, dry matter; CP , crude protein; NDF, neutral detergent fibre; MM, mineral matter; OM, organic matter; CHO, soluble carbohydrates; S.E.M., standard error of the mean.
Means followed by different letters in the row are statistically different from each other by the Tukey's test $P < 0.05$; *significant at $P < 0.05$; ^{ns}non-significant at $P > 0.05$.

Table 3. Quantification of O_2, CO_2 and internal temperature of the haylage of Marandu grass according to different contents of DM and storage periods

DM content	Days of storage (DS)						Mean
	0	7	15	30	45	90	
O_2 (%)							
In natura	22.2	1.7	0.6	1.4	1.9	1.3	4.8A
30–40% DM	21.6	1.2	0.6	1.2	1.4	1.1	4.5B
40–50% DM	21.7	1.4	0.8	1.2	1.3	1.2	4.6AB
50–60% DM	21.9	1.7	1.2	1.0	1.5	1.0	4.7AB
Mean	21.8a	1.5b	0.8c	1.2b	1.5b	1.1bc	
CO_2 (%)							
In natura	0.2	38.5	32.0	18.0	18.7	14.2	20.2AB
30–40% DM	0.0	37.7	23.2	14.2	15.0	14.0	17.3B
40–50% DM	0.0	33.0	27.0	17.5	16.5	12.5	17.5AB
50–60% DM	0.2	44.2	32.2	20.5	16.5	16.7	21.7A
Mean	0.1d	38.2a	28.6b	17.5c	16.6c	14.3c	
Temperature (°C)							
In natura	33.5bC	30.0cB	34.1aA	27.6dA	26.6eB	26.5eB	29.7
30–40% DM	33.8aBC	30.0bB	34.0aAB	27.6dA	27.1eA	28.2cA	30.1
40–50% DM	34.3aA	30.2cAB	33.7bB	27.6eA	27.1fA	28.1dA	30.2
50–60% DM	34.0aAB	30.5bA	34.0aAB	27.4dA	27.0eA	28.0cA	30.2
Mean	33.9	30.2	33.9	27.5	26.9	30.2	

Analysis of variance	*P*-value			
	DM content	DS	DM content × DS	S.E.M.
O_2	0.02	<0.01	0.37	0.07
CO_2	0.02	<0.01	0.84	1.43
Temperature	<0.01	<0.01	<0.01	0.03

S.E.M., standard error of the mean.
Means followed by different letters in the column are statistically different from each other by the Tukey's test $P < 0.05$; *significant at $P < 0.05$; ^{ns}non-significant at $P > 0.05$.

well as a difference was observed between the storage days for these gases (Table 3). The presence of O_2 in the haylage of Marandu grass was greater in the *in natura* material, which was 4.8 ± 0.07%. As for the storage days, on the day the bales were wrapped (day 0) the haylage had a greater amount of O_2, which was 21.8 ± 0.07%, reducing to values less than 2% after 7 days of storage, showing no difference ($P = 0.01$) between 30, 45 and 90 days of storage. The highest amount of CO_2 in the bales

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($P = 0.02$) was found in the treatment 50–60% DM, which was 21.7 ± 1.43%, as well as the highest value was found after 7 days of storage, which was 38.2 ± 1.43% CO_2 in the haylage of Marandu grass.

An effect of interaction ($P = 0.01$) between the DM content of the plant and storage days was found on the internal temperature of the haylage of Marandu grass (Table 3). The internal temperature of the haylage with content of 40–50% DM was superior to the other treatments at 0 days of storage, which was 34.3 ± 0.03 °C. The haylage stored for 30, 45 and 90 days after bailing showed temperatures below 29 °C in all treatments.

Microbiological population of the plant and haylage

In the evaluation of the haylage of Marandu grass, no variation was observed between the different treatments in the population of lactic acid bacteria (LAB), which presented an average of 6.98 $\log_{10}$ CFU/g (Fig. 1). No yeast populations were found in the haylage of Marandu grass, regardless of the DM content in the storage.

A greater number of moulds was observed in the haylage stored with 50–60% DM, which was 7.67 $\log_{10}$ CFU/g. Also, the haylage of Marandu grass stored with 50–60% DM showed a higher concentration of enterobacteria, which was 5.00 $\log_{10}$ CFU/g.

VFA content

Only acetic acid was affected ($P = 0.05$) by the DM content in the haylage of Marandu grass (Table 4), while there was no significant effect of the DM contents on propionic, isobutyric, butyric, isovaleric and valeric acids.

When the grass was stored with DM contents of 30–40 and 50–60%, the haylage presented higher concentrations of acetic acid, which were 55.92 and 46.44 g/kg DM, respectively. The lowest concentration of acetic acid was observed in the haylage of *in natura* material (±28% DM), which was 8.58 g/kg DM.

Aerobic stability, pH and N-NH_3 of the haylage

There was an effect of interaction ($P = 0.01$) between the DM content and the time of exposure of the haylage to air on pH (Table 5). For the surface temperature, there was a linear positive effect ($P = 0.01$) according to the time of air exposure. For the

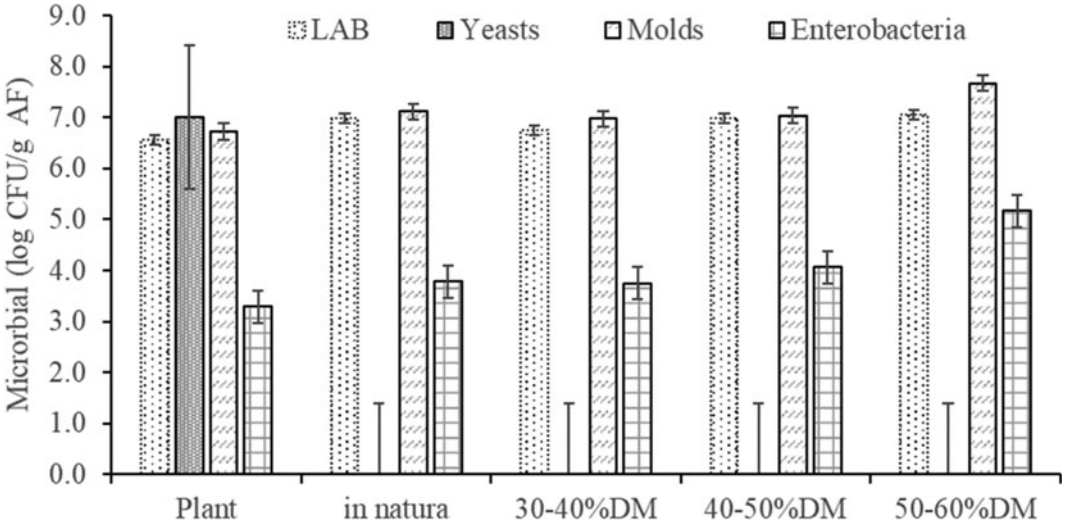

Fig. 1. Evaluation of the microbial population of the Marandu grass plant and its haylage according to different contents of DM in the storage (*in natura*, 30–40 DM, 40–50 DM and 50–60% DM).

Table 4. Contents of VFAs in the haylage of Marandu grass according to different contents of DM in the storage

	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric
			g/kg DM			
In natura	8.58C	7.46	4.48	2.05	2.83	9.43
30–40% DM	55.92A	4.68	1.74	2.53	3.06	5.34
40–50% DM	21.72B	2.06	0.0	1.04	0.0	0.0
50–60% DM	46.44A	1.24	0.0	0.00	0.0	0.0
P-value	<0.05	0.17	0.50	0.41	0.50	0.19
S.E.M.	5.11	4.81	8.95	1.95	5.79	14.01

S.E.M., standard error of the mean.
Means followed by different letters in the column are statistically different from each other by the Tukey's test $P < 0.05$; *significant at $P < 0.05$; ^{ns}non-significant at $P > 0.05$.

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Table 5. Aerobic stability of the haylage of Marandu grass according to different contents of DM in the storage

	Hours							*P*-value	
DM content	0	24	48	72	96	120	Mean	*X*	R^2
Room temperature (°C)									
	24.0	24.2	24.1	24.2	24.6	24.5			
Surface temperature (°C)									
In natura	20.6	23.0	21.7	22.7	24.1	24.4	22.7	–	–
30–40% DM	20.9	25.5	21.8	22.3	24.0	24.1	23.1	–	–
40–50% DM	20.9	22.7	21.5	23.2	24.2	24.2	22.8	–	–
50–60% DM	21.5	23.1	22.2	23.1	24.4	24.4	23.1	–	–
Mean	21.5	22.1	22.6	23.2	23.7	24.3		<0.01	58.9
Internal temperature (°C)									
In natura	21.7	24.2	24.0	23.2	24.5	25.2	23.8A	–	–
30–40% DM	22.0	24.0	23.5	23.5	23.7	24.7	23.5AB	–	–
40–50% DM	21.7	23.5	22.2	23.0	24.5	24.5	23.2B	–	–
50–60% DM	22.0	24.2	22.5	23.5	24.5	25.2	23.6AB	–	–
Mean	22.4	22.8	23.3	23.8	24.2	24.7		<0.01	67.0
pH									
In natura	5.6C	6.0AB	5.9BC	6.4A	6.0BC	6.5A	6.1	<0.01	64.3
30–40% DM	5.7BC	6.0AB	6.1AB	6.1B	5.8C	6.2B	6.0	<0.01	29.3
40–50% DM	6.0AB	5.8B	5.8C	6.0B	6.2AB	6.1B	6.0	<0.01	51.5
50–60% DM	6.2A	6.1A	6.2A	6.2AB	6.4A	6.3AB	6.2	<0.01	54.8
Mean	5.9	5.9	6.0	6.1	6.2	6.2		–	–
N-NH_3 (%)									
In natura	1.85	1.35	1.30	1.15	0.85	0.50	1.26B	–	–
30–40% DM	2.95	2.00	1.50	1.80	1.00	0.50	1.62A	–	–
40–50% DM	1.60	1.05	1.00	1.20	0.55	0.45	0.97C	–	–
50–60% DM	1.45	1.35	0.95	1.25	0.40	0.35	0.95C	–	–
Mean	1.96	1.43	1.18	1.35	0.70	0.45		<0.01	89.7

Analysis of variance	*P*-value			S.E.M.
	DM content	Hours	DM × Hours	
Haylage surface temperature	0.44^{ns}	<0.01	0.17^{ns}	0.21
Haylage internal temperature	<0.01	<0.01	0.06^{ns}	0.11
pH	<0.01	<0.01	<0.01	0.02
NH_3	<0.01	<0.01	0.10^{ns}	0.07

S.E.M., standard error of the mean.
Means followed by different letters in the column are statistically different from each other by the Tukey's test $P < 0.05$; *significant at $P < 0.05$; ^{ns}non-significant at $P > 0.05$; × = linear effect.

internal temperature and NH_3 there was effects ($P = 0.01$) of both the DM content of the haylage material and the time of air exposure. There was no break in the aerobic stability of the haylage of Marandu grass in any of the treatments.

The pH values according to the different DM contents of the stored material had an increasing linear effect of the hours of air exposure, with higher values at 96 and 120 h. The greatest variation in pH was observed in the *in natura* material, which ranged from 5.6 to 6.5. As for N-NH_3, there was a greater amount in the treatment with 30–40% DM, which was 1.62 ± 0.07%, while the haylages with 40–50 and 50–60% DM presented values lower than 1.0%.

Discussion

Chemical composition of the haylage

A difference was observed between the treatments regarding the DM contents of the haylage of Marandu grass (Table 2), which was expected, since the treatments consisted of baling the

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Marandu grass with different DM contents (Table 1). Müller *et al.* (2011) evaluated the quality of haylage through the chemical and microbiological composition, and found a DM content of 653 ± 89.9 g/kg which is close to what was found in the haylage of Marandu grass with 50–60% DM, and this value might be related to the moisture content of the grass used at the moment of baling, which shows that DM values close to 600 g/kg, are adequate for grass haylage. Higher DM contents of the material used for haylage making can provide better conditions to the forage mass for conservation. According to Müller (2018) the DM content of the plant at harvest directly influences the haylage fermentation, because as the plant dehydrates during the field drying period, the DM increases.

The haylage with the highest DM content (50–60% DM) showed greater conservation of CHO, while the haylage with more moisture in the material had lower CHO content. The reduction in the content of CHO occurs due to their conversion into organic acids by microorganisms, which, found in ideal environment, proliferate, providing a suitable space for the fermentation of the ensiled material (Ferreira *et al.*, 2019). CHO are used by microorganisms during fermentation in bale-preserved forages (McDonald *et al.*, 1991), and lower amounts of CHO in the haylage can be an indicative of undesirable fermentations. Nath *et al.* (2018) found 32.29 g/kg DM of CHO in haylage of Tifton 85, while in the haylage of Marandu grass with 50–60% DM, higher values were observed. According to Silva *et al.* (2021) and Amorim *et al.* (2020), in order to obtain an adequate fermentation, a water-CHO content between 60 and 150 g/kg DM is needed. Although the CHO content in the plant was 119.1 g/kg DM (Table 1), it had high moisture, thus the haylage from *in natura* material had the lowest amount of CHO (Table 2).

The CP contents of the haylage of Marandu grass were greater than 80 g/kg, with no difference between treatments. However, despite the low value of CP, this amount is considered to be adequate for ruminant nutrition, as it is within the minimal range of 60.0–80.0 g/kg DM for ruminants, thus meeting the microbial requirements for nitrogen compounds, obtaining the ideal effect on rumen fermentation as suggested by Van Soest (1994). For instance, a CP level of 80 g/kg DM is sufficient for a dry beef cow, but totally inadequate for a growing steer or dairy cow, which would need supplementation. It was also not observed influence of the DM contents of the haylage of Marandu grass on NDF, MM and OM contents. The average NDF contents found in the haylage of Marandu grass are below those observed by Ferreira *et al.* (2019) which was 743.8 g/kg. Lower values of MM and OM were observed in the haylage of Marandu grass than those observed by Bergamaschine *et al.* (2006) who found MM content of 75.6 g/kg and OM content of 924.4.4 g/kg. These contents of the chemical composition of the haylage of Marandu grass were not affected due to the preservation of these substances in the conservation of the material. According to Nascimento *et al.* (2021) the non-alteration of the chemical composition in a fermentation process is a positive fact in forage conservation.

Effects on O_2, CO_2 and internal temperature of the haylage

The final O_2 concentration stabilized from the 7th day onwards at values <2% regardless of the DM content of the stored material. It is important to point out that, when the material is wrapped for haylage making, it creates an aerobic environment and the O_2 present inside the bale is consumed by aerobic microorganisms up to a reduction to levels below 2%. In the first 23 h of ensiling, respiration continues in the ensiled grass, with losses of CHO and increase in temperature, as well as aerobic microorganisms in the forage (Jobim, 2010). In wrapped environments, there is a low amount of O_2, which creates the ideal conditions for the action of microorganisms that are beneficial to the fermentation desirable for conservation, leaving the feed in a good state of conservation, preserving its nutritional value. According to Costa *et al.* (2020) this occurs because CHO are converted into organic acids by the action of these microorganisms and that is due to the consumption of O_2 by aerobic microorganisms and stabilization of the environment through the reduction in the amount of O_2. No higher values of O_2 were found, due to the DM values of the material at the moment of wrapping, which is characteristic of the haylage not to be totally dry as in hay, thus O_2 decreases and CO_2 increases quickly.

The haylage made from *in natura* material presented a higher concentration of O_2, increasing the aerobic phase inside the bales. The aerobic phase occurs immediately following haylage wrapping, which favours the proliferation and growth of opportunistic and undesirable microorganisms that are present in the forage, such as yeasts, fungi and aerobic bacteria, and they consume nutrients and release CO_2, which is a process that can cause losses in the nutritional value of the haylage, compromising the intake by the animals (Lindgren *et al.*, 1985; Mantilla *et al.*, 2010*a*).

The highest concentration of CO_2 observed after 7 days of storage of the haylage of Marandu grass was due to fermentation processes of the forage after aerobic fermentation, when there is an intense microbial activity, as these microorganisms consume a large part of the CHO and the O_2 present there, and in return release CO_2 and acids, with heat generation (Kung and Ranjit, 2001). This shows that anaerobiosis produced relatively little heat in comparison with aerobic respiration in the haylage. According to Ashbell and Lisker (1988), it should be noted that if the haylage package presents any leakage point, there may be outflow of CO_2, which will be replaced by O_2 and N_2. In this experiment there was a reduction in the CO_2 produced and the amount of O_2 remained below 2% after 7 days of storage. The haylage with 50–60% DM had the highest concentration of CO_2, which may contribute to preserving the quality of the conserved material, since this gas has properties to control the action of microorganisms that are undesirable to fermentation. According to Mantilla *et al.* (2010*b*) the increase in the conservation time of feeds is due to the inhibitory effect of CO_2) on different microbial types and the reduction or removal of O_2 from the interior of the package.

The temperature reduction according to the haylage storage period occurs due to the stabilization of the activity of microorganisms. Heat losses in the stored material depend on the oxidation of DM promoting losses in the form of CO_2, as reported by Hill and Leaver (2002). The highest temperatures were observed in the first 15 days of storage in all treatments due to the intensity of the aerobic microbial activities in that period. At the beginning of the haylage storage process, loss will always occur due to the presence of O_2, thus, according to Silva *et al.* (2020) the longer the aerobic phase, the greater the nutritional losses in the material.

During fermentative activities inside the haylage bales, the level of O_2 is reduced and CO_2 is raised by microorganisms,

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which depends on the species, extent of plant respiration, harvest processes, material wrapping and DM content (Costa *et al.*, 2018a).

Microbial population of the plant and haylage

The amount of LAB in the haylage was greater than that in fresh Marandu grass, as the plant is in an aerobic environment, whereas the haylage is in an environment with low concentrations of O_2. The Marandu grass presented an average LAB population of 6.57 $\log_{10}$ CFU/g, which, when compared to the treatment with 50–60% DM there was an increase of only 7% of LAB at the opening of the bales. Nath *et al.* (2018) evaluated haylage of Tifton 85 grass with four layers of packaging, and found a LAB concentration of 5.83 logs CFU/g, which is a value lower than what was found in the haylage of Marandu grass, that was higher than 6.00 $\log_{10}$ CFU/g. According to Muck and Albrecht (1991), a LAB population greater than 5.00 $\log_{10}$ CFU/g is an indicative of good fermentation. The increase in LAB is relatively small in crops (<10 CFU/g) (Jaster, 1994), however, the harvest of the forage plant causes the release of plant saps, in addition to the species and plant maturity stage, that causes increase in LAB population prior to the haylage making (Santos *et al.*, 2013), favouring the fermentative process, which is similar to what was observed in the present study.

The lack of yeasts in the haylage of Marandu grass is probably due to the high pH in the medium obtained, regardless of the treatment. Yeasts become important in the conservation process through fermentation when there are very high amounts of carbohydrates, associated with a very acidic pH below 3.0, with predominantly alcoholic fermentation, which result in substantial losses in the form of CO_2 (Santos *et al.*, 2019), which was not observed in this experiment. Yeasts indicate aerobic deterioration, consuming sugars and fermentation acids and increasing temperature and pH (Moraes *et al.*, 2017). Yeasts convert sugars into alcohol, which is good fermentation, where about 95% of sugar is converted to ethanol and CO_2, 1% is converted to yeast cell material and 4% is converted to other products such as glycerol (Vohra *et al.*, 2016; Costa *et al.*, 2018b).

Among the treatments, the haylage of Marandu grass with 50–60% DM showed the largest mould population. The presence of moulds in haylage can indicate the degradation of residual sugars and lactic acid produced in the anaerobic phase. Anaerobic fermentation begins with competition between epiphytic microorganisms to use CHO and produce fermentation products (McDonald *et al.*, 1991; Ridwan *et al.*, 2015). In the visual observation of the haylages, moulds were not observed in any of the treatments.

The haylage of Marandu grass with 50–60% DM presented the largest population of enterobacteria. In fact, enterobacteria are one of the main competitors of LAB for the use of CHO present in the forage (Schenck and Müller, 2013). In the fermentation of enterobacteria, the main product is acetic acid, which has a positive effect on aerobic stability, thus, haylages with pool aerobic stability are those with high-residual sugar and lactic acid contents (Collins *et al.*, 2017).

VFA content

When the Marandu grass was stored with 30–40% DM and 50–60% DM it showed higher concentrations of acetic acid, probably due to the presence of enterobacteria during fermentation (Nath *et al.*, 2018), which was observed in the treatment with 50–60% DM where a high population of these bacteria was found. The greater amount of acetic acid helps in the reduction and proliferation of bacteria of the genus *Clostridium* (Santos *et al.*, 2019) and the quality of the haylage is maintained for a longer period, mainly after opening the bale.

Propionic, isobutyric, butyric, isovaleric and valeric acids in the haylage of Marandu grass were not influenced by the DM contents (Table 4), which is considered important, since these are undesirable for fermentation, as they promote fast deterioration of the haylage. Butyric acid is related to the lowest rates of decrease and highest final pH values. Heterofermentative microorganisms use lactic acid and glucose as substrates for the production of acetic and propionic acids, which are effective at controlling fungi under low pH (Zopollatto *et al.*, 2009).

Aerobic stability, pH and N-NH_3 of the haylage

There was no break in aerobic stability during 120 h of air exposure of the haylage with different DM contents. The break in aerobic stability is obtained when the material, after exposure to air, presents an increase of 2 °C in relation to the room temperature, according to Taylor and Kung (2002). The haylage temperatures can be considered stable, since the changes were minimal, in addition, they did not exceed 2 °C above the room temperature.

Bernardes *et al.* (2008) evaluated the fermentation profile, aerobic stability and nutritional value of Marandu grass silages ensiled with additives, and found that the temperature of the studied silage did not exceed 2 °C in relation to the room temperature during 6 days of air exposure. Araújo *et al.* (2020) found that the aerobic stability lasted up to 62 h in silages that were made right after harvest, showing that the longer time of the ensiling process can lead to lower aerobic stability of the silages. It can be inferred that there was no break in stability, as they are pasture grasses, with high moisture content, low levels of CHO and high content of acetic acid.

The haylage with 50–60% DM at 0 h, after opening the bale, presented the highest pH value when compared to the other treatments that presented pH values above 5.5. The high pH explains the larger population of enterobacteria observed in this treatment. For material stored in controlled environments, conventionally conserved, a high pH is an indicative of greater production of butyric and acetic acids, which are characteristic of undesirable fermentation processes (Van Soest, 1994). According to Santos *et al.* (2019), when the amount of substrate in the medium is not enough for an intense fermentation, there will be a predominance of enterobacteria, when the pH remains above 6.0. High pH values are frequent in haylage.

The haylages with 40–50 and 50–60% DM had lower N-NH_3 values. The value obtained in this study was similar to what was found by Costa *et al.* (2018*a*) which was 1.25% and by Guimarães *et al.* (2019) which was 1.10%, in haylage of Tifton 85 at 56 days of evaluation.

Conclusion

Marandu grass (*B. brizantha*) with a DM content of 50–60% is recommended for storage in the form of haylage, as it has higher contents of DM, CHO, acetic acid and CO_2, lower contents of ammonia nitrogen, and demonstrate no break in aerobic stability.

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Conflict of interest. The authors declare that there are no conflicts of interest.

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