Quality of Tanzania grass (*Panicum maximum*) haylage in relation to plant dry matter content

Calidad del pasto Tanzania (*Panicum maximum*) almacenada como henolaje según la materia seca de la planta

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Originales: *Recepción:* 25/10/2020 - *Aceptación:* 13/04/2023

Abstract

This study aimed to evaluate the quality of Tanzania grass (*Panicum maximum*) haylage with varying contents of dry matter (DM) and stored for 90 days. The quality of this grass was evaluated through the lens of a variety of physiochemical properties (*e.g.*, chemical composition, aerobic stability, pH, microbial profile, etc.). A completely randomized design was used with four treatments (*in natura*, 400, 500, and 600 g kg-^{1} DM) and five replicates. Treatment with 600 g kg^{-1} DM yielded the highest DM haylage ($p < 0.01$) and soluble carbohydrate content ($p < 0.01$). Treatment *in natura* resulted in the highest O_2 concentration inside the bales ($p < 0.01$), whereas treatments with 500 and 600 g kg^{-1} DM resulted in the highest CO_2 values. The highest acetic acid concentrations of 36.4 ± 1.6, 38.2±1.6, and 48.9 ± 1.6 g kg^{-1} DM ($p < 0.01$) were observed post the *in natura*, 500 g kg^{-1} DM, and 600 g kg^{-1} DM treatments, respectively. Treatment with 600 g kg^{-1} DM yielded the highest pH value at hour zero ($p < 0.01$). Tanzania grass with 500 and 600 g kg^{-1} DM produced the highest quality haylage.

Keywords
conservation • haylage • grasses • microbiology • moisture • Tanzania grass

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Resumen

El objetivo fue evaluar la calidad del henolaje del pasto Tanzania (*Panicum maximum*) con diferentes contenidos de materia seca y un almacenamiento de 90 días, a través de la composición química, cuantificación de gases, ácidos grasos volátiles, perfil microbiológico, estabilidad aeróbica, pH y nitrógeno amoniacal. El diseño experimental utilizado fue completamente al azar con cuatro tratamientos y cinco repeticiones. Los tratamientos consistían de cuatro contenidos de materia seca (MS) de la planta en el momento de la producción del henolaje del pasto Tanzania siendo: en materia fresca (sin deshidratación), 400, 500 y 600 g kg^{-1} de MS (deshidratados en pleno sol). El tratamiento con 600 g kg^{-1} de MS de la planta proporcionó la mayor estimación ($p < 0,01$) de MS en el henolaje con 581,6 ± 15,4 g/kg, y el mayor ($p < 0,01$) contenido de carbohidratos solubles con 45,4 ± 1,24 g/kg MS. Después de 90 días de almacenamiento, el tratamiento en materia fresca presentó mayores ($p < 0,01$) cantidades de O_2 en el interior de los fardos. En relación al CO_2 los mayores índices fueron observados para los tratamientos con 500 y 600 g kg^{-1} de MS. También se observó el mayor ($p < 0,01$) contenido de ácido acético en los tratamientos materia fresca y con 500 y 600 g kg^{-1} de MS de la planta, con 36,4 ± 1,6, 38,2 ± 1,6 y 48,9 ± 1,6 g kg^{-1} de MS, respectivamente. Para el ácido butírico se obtuvo la mejor ($p < 0,01$) valoración de 27,0 ± 0,5 g kg^{-1} de MS en el tratamiento en materia fresca. El tratamiento con 600 g kg^{-1} de MS mostró mayor ($p < 0,01$) valor de pH en la hora cero con 6,36 ± 0,03. El tratamiento en materia fresca presentó mayor valor de N_{-NH3} en la hora cero de exposición al aire con 4,65 ± 0,12. El pasto Tanzania con 500 y 600 g kg^{-1} de MS, presenta el henolaje de mejor calidad.

Palabras clave
conservación • henolaje • gramíneas • microbiología • humedad • pasto Tanzania

Introduction

The storage of forage plants in the form of haylage is in line with the sustainable use of leguminous and grass forage species. For example, oats (*Avena sativa*) and ryegrass (*Lolium multiflorum*) are suitable for the production of haylage in temperate regions, whereas species in the genera *Brachiaria*, *Cynodon*, *Panicum*, and *Pennisetum* are better suited for the production of haylage in tropical regions (27). Tanzania grass (*Panicum maximum*) has shown great potential within the context of haylage production, as it has a high yield, a large number of leaves, and a high nutritional value (9, 54).

Haylage can be defined as stored pre-dried forage with a dry matter (DM) content of approximately 400 to 800 g/kg (7, 43). It is stored in the form of bales wrapped in a plastic cover, providing ideal conditions for the growth of lactic acid bacteria (LAB) that are beneficial for the conservation and storage of forage. This forage would then be used as animal feed; this is especially important when resources are scarce (*e.g.*, during droughts) (22).

The preservation of grass in the form of haylage is an option for forage grasses with high moisture content because dehydration of the material increases DM content, which reduces proteolysis, secondary fermentation, and pH buffering in the stored material (23). The moisture content of the forage plant is one factor that influences the microbial profile of the forage mass preserved by fermentation (61). When harvested, tropical grasses have a high moisture content accompanied with low levels of soluble carbohydrates (CHO) (49), which favors the occurrence of undesirable fermentation as the grass is preserved.

An alternative to adjusting the DM content in tropical grasses is dehydration in the field after cutting (10). This process increases the DM content of the forage mass, facilitating the preparation of the material for undergoing preservation via fermentation. The DM percentage of haylage influences the quality of the stored material (40).

No exact recommendations are available for the DM content of tropical grasses for conservation as haylage, and no studies have been conducted within the context of determining the DM content of Tanzania grass. Therefore, this study aimed to evaluate the quality of Tanzania grass haylage stored with different DM contents based on its chemical composition, gas quantification, volatile fatty acids, microbiological profile, and aerobic stability.

Material and methods

Study Area

A pasture area established in 2013 was used for haylage production. The study area is in Alvorada do Gurgueia, Piauí, Brazil, at latitude 08°25'28" South, longitude 43°46'38" West, and an altitude of 281 m. According to the Köppen classification (1936), the climate of the region is classified as BSh, hot semi-arid, with rainy summers and dry winters, as described Medeiros *et al.* (2013) and Alvares *et al.*(2013).

The area of the pasture was determined to be 0.5 hectares, and it had no artificial irrigation systems. A standardization cut was made 30 cm from the ground at the beginning of the experimental period for haylage production, according to the recommendation of Braz *et al.* (2017). Fertilization was performed according to the soil analysis and recommendations for highly demanding species (30).

Experimental design

To assess the chemical composition and volatile fatty acids of the Tanzania grass haylage, a completely randomized design with four treatments and five replicates was adopted. The treatments consisted of four groups of haylage that varied in terms of DM content as follows: *in natura* plant (not dehydrated), 400, 500, and 600 g kg^{-1} DM (dehydrated in the field until reaching the DM content of the treatment).

A completely randomized design in a 4 × 6 factorial scheme, with five replications, was adopted for the gas assessment of the Tanzania grass haylage. The factors were four levels of DM of the plant for haylage production and six gas evaluation times: 0, 7, 15, 30, 45, and 60 d after wrapping the haylage bales.

To assess the aerobic stability of Tanzania grass haylage, a completely randomized design in a 4 × 6 factorial scheme, with five replications was adopted. There were four levels of plant DM for haylage production and six evaluation times: 0, 24, 48, 72, 96, and 120 h after opening the bales.

Haylage production

Tanzania grass was harvested right before it flowered; at this point, the pasture had a height of 90 cm (30 days), as recommended by Euclides *et al.* (2014). The extracted material was left in the field for pre-drying until it reached the determined DM content (400, 500, and 600 g kg^{-1} DM), except for the material of the *in natura* treatment, which was not dehydrated and immediately baled. For treatments with pre-dried forage, the forage mass was revolved to standardize dehydration. The forage was collected and sealed when it reached the predetermined DM level. The DM content was determined using the microwave method as previously described (55).

The bales were made in manual balers and then manually wrapped in plastic film (SSFILM SSilage Xtreme®), with eight rounds per bale, as recommended previously (38), to minimize gas exchange. The haylage bales weighed approximately 3 kg and were stored for 90 d in a ventilated shed with no sunlight exposure.

To characterize the quality of the haylage, both *in natura* forage and haylage were assessed using the following variables: chemical composition, gas quantification, volatile fatty acids, microbiological profile, aerobic stability, pH, and ammonia ($N\text{-}NH_3$). The analyses were conducted in the Animal Nutrition Laboratory and Microbiology Laboratory of the Federal University of Piauí, located in the Bom Jesus, Piauí, Brazil.

Determination of chemical composition and gases

The samples used for the chemical composition analysis of Tanzania grass before the production of the haylage (table 1, page 41) (*i.e.*, after 90 days of storage) were dried in a circulation and air renewal oven, at a maximum temperature of 55 °C, until they reached a constant weight. They were then ground in a Thomas Willey stationary mill through a 1-mm-mesh sieve. The contents of DM (n°. 934.01), crude protein (CP n°. 981.10), mineral matter (MM n°. 934.05), and organic matter (OM n°. 934.05) were determined using the methods described previously (4), whereas neutral detergent fiber (NDF) was determined using the methodology proposed by Van Soest *et al.* (1991).

Table 1. Chemical composition of Tanzania grass across varying dry matter (DM) contents, expressed as g kg^{-1} DM, prior to the production of haylage.

Tabla 1. Composición química de la planta de pasto Tanzania de acuerdo con la deshidratación, expresada como g kg^{-1} DM antes de la producción de henolaje.

Variables	DM Content (g kg^{-1})			
	in natura	400	500	600
Dry matter (g kg^{-1})	238.7	381.2	486.0	575.3
Crude protein (g kg^{-1} DM)	106.2	121.2	123.1	130.2
NDF	724.3	749.0	657.8	613.0
MM	60.0	69.4	65.0	67.4
OM	939.9	930.6	934.9	946.2
CHO	80.9	73.8	67.7	54.1

NDF: neutral detergent-insoluble fiber. MM: Mineral matter. OM: Organic matter. CHO: Soluble carbohydrate.

NDF: Fibra insoluble en detergente neutro. MM: Materia mineral. OM: Materia orgánica. CHO: Carbohidratos solubles.

The total CHO (TCHO) content was determined using the concentrated sulfuric acid method described previously (17) with adaptations of Corsato *et al.* (2008). The TCHO content was calculated as g 100 ml^{-1} based on the solution and subsequently adjusted based on the DM of each sample used.

To evaluate the gases produced in the haylage, the levels of O_2 and CO_2 were measured. Assessments were performed on the haylage on days 0, 7, 15, 30, 45, and 90, after it was wrapped. Haylage was assessed on day 0, immediately after wrapping. The readings were acquired through two valves (PVC pipes) that were inserted into each bale and sealed for the duration of the established days'. For the gas analysis, an O_2 meter Instrutherm® (model MO-900) was used, which also measured the internal temperature of the bales, while CO_2 was measured by a CO_2 analyzer Testoryt® (White).

Quantification of volatile fatty acids and microorganisms

To quantify the contents of volatile fatty acids (*i.e.*, acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids) of Tanzania grass haylage after 90 days of storage, only portions of each sample were used for analysis through the method mentioned by Kung Jr and Ranjit (2001), where the juice was extracted using a manual press. The samples were centrifuged, and subsequently, the analysis of organic acids was performed using high-resolution liquid chromatography using a high-performance liquid chromatograph (HPLC) detector model SPD-10ª VP, coupled to the ultraviolet detector (UV), using a wavelength of 210 nm. The boiling alcohol content was determined using an ebulliometer, as recommended previously (28). Analyses were performed at the Laboratory of the Luís de Queiroz College of Agriculture.

Microbiological evaluation was performed according to the recommendations of González *et al.* (2003) by collecting 25 g of fresh sample, adding 225 mL of distilled water, and processing in a blender for approximately 1 min. One milliliter of the mixture was pipetted at the appropriate dilution (10^{-1} 10^{-9}). Plating was performed in duplicates for each culture medium. The populations were determined by the selective technique of culturing in anaerobic media. Rogosa Agar medium was used for counting lactobacilli (after incubation of 48 hours in an oven at 37°C); BDA Agar medium (Potato Dextrose Agar) acidified with 1% tartaric acid, for the counting yeasts and molds (after 3-7 days of incubation at room temperature); and Brilliant Green Bile Agar medium, for counting the enterobacteria (after incubation of 24 hours at 35°C).

Plates with values between 30 and 300 colony-forming units (CFU) in the Petri dish were considered acceptable for counting. Plaque averages of the selected dilutions were considered.

Evaluation of aerobic stability, pH, and ammonia nitrogen

When the haylage bales were opened, the forage mass was exposed to air under a controlled room temperature (25°C); this approach was similar to that applied in evaluations conducted in Johnson *et al.* (2002). Room temperature was controlled using an INCOTERM® room thermometer. The internal temperature of the haylage was measured using an

INCOTERM® digital skewer thermometer, and the surface temperature was measured using a BENETECH® infrared digital thermometer with laser aim (-50 to 420°C). Temperature was measured at 0, 24, 48, 72, 96, and 120 h. The aerobic stability break was defined as an increase of 2°C in the temperature of the haylage in relation to room temperature after opening the bales (35). During the evaluation period, samples from each treatment were collected (approximately 100 g) at different time points (0, 24, 48, 72, 96, and 120 h) to assess pH and ammonia ($N\text{-}NH_3$) levels, as per a previously described methodology (33).

Statistical analysis

The data were subjected to an analysis of variance. Means were compared using Tukey's test and linear regression, and all analyses were performed at a significance level of $p < 0.05$. The data were analyzed using the SISVAR software (version 5.0; 19).

Tukey's test was used to analyze the chemical composition and volatile fatty acid data. The adopted statistical model was:

$$\mathbf{Y_{ij} = \mu + T_i + \varepsilon_{ij}}$$

where:
Y_{ij} = record of the DM content i
μ = general constant
T_i = effect of the DM content i
with i = 1-4; ε_{ij} = random error associated with each DM content Y_{ij}

Gas data were analyzed using Tukey's test for plant DM and evaluation times. The following statistical model was adopted:

$$\mathbf{Y_{ijk} = \mu + A_i + T_j + AT_{ij} + \varepsilon_{ijk}}$$

where:
Y_{ijk} = record k, referring to the DM content i evaluated at time j
μ = general constant
A_i = effect of DM content i, i = 1-4
T_j = gas evaluation time j, j = 0-120
AT_{ij} = interaction between DM content i and gas evaluation time j
ε_{ijk} = random error associated with each Y_{ijk} record

To evaluate the aerobic stability data, Tukey's test was used for plant DM, and linear regression analysis was used for the evaluation times. The following statistical model was adopted:

$$\mathbf{Y_{ijk} = \mu + A_i + T_j + AT_{ij} + \varepsilon_{ijk}}$$

where:
Y_{ijk} = record k, referring to the DM content i, evaluated at time j
μ = general constant
A_i = effect of the DM content i, i = 1-4
T_j = stability evaluation time j, j = 0,..., 120
AT_{ij} = interaction between the DM content i and stability evaluation time j
ε_{ijk} = random error associated with each Y_{ijk} record

Data referring to the quantification of microbial groups (logarithmic units, log 10) were analyzed descriptively.

Results

Chemical composition and gases

The chemical composition of Tanzania grass haylage according to plant DM and CHO contents was affected ($p < 0.01$) after 90 days of storage (table 2).

Table 2. Chemical composition of Tanzania grass haylage according to the dry matter content.

Tabla 2. Composición química del henolaje de pasto Tanzania según el contenido de materia seca (MS) de la planta.

Variables	DM Content (g kg^{-1})				Mean	SEM	*–p-value*
	in natura	400	500	600			
Dry matter (g kg^{-1})	267.6^{c}	397.7^{b}	480.3^{b}	581.6^{a}	406.5	15.4	< 0.01
Crude protein (g kg^{-1} DM)	96.9	87.5	94.8	99.4	94.6	3.58	0.18
NDF	692.0	650.5	721.3	653.9	679.5	54.7	0.77
MM	74.1	74.2	73.8	71.0	59.3	3.71	0.83
OM	925.8	925.7	926.1	943.6	920.3	53.6	0.09
CHO	35.0^{b}	36.2^{b}	42.6^{a}	45.4^{a}	39.8	1.24	< 0.01

Means followed by different letters in a row indicate statistical differences according to Tukey's test at p <0.05; NDF: Neutral Detergent Insoluble Fiber. MM: Mineral matter. OM: Organic matter. CHO: Soluble carbohydrate. SEM: standard error of the mean.

Medias seguidas de letras diferentes en la fila son estadísticamente diferentes según la prueba de Tukey con $p < 0,05$. NDF: Fibra insoluble en detergente neutro. MM: Materia mineral. OM: Materia orgánica. CHO: Carbohidratos solubles. SEM: error estándar de la media.

The highest DM and CHO contents were observed in the haylage treated with 600 gDM/kg (581.6 ± 15.4 gDM/kg and 45.4 ± 1.24 gCHO /kg DM, respectively). The other chemical composition variables were not significantly different among the treatments, yielding mean values of 94.6 ± 3.58 gCP/kg DM, 679.5 ± 54.7 gNDF/kg DM, 59.3 ± 3.71 gMM/kg DM, and 920 ± 53.6 gOM/kg DM. The desired DM contents after the 400, 500, and 600 gDM/kg treatments were very similar between plants (381.2, 486.0, and 575.3 g/kg, respectively; table 1, page 41) and haylage (397.7, 480.3, and 581.6 g/kg; table 2).

The quantification of O_2 and CO_2 gases in the Tanzania grass haylage revealed a significant interaction effect ($p < 0.01$) between the plant DM and number of days during which the gas composition of the grass was evaluated during storage (table 3, page 44). Treatment with 400 g kg^{-1} DM resulted in the lowest value of O_2 on day 0, whereas the treatment *in natura* resulted in the highest amount of O_2 inside the bales when they were opened after 90 days of storage. There was a reduction in the amount of O_2 inside the Tanzania grass haylage bales after 7 days of storage after all the treatments, and, after 90 days of storage, the O_2 content was found to be less than 2.5% inside all the bales.

The lowest CO_2 values were observed on day 0. CO_2 increased between days 7th and 15th days of storage and after 90 days of storage. The treatments 400 and 500 g kg^{-1} DM resulted in the highest CO_2 concentrations, which were 16.7 ± 1.0% and 16.2 ± 1.0%, respectively. There was a significant effect ($p < 0.05$) of the storage period on the internal temperature of the haylage, which reduced to 7.3 ± 0.21°C after 45 days, and the highest temperatures were observed on days 0 and 15.

Volatile fatty acids and microorganisms

The highest acetic acid value of 36.4 ± 1.6 g kg^{-1} DM was obtained for the 600 g kg^{-1} DM treatment, followed by the values of 38.2 ± 1.6 and 48.9 ± 1.6 g kg^{-1} DM for the *in natura* and 500 g kg^{-1} DM treatments, respectively. As for butyric acid, the highest value (27.0 ± 0.5 g kg^{-1} DM) was observed for the *in natura* treatment (table 4, page 44).

Table 3. Gas and temperature quantification in Tanzania grass haylage based on the plant dry matter (DM) during the various storage times

Tabla 3. Cuantificación de los gases y la temperatura de henolaje de hierba de Tanzania según la materia seca de la planta a través de los tiempos de almacenamiento.

DM Content ($g\ kg^{-1}$)	Days						Mean
	0	7	15	30	45	90	
Oxygen (O_2)							
in natura	21.7aA	1.1cdB	1.1cdA	1.0dA	1.9bcA	2.4bA	4.9
400	20.8aB	1.4AbcB	0.8cA	1.0bcA	1.6bcA	1.9bAB	4.6
500	21.5aAB	2.2bA	0.7cA	0.8cA	1.8bA	1.4bcB	4.7
600	22.1aA	1.3bB	1.0bA	0.8bA	1.7bA	1.3bB	4.7
Mean	21.5	1.5	0.9	0.9	1.7	1.7	
CO_2							
in natura	1.8bA	18.7aC	20.7aB	16.7aA	13.5aA	12.5AaB	14.0
400	1.2cA	29.5aB	20.0bB	15.0bA	14.2bA	14.6bB	15.7
500	1.5dA	29.5aB	16.0bC	13.2bcA	12.7bcA	16.7cA	13.3
600	0.0cA	37.2aA	29.5aA	18.5bA	16.5bA	16.2bA	19.6
Mean	1.1	28.7	21.5	15.8	14.2	12.5	
Internal temperature							
in natura	34.4	29.0	33.5	26.8	26.3	26.5	29.4
400	32.1	28.6	33.6	27.0	26.3	27.2	29.1
500	34.2	29.0	33.8	27.2	26.5	27.6	29.7
600	34.1	29.4	33.9	27.4	26.6	27.9	29.9
Mean	33.7a	29.0b	33.7a	27.1c	26.4c	27.3c	
Analysis of variance	***-p-value***						
	DM Content		**Days**	**DM Content × Days**			**SEM**
Oxygen	0.08		< 0.01	< 0.01			0.10
CO_2	< 0.01		< 0.01	< 0.01			1.00
Temperature	0.07		< 0.01	0.70			0.21

Means followed by different letters in a column and row indicate statistical differences according to Tukey's test at $p < 0.05$. SEM: Mean Standard error.

Medias seguidas de letras diferentes en la columna y en la fila son estadísticamente diferentes según la prueba de Tukey con p <0,05. SEM: Error estándar de la media.

Table 4. Concentration of volatile fatty acids in Tanzania grass haylage based on the plant dry matter (DM) content, expressed as g/kg DM.

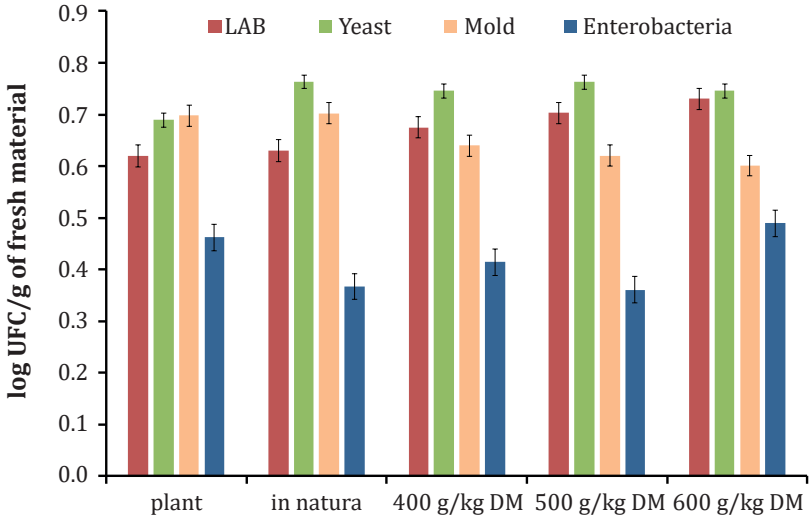
Tabla 4. Concentración de ácidos grasos volátiles en henolaje de pasto Tanzania según la materia seca de la planta, expresada como g/kg MS.

DM Content ($g\ kg^{-1}$)	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric
in natura	36.4^{b}	3.2	0.5	27.0^{a}	9.1	9.6
400	11.1^{c}	2.7	0.0	1.4^{b}	0.0	0.0
500	38.2^{b}	0.7	0.0	2.5^{b}	3.0	4.5
600	48.9^{a}	4.1	2.0	3.2^{b}	6.1	1.2
Mean	38.2	2.7	0.6	8.59	4.5	3.8
SEM	1.5	1.8	0.4	0.5	2.4	2.4
P - value	<0.01	0.65	0.12	<0.01	0.22	0.15

Means followed by different letters in a column indicate statistical differences according to Tukey's test at $p < 0.05$. SEM: Mean Standard error.

Medias seguidas de letras diferentes en la columna son estadísticamente diferentes según la prueba de Tukey con p <0,05. SEM: Error estándar de la media.

In the assessment of the microbial composition of Tanzania grass and its resultant haylage (figure 1), an increase in the concentration of LAB was shown to be associated with an increase in the DM content. LAB populations were found in the haylage at 6.9, 7.0 and 7.5 log CFU/g for the 400, 500 and 600 g kg^{-1} DM treatments, respectively.



LAB: lactic acid bacteria. FM: fresh material.

LAB: bacteria del ácido láctico. FM: material fresco.

Figure 1. Microbial composition of the plant and haylage of Tanzania grass stored with different levels of dry matter.

Figura 1. Población de microorganismos de la planta y henolaje de pasto Tanzania almacenado con diferentes niveles de materia seca.

No difference was found in the yeast population at the different plant DM contents used for haylage production. Tanzania haylage had the smallest yeast population of 6.9 log CFU/g. The 400, 500, and 600 g kg^{-1} DM treatments yielded yeast populations of 7.0, 7.6, and 7.2 log CFU/g in the haylage, respectively.

The lowest amounts of mold, at 6.2 and 6.0 log CFU/g, were observed in the haylage for the 500 and 600 g kg^{-1} DM treatments, respectively. While the smallest amounts of enterobacteria, at 4.5 and 3.5 log CFU/g, were found in haylages of the *in natura* and 500 g kg^{-1} DM treatments, respectively.

Aerobic stability, *pH*, and ammonia nitrogen

Aerobic stability was affected by the interaction between the different plant DM contents and hours of exposure of the Tanzania grass haylage to air after opening the bales; this process was largely driven by surface temperature, internal temperature, pH, and N-NH_3 (table 5, page 46).

Haylage surface temperature had a linear relationship ($p < 0.01$) with the length of time the materials were exposed to air. Specifically, between 0 and 120 hours of exposure to air, increases of 2.7 ± 0.06°C, 2.4 ± 0.06°C, 1.7 ± 0.06°C, and 1.6 ± 0.06°C were observed for the *in natura*, 400 g kg^{-1} DM, 500 g kg^{-1} DM, and 600 g kg^{-1} DM treatments, respectively. The 600 g kg^{-1} DM treatment yielded the highest surface temperatures of the haylage, which were 21.9, 23.4, 21.5, 22.4, and 23.8 ± 0.06°C for the exposure to air times of 0, 24, 48, 72, 96, and 120 hours, respectively. The in natura, 400 g kg^{-1} DM, and 600 treatments had an increasing linear effect ($p < 0.01$) on internal temperature over the hours of haylage exposure to air.

The in natura treatment yielded the highest temperature of 25 ± 0.82°C in the haylage after 48 hours of exposure to air, and the highest room temperature recorded was 24.6°C. During exposure to air, there was no increase of 2% in the surface and internal temperatures of the haylage compared to the room temperature (table 5, page 46).

Table 5. Aerobic stability of Tanzania grass haylage based on the plant dry matter (DM).
Tabla 5. Estabilidad aeróbica de henolaje de pasto Tanzania según la materia seca de la planta.

DM Content (g kg^{-1})	Hours						Mean	*-p-value*	R^2
	0	24	48	72	96	120		x	
Room temperature (°C)									
	24.0	24.2	24.1	24.2	24.6	24.5			
Surface temperature (°C)									
In natura	21.0B	22.7B	21.1A	21.9AB	23.1B	23.7A	22.3	< 0.01*	56.2
400	21.4AB	23.0AB	21.0A	21.4B	23.2AB	23.8A	22.3	< 0.01*	35.0
500	21.7A	23.2AB	21.2A	21.7B	23.2AB	23.4A	22.4	< 0.01*	21.2
600	21.9A	23.4A	21.5A	22.4A	23.8A	23.5A	22.7	< 0.01*	37.4
Mean	21.6	21.9	22.3	22.6	22.9	23.3			
Internal temperature (°C)									
In natura	22.2A	23.5A	25.0A	22.1A	23.2A	24.7A	23.5	0.03*	19.6
400	22.5A	23.0A	21.3B	22.0A	23.6A	24.2A	22.7	0.01*	31.1
500	23.0A	23.2A	21.5B	21.7A	23.5A	24.1A	22.8	0.11^{ns}	-
600	23.2A	23.1A	22.0B	22.2A	24.5A	24.5A	23.2	0.01*	30.0
Mean	22.4	22.6	22.9	23.2	23.5	23.8			
pH									
In natura	5.97B	6.16A	6.19A	6.17A	6.96A	6.95A	6.40	< 0.01*	79.3
400	5.91B	5.98A	6.24A	6.06A	6.20A	6.30B	6.11	< 0.01*	70.2
500	6.00B	6.28A	6.23A	6.15A	6.42A	6.51B	6.26	< 0.01*	70.4
600	6.36A	6.23A	6.42A	6.23A	6.52A	6.48B	6.37	0.12^{ns}	-
Mean	6.04	6.1	4	6.34	6.44	6.54			
N-NH_3 (%)									
In natura	4.65A	1.60A	2.25A	1.60A	1.10A	0.20A	1.90	< 0.01*	74.6
400	2.40AB	2.25A	1.60A	1.50A	0.90A	0.70A	1.55	< 0.01*	96.7
500	3.35B	1.40A	1.90A	2.25A	1.00A	0.65A	1.75	< 0.01*	62.2
600	1.85C	1.15A	1.50A	1.55A	0.90A	0.35A	1.21	< 0.01*	66.3
Mean	2.67	2.24	1.82	1.39	0.96	0.54			

Analysis of variance	*-p-value*			
	DM Content	Hours	DM Content × Hours	SEM
Surface temperature of the haylage	< 0.01	< 0.01	< 0.01	0.06
Internal temperature of the haylage	0.06	< 0.01	0.03	0.82
pH	< 0.01	< 0.01	< 0.01	0.03
NH_3	< 0.01	< 0.01	< 0.01	0.12

Means followed by different letters in a column indicate statistical differences according to Tukey's test at p < 0.05. * Significant at *P* < 0.05. ^{ns} not significant at *p* > 0.05. x: linear effect; SEM: Mean Standard error.

Medias seguidas de letras diferentes en la columna son estadísticamente diferentes según la prueba de Tukey con p < 0,05. * significativo a p < 0,05. ^{ns}no significativo a pb> 0,05. x: efecto lineal; SEM: Error estándar de la media.

The *in natura*, 400 g kg^{-1} DM, and 500 g kg^{-1} DM treatments had an increasing linear effect (p < 0.01) on the pH of haylage during air exposure. In all treatments, the highest pH values were recorded after 120 hours of exposure of the haylage to air, and these values were 6.95 ± 0.03, 6.30 ± 0.03, 6.51 ± 0.03, and 6.48 ± 0.03 for the *in natura*, 400 g kg^{-1} DM, 500 g kg^{-1} DM, and 600 g kg^{-1} DM treatments, respectively. The 600 g kg^{-1} DM treatment

showed the highest pH value of 6.36 ± 0.03 at hour 0, while the *in natura* treatment showed the highest pH value of 6.95 ± 0.03 after 120 hours of exposure to air.

There was a decreasing linear effect ($p < 0.01$) of the hours of exposure to air on the $N\text{-}NH_3$ of Tanzania grass haylage in all treatments at the time of baling. The *in natura* treatment showed the highest $N\text{-}NH_3$ value at hour 0 of exposure to air (4.65 ± 0.12%).

Discussion

Chemical composition and gases

DM increased as the dehydration of the Tanzania grass continued in the field; thus, the higher DM content of the haylage obtained in the 600 g kg^{-1} DM treatment, as compared to that obtained after the other treatments, was due to the grass being dehydrated to a greater extent during this treatment before it was baled (table 2, page 43). The higher DM content in the stored material optimizes the fermentation of the forage, shaping its preservation as haylage. Nath *et al.* (2018) obtained DM values for Tifton 85 grass haylage with different additives and storage times, with an average of 531.10 g/kg. Haylage is a technique that can be used for the storage of grasses because dehydration reduces the probability of secondary fermentation, which causes DM loss (31, 44, 58).

The DM values obtained for each treatment through dehydration, both before and after the production of haylage, were nearly adequate according to each treatment (400, 500, and 600 g kg^{-1} DM), demonstrating that the method used to determine the DM content through a microwave is a viable alternative to quickly obtain the DM value of forage plants on the farm (55) and can be used to determine the plant DM for haylage production. The plant DM at harvest directly influences haylage fermentation (26, 40).

The haylage in the 600 g kg^{-1} DM treatment group had the highest crude protein (CP) content; however, all haylage groups had a CP content greater than 70 g kg^{-1} DM, which is suggested by Van Soest (1994) as the ideal amount for the growth of rumen microorganisms. The high CP content in the haylage was due to the high CP content of the Tanzania grass before storage (table 1, page 41). Castro *et al.* (2010) evaluated the chemical composition of Tanzania grass at day 42 of storage and obtained a CP content of 97.7 g/kg DM.

The results also indicated that the storage of haylage preserved the CP content at levels suitable for animal feeding. The high CP content indicates that when haylage is stored with adequate amounts of DM, it produces conditions suitable for the growth of LAB (53) and inhibits the growth of undesirable microorganisms (44) that deteriorate CP.

The content of neutral detergent fiber (NDF) was higher than the maximum limit of 550 g/kg DM recommended for good digestibility of the mass, which occurs in silages with NDF levels as described previously (58). High NDF content may be related to the loss of cellular content during the fermentation period (31), which negatively influences feed intake due to rumen filling (8). The NDF content obtained in the haylage of the 500 g kg^{-1} DM treatment group was lower than that found by (6) in haylage of Tifton 85 grass, which was 723.6 g/kg DM, indicating the high quality of Tanzania grass when harvested before flowering. Since NDF constitutes the cell wall of plants (58), having haylages with NDF content similar to that of the original plant suggests the adequate preservation of nutrients (table 2, page 43).

Lower contents of mineral matter (MM) were observed in the Tanzania grass haylages in all treatments (table 1, page 41) than in the material before storage (table 2, page 43). Low MM content is an indicator of better forage conservation because when inadequate fermentation occurs, the loss of organic material increases the amount of MM in the DM. The values obtained for MM in this study were lower than those found by AOAC (1990) in the haylage of Tifton 85 grass containing a bacterial inoculant.

The higher DM content treatments (500 and 600 g kg^{-1} DM) also yielded higher CHO content, which is an important substrate for the fermentation and conservation of forage in the form of haylage. The increase in dehydration of Tanzania grass increased its CHO content, indicating that in treatments with higher moisture, there was a greater use of CHO by microorganisms responsible for driving fermentation (50). In a previous study (14), it was observed that the haylages of two cultivars of perennial ryegrass (*i.e.*, AberDart and Fennema) fermented better when the DM had a higher concentration of CHO.

Most tropical forage grasses do not have adequate levels of DM, CHO, or buffering capacity to allow for fermentation to occur efficiently, resulting in losses due to secondary fermentation, effluent production, and aerobic deterioration, which are obstacles in the conservation of tropical grasses (10). Thus, the high levels of CHO in the haylage after 90 days of storage, as obtained in the 500 and 600 g kg^{-1} DM treatments, showed that fermentation was well controlled, resulting in good-quality forage for animal feeding.

A high amount of oxygen was observed at the time of storage (0 days), which was subsequently reduced after the 7th day of storage. The presence of oxygen during storage favors the growth of microorganisms that release energy in the form of heat, and fermentation by this microbial mass results in the degradation of the roughage. Therefore, oxygen must be eliminated before fermentation; in its absence, there is a decrease in fungal and yeast growth, as anaerobic conditions are not optimal for the growth of these organisms (20).

The increase in CO_2 after the first days of storage was due to it being released by aerobic microorganisms inside the bales during fermentation. According to Paula *et al.* (2016), the respiration of aerobic microorganisms occurs in the aerobic phase. These microorganisms use some of the desirable substrates for energy production, causing DM consumption and CO_2 production, which can be considered as one of the main factors that influence the quality of haylage.

The low levels of oxygen observed in the 500 and 600 g kg^{-1} DM treatments with 90 d of storage were indicative of higher levels of anaerobic fermentation inside the haylage bales, especially if it was associated with high amounts of CO_2, as was observed in the haylage with 600 g kg^{-1} DM. Low amounts of oxygen and high amounts of CO_2 are desirable parameters that guarantee adequate anaerobic fermentation and yield products of good nutritional quality. The activity of certain microorganisms can be controlled using a controlled atmosphere or packaging in a modified atmosphere (52). According to Müller (2005), the greater the number of wrapping layers in the haylage bales, the greater the CO_2 concentration. According to Mantilla *et al.* (2010), the increase in food conservation time was due to the inhibitory effect of carbon dioxide (CO_2) on different microbial types and the reduction or removal of oxygen (O_2) from inside the bale.

It was observed that during storage, the low CO_2 concentration increased from the 7th day, and this occurred because the aerobic microorganisms and optional aerobes began to consume the available CHO, increasing the production of gases through respiration and fermentation (carbon dioxide and ethanol). After the 30th day, it was observed that the microbial activity stabilized, decreasing respiration, and consequently, the production of gas, as previously noted (60).

The internal temperature of the haylages increased during the first few days of storage (0, 7, and 15 days), whereas the amount of O_2 decreased and that of CO_2 increased in this time period. According to Mcdonald *et al.* (1991), in the first few days of storage until the end of the aerobic phase, it is common to observe heating of the material, which can last from 48 to 144 h.

Volatile fatty acids and microorganisms

A greater amount of acetic acid was observed in the 600 g kg^{-1} DM treatment as compared to the other treatments, indicating that the lower moisture content caused an increase in the activity of acetic acid-producing microorganisms in the haylage of Tanzania grass. The presence of acetic acid is indicative of the action of heterofermentative LAB and enterobacteria. High levels of this acid promote greater aerobic stability of haylage after prolonged storage because it can inhibit yeast growth (1). Low concentrations of strong acids in haylage do not imply poor fermentation (38), which may be due to the high DM content of the material.

The higher concentration of butyric acid in the *in natura* treatment as compared to the other treatments is due to the higher moisture content of the plant, which favors the growth of bacteria of the genus *Clostridium* (49). No difference was observed for the other acids, and this indicates that the DM content greatly influences the production of haylage within the context of Tanzania, and its presence is shaped by the action of heterofermentative LAB, enterobacteria, and clostridia.

LAB concentrations increased according to increasing DM content of the plants used for haylage production (figure 1, page 45). The larger population of homofermentative LAB tends to reduce pH more quickly, reducing the action of undesirable microorganisms and preserving a greater amount of carbohydrates, with the increase in the DM content (36); this phenomenon was not observed in this experiment that obtained high pH values. The haylages produced had LAB populations greater than the minimum limit of 5 log CFU g^{-1} recommended by Pahlow (1986) and Muck *et al.* (1991) and t required for a good fermentation process.

The increased presence of yeast populations is concerning because of their potential to rapidly multiply, but no difference was observed in the yeast population with respect to the different DM contents of the plant used for haylage-making. Notably, several types of yeasts may predominate during the haylage-making process, and the yeast species present are not necessarily aerobic. This may explain the absence of a difference in the counts of these microorganisms between treatments. Low yeast populations are desirable for preserving the material during fermentation and after bales opening (45). The yeast count of the Tanzania grass haylages was higher than that found by Müller and Johansen (2020) in reallocated haylage (5.31 log CFU g^{-1}) and that observed by Müller *et al.* (2011) in the haylages of horse farms (4.57 log CFU g^{-1}).

A lower count of molds was observed in the haylage of the 500 and 600 g kg^{-1} DM treatments, which is related to the plant DM during storage. Generally, the population of microorganisms is strongly affected by the moisture content and temperature recorded during storage (37). The presence of fungi causes a reduction in nutritional value and palatability due to the associated protein degradation (16). Some mycotoxin-producing molds were observed in the haylages, but they only occurred at low concentrations; this was in line with the results of a study by Müller *et al.* (2011). The presence of such molds can be reduced through the use of additives (38).

The 600 g kg^{-1} DM treatment yielded the highest amount of enterobacteria in the haylage of Tanzania grass. The large number and prevalence of these microorganisms are undesirable, as they cause protein degradation by performing secondary fermentation and producing compounds such as acetic and butyric acids, impairing conservation (51). However, these bacteria produce acetic acid, which, in the absence of lactic acid, can help conserve the material and increase its aerobic stability. Enterobacteria compete for water-soluble carbohydrates with LAB, and the component with the highest concentration at the end of this process is acetic acid, which has a positive effect on aerobic stability. Haylage with poor aerobic stability has high levels of residual sugar and lactic acid (14).

Aerobic stability, *pH,* and ammonia nitrogen

The temperature increase observed in Tanzania grass haylages with different DM levels throughout exposure to air was not sufficient to negatively impact aerobic stability across the evaluation period of 120 h. The aerobic stability of the haylage, regardless of treatment, was likely maintained because of the high acetic acid concentration in the 600 g kg^{-1} DM treatment and the low amount of CHO in the other treatments. These characteristics inhibit the growth of deteriorating microorganisms (16). Müller (2009) did not observe a change in the aerobic stability of the haylage of plants harvested at different times.

Haylage treated with 600 g/kg DM had the highest surface temperature, probably because of the high amounts of CHO. Better haylage fermentation patterns with higher DM content provide a greater number of available substrates for the consumption of microorganisms in the aerobic phase (61).

The variation in the internal temperature of the Tanzania grass haylage was not enough to overcome the room temperature at 2°C in all treatments and times. Neres *et al.* (2013) assessed the aerobic stability of Tifton 85 grass silage and observed that the room temperature was lower than the ensiled mass temperature during the seven days of aeration, which contributed to good preservation of the roughage and inhibition of the growth of undesirable microorganisms. The aerobic stability of haylage can also be influenced by the production of acetic acid, which varies according to pH and temperature increases in the respective pre-dried forage masses (56).

Haylage stored with 600 g kg^{-1} DM showed no difference in pH during exposure to air. This occurred because of the lower moisture content, which provided greater resistance to the pH drop because of the lower activity of microorganisms. Belém *et al.* (2016) reported that the limited activity of bacteria owing to moisture has a direct effect on aerobic fermentation.

The pH values observed in the Tanzania grass haylages were higher than those observed by Coblentz *et al.* (2016) in the alfalfa haylage (5.1), which was almost similar to those observed by Nath *et al.* (2018) in the haylage of Tifton 85 grass (5.72) and lower than those obtained by Weirich *et al.* (2018) in the Tifton 85 haylage (7.38). The high pH values observed in the haylage of tropical grasses may be due to the low concentration of organic acids in the masses of these species (3). Müller *et al.* (2007) compared silage to haylage and observed that haylage had a higher pH owing to lower concentrations of fermentative products.

The N-NH_3 content of Tanzania grass haylage decreased as its exposure to air progressed. As the times of aerobic exposure advanced, the Tanzania grass haylages showed a reduction in the average levels of ammoniacal nitrogen, probably due to evaporative processes and a decrease in the enterobacteria population (48).

The *in natura* treatment produced the highest amount of N-NH_3, indicating the high intensity of proteolysis during the fermentation process. However, it is important to note that all haylages were classified as those of good quality. Monteiro *et al.* (2011) classified haylages as good quality haylages when the fermented materials had levels of N-NH_3 below 12%. This was also indicative of low proteolysis intensity during fermentation (57).

Conclusions

Higher plant DM yields Tanzania grass haylage of high quality. Tanzania grass with 500 and 600 g kg^{-1} DM for haylage production had a high content of CHO, a better concentration of gases, and a greater amount of volatile fatty acids and beneficial microorganisms that facilitate preservation. Additionally, these haylages showed sustained aerobic stabilities.

It is necessary to conduct further studies on plant DM using other tropical grasses to produce high-quality haylage.

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Acknowledgments

Tanks CNPq (Grant No.424941/2016-3) for financial support.

Disclosure statement

The authors declare no conflicts of interest associated with this paper. The authors alone are responsible for the content and writing of this manuscript.