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RESEARCH ARTICLE

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Qualitative evaluation of total mixed ration silage containing forage cactus and guinea grass as a nutritional alternative for feedlot-finished sheep

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ABSTRACT

This study aimed to evaluate the fermentation characteristics, microbial populations, losses, aerobic stability and chemical composition of total mixed ration based on forage cactus and different ratios of Guinea grass at four storage times (7, 15, 60 and 100 days). Five diets were formulated with forage cactus as the main ingredient, with varying Guinea grass inclusion ratios: 0, 10, 20, 25 and 30% on a dry matter basis. The experimental design used was a completely randomised design with a factorial arrangement and three replicates. The silages had pH values of approximately 4.0. Lactic acid bacteria were predominant in all silages, and this predominance increased after 7 days of ensiling in all diets, ranging from 8.0–10.1 log10 cfu g^{-1} of silage. Silages with 10% and 20% guinea grass showed aerobic deterioration, while the others remained stable throughout the aerobic stability test. Guinea grass in combination with forage cactus can be added in total mixed ration silages up to a level of 30%, without compromising the chemical composition, silage losses or fermentative profile. However, considering aerobic stability, the addition of 10% and 20% guinea grass resulted in greater deterioration after 48 h of exposure to air.

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Ensiling; *Megathyrsus maximus*; *Nopalea cochenillifera*; fermentation profile; organic acids

Introduction

Guinea grass (*Megathyrsus maximus*) is a grass suitable for pasture, cut-and-carry, silage and hay, with a high nutritional value, including a crude protein (CP) content usually between 55.0 and 181.0 g kg^{-1} of dry matter (DM) and an in vitro DM digestibility of approximately 660.0 g kg (Santos et al. 2014; Tomaz et al. 2018). However, Guinea grass (GG), like other tropical forage grasses, does not have adequate DM, water-

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soluble carbohydrates (WSC) and buffering capacity (BC) to ensure an efficient fermentation process to produce high-quality silage (Reis et al. 2013).

On the other hand, the potential of forage cactus (*Nopalea cochenillifera*) was recently evaluated as a source of substrate when ensiling plants and total mixed-ration silages (TMRS) in order to overcome fermentation problems due to lack of WSC (Macêdo et al. 2018; da Silva Brito et al. 2020; Santos et al. 2020; Pereira et al. 2021) and to improve the fermentative profile of silages (Nogueira et al. 2019; Sá et al. 2020). This has been possible due to the high concentration of WSC in forage cactus cladodes, which ensures good heterolactic fermentation, increasing the stability of these silages during air exposure (Pereira et al. 2019). Forage cactus, however, also has a high BC and an especially high moisture content, which could make the mixed ensilage of cactus and GG undesirable, with high effluent losses and undesirable fermentations (Sá et al. 2020).

Considering the worldwide demand for novel technologies to improve the use of feed resources and intensify the production of ruminants, the use of mixed total feed silages becomes a viable alternative for formulating diets based on cactus pear and GG. Commonly known as TMRS, the process consists of a homogeneous mixture through the combination of ingredients in the silo, which minimises the occurrence of the selection of ingredients by animals, optimising the ensilage of high-moisture and/or low-WSC ingredients, improving the performance of herds and reducing costs in relation to conventional rations (Santos et al. 2020).

In the case of TMRS based on Cactus and Guinea grass, this technique takes advantage of co-ensiling both cactus and grass, which balances the fibre, energy and protein requirements of the ruminant, thus minimising the occurrence of nutritional disorders and optimising the production system from a nutritional perspective.

Recently, Macêdo et al. (2018), evaluated the TMRS based on forage cactus and different buffelgrass (*Cenchrus ciliaris*) inclusion ratios and obtained satisfactory results, with dry matter recovery (DMR) values ranging from 960 g kg^{-1}–990 g kg^{-1}. This result was associated with the WSC present in cactus pear that served as substrates for lactic acid bacteria (LAB), which converted them into organic acids, providing a reduction in the pH of the ensiled mass, which ensured good preservation and high DMR. Thus, the authors concluded that TMRS based on cactus pear can be recommended for production.

Nonetheless, due to a scarcity of information about TMRS based on forage cactus and guinea grass, studies are needed to assess nutritional and fermentative quality at different storage times. In this way, research addressing this theme becomes indispensable for the development of tried-and-true nutritional strategies aimed at increasing the productivity of animals on drylands in a sustainable way. Our hypothesis is that the inclusion of Guinea grass in forage-cactus-based TMRS optimises the heterolactic fermentation profile, extending the aerobic stability time without compromising the nutritional quality of the ensiled mass while also increasing the levels of neutral detergent fibre (NDF).

The objective of this study was to evaluate the fermentation characteristics, losses, microbial populations and chemical composition of total mixed ration silages based on forage cactus and different ratios of GG for four storage times and during aerobic stability.

Materials and methods

Localisation, silage material and treatments

The research was conducted in the Forage Production Sector, belonging to the Department of Animal Science of the Federal University of Paraíba (UFPB), located in Areia - Paraíba, Brazil.

The forage cactus *Nopalea cochenillifera* Salm Dyck, was used, with a regrowth age of two years from an already established crop. Based on the Köppen classification, the climatic type of the region is BSh, which is hot and semiarid, with rainfall from January to April, an average annual temperature of 24°C, relative humidity of approximately 68%, an average annual rainfall of approximately 400 mm and drought conditions during almost the entire year.

Guinea grass (*Megathyrsus maximus*) was obtained from an already established area, where it was harvested using a manual brush cutter at a height of 20 cm from the ground. The material was harvested 40 days after a uniformization cut of the area.

Five TMRS were formulated with forage cactus as the main ingredient, where the ratios of the dietary ingredients varied according to the Guinea grass inclusion ratios, which were approximately 30%, 25%, 20%, 10% and 0%, on a DM basis (Tables 1 and 2). The diets were formulated to meet the 200 g requirement of daily weight gain in sheep with 30 kg of live weight and of undefined breed (NRC 2007).

The Guinea grass and the forage cactus were harvested manually. Subsequently, the plants were processed in a stationary forage machine, and particles with an average size of approximately 2 cm were obtained. Then, all ingredients were mixed with forage cactus according to treatments corresponding to the different ratios of Guinea grass.

The silages were produced in 60 experimental PVC silos (polyvinyl polychloride; 30 cm height and 10 cm diameter) for evaluation of microbial populations, the fermentation profile, fermentation losses and chemical composition of silages at different storage times i.e. 7, 15, 60 and 100 days after ensiling.

The silages were produced in 15 experimental PVC silos (30 cm height and 15 cm diameter), which were fitted with a *Bunsen* valve for release of the gases resulting from fermentation. To capture the effluents, 1 kg of dry sand was added to the bottom of each experimental silo and covered with a TNT screen (nonwoven fabric). At the end of this process, the silos were closed, and they were opened at 100 days after ensiling.

An experimental period of up to 96 h was considered for the aerobic stability test, and the microbial populations, fermentation profile and DMR of the silages were evaluated at 0, 24, 48 and 96 h of air exposure.

Table 1. Chemical composition of feed ingredients in g/kg of dry matter.

Item	Forage cactus	Guinea grass	Soy bran	Corn bran	Wheat meal	Urea
Dry matter (g $kg^{-1)a}$	112.67	222.53	918.11	915.88	896.34	99.00
Organic matter	87.55	88.64	853.73	863.41	943.66	-
Crude protein	43.11	108.29	461.85	89.37	167.83	281.00
Ether estract	17.99	22.31	25.22	46.92	35.71	-
Neutral detergent fibre	30.32	732.11	144.94	134.09	422.11	-

[a]On a fresh matter basis

Table 2. Ingredients used and chemical composition of the experimental diets.

	Inclusion of guinea grass in TMR				
Item	0%	10%	20%	25%	30%
Ingredients used (g kg^{-1} DM)‡					
Forage cactus	191.5	192.5	189.6	188.6	187.5
Guinea grass	0.00	98.5	191.6	238.0	283.9
Soybean meal	132.8	135.6	146.3	146.1	144.6
Corn meal	361.3	351.6	348.9	345.6	343.6
Wheat meal	295.0	198.8	98.3	48.9	0.00
Urea	2.7	6.2	8.1	10.1	12.1
Mineral mix	6.8	6.9	7.4	13.0	18.5
Ammonium chloride	9.9	9.9	9.8	9.7	9.8
Chemical composition (g kg^{-1} DM)					
Dry matter (g kg^{-1} FM)‡	279.73	272.60	240.87	269.84	252.23
Crude protein	191.66	195.33	194.91	193.73	196.17
Water-soluble carbohydrates	65.85	59.90	55.19	58.34	107.55
Neutral detergent fibre	268.0	366.1	380.8	411.9	447.3

‡DM, Dry matter; FM, Fresh matter.

At each evaluation time, in both experiments, the pH values were determined by Bolsen et al. (1992), and silage samples of approximately 400 g were collected for future evaluations.

Microbial populations and fermentation profile

The microbial populations were quantified in the diets, prior to ensiling and in the silages using elective culture media for each microbial group: MRS agar (Man, Rogosa and Sharpe) containing 1.5 ml/L of acetic acid for lactic acid bacteria (LAB) and potato dextrose agar containing 1 of 10% tartaric acid for moulds and yeasts (MY).

The microbial groups were quantified using 10 g of a sample composed of the replicates of each diet, to which 90 mL of sterile distilled water was added and the samples homogenised for 1 min to obtain a 10^{-1} dilution. Then, serial dilutions were prepared to obtain dilutions ranging from 10^{-1}–10^{-9}, and culture was performed in sterile disposable Petri dishes. The petri dishes were incubated using incubation temperatures specific for each microbial group. Petri dishes with values between 30 and 300 colony forming units (CFU) were considered appropriate for counting (González and Rodríguez 2003).

The organic acids (lactic, acetic, propionic and butyric acids) and ethanol were determined using the methodology described by Kung and Ranjit (2001). The ammoniacal nitrogen (NH_3-N) and the BC of the silages were evaluated using the methods described by Bolsen et al. (1992) and Playne and McDonald (1966), respectively.

Part of the silage samples collected, approximately 300 g, was dried in a forced-air oven (55°C) until the sample weight stabilised, processed in a Wiley mill with a 1-mm sieve and analysed according to the AOAC (2016) for determination of the DM (method 930.15) and crude protein (CP) (method 954.01) contents. The WSC content was determined using the concentrated sulfuric acid method as described by DuBois et al. (1956) and modified by Corsato et al. (2008). The methodologies described by Van Soest et al. (1991) were used to determine the NDF levels.

The dry matter losses in the silages in the form of gas and effluent were quantified by weight difference using the equations described by Zanine et al. (2010):

where G = the gas losses (% of dry matter), WFc = the weight of the filled silo at closing (kg), WFo = the weight of the filled silo at opening (kg), FMc = the forage/feed mass at silo closing (kg) and DMc = the feed dry matter concentration at silo closing (%).

$$E = \frac{[(WEf - Tb) - (WEi - Tb))}{FMi} \ x\ 100$$

where E = the effluent losses (kg ton^{-1} of fresh matter), WEi = the weight of the empty silo + sand at closing (kg), WEf = the weight of the empty silo + sand at opening (kg), Tb = the weight of the empty silo (kg) and FMi = the feed mass at silo closing (kg).

The DMR was estimated by the difference in feed weight and DM concentration before and after ensiling using the equation described by Zanine et al. (2010):

$$DMR = \frac{(FMo\ x\ DMo)}{(FMc\ x\ DMc)} \ x\ 100$$

where DMR = the dry matter recovery rate (%), FMo = the feed mass at silo opening (kg), DMo = the feed dry matter concentration at silo opening (%), FMc = the feed mass at silo closing (kg) and DMc = the feed dry matter concentration at silo closing (%).

At 100 days of ensiling, the aerobic stability of the silages (expressed in hours) was evaluated by monitoring the surface and internal temperatures of the silages exposed to air. The silage samples were placed without compaction in unlidded experimental PVC silos and kept in a temperature-controlled closed environment (25°C). Temperatures were recorded at each hour using thermometers (digital laser and digital immersion), according to the methodology described by Ranjit and Kung (2000).

Statistical analysis

For the experiment, a completely randomised experimental design was adopted in a 5 × 4 factorial scheme (five diets and four opening times) with four replications for the variables chemical composition, microbiology and the fermentative profile. RMS, GL, EL and aerobic stability were evaluated 100 days after ensiling. Data were analysed by analysis of variance (ANOVA), and when significant, the means were compared by Tukey's test at a significance level of 5% using SISVAR software (Ferreira 2008). The following statistical model was applied:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk}$$

Where μ is the population mean, α_i is the total mixed ration effect, β_j is the time effect, γ_{ij} is the interaction diet × time and ε_{ijk} is the residual error.

The aerobic stability test and the quantification of the microbial groups were analysed descriptively for both experiments.

Results

We observed an interaction effect of GG levels and opening times on DM ($P = 0.0250$), CP ($P = 0.0094$), WSC ($P = 0.0498$) and NDF ($P = 0.0001$) (Table 3).

A reduction in MS content was observed as the GG content increased in the TMRS, with the highest averages being found at 0 and 10% and the lowest at 20, 25 and 30%

Table 3. Means resulting from the effect of the interaction treatment x storage time for dry matter, crude protein, water-soluble carbohydrates and neutral detergent fibre of the total mixed ration silages based on forage cactus and different ratios of Guinea grass.

	Inclusion of guinea grass in TMR						
Item	0%	10%	20%	25%	30%	SEM‡	*P* value
Dry matter (g kg^{-1})							
7 days	343.8^{ABa}	324.4^{Aab}	315.8^{Abc}	290.6^{Ac}	288.9^{Ac}	0.37	0.0250
15 days	338.0^{Aba}	297.4^{Bb}	291.9^{BCb}	293.8^{Ab}	293.1^{Ab}		
60 days	346.4^{Aa}	317.4^{ABb}	309.6^{ABb}	272.1^{Ac}	277.2^{ABc}		
100 days	322.0^{Ba}	295.8^{Bb}	275.0^{Cbc}	273.3^{Ac}	260.5^{Bc}		
Crude protein (g kg^{-1} DM)							
7 days	178.2^{ABab}	187.9^{Aa}	195.3^{Aa}	184.3^{Aa}	161.9^{Bb}	0.12	0.0094
15 days	189.8^{Aa}	181.2^{Aa}	187.2^{Aa}	183.3^{Aa}	187.7^{Aa}		
60 days	183.4^{Aba}	181.9^{Aa}	179.4^{Aa}	182.3^{Aa}	180.1^{Aa}		
100 days	171.9^{Ba}	172.5^{Aa}	185.9^{Aa}	183.7^{Aa}	181.2^{Aa}		
Water-soluble carbohydrates (g kg^{-1} DM)							
7 days	ND	ND	ND	ND	ND §	0.49	0.0498
15 days	80.8^{Bab}	139.2^{Aa}	72.3^{Bb}	88.1^{Aab}	90.5^{Aab}		
60 days	91.6^{Bb}	149.9^{Aa}	136.5^{Aab}	127.7^{Aab}	115.6^{Aab}		
100 days	143.9^{Aa}	136.5^{Aa}	144.6^{Aa}	130.9^{Aa}	97.2^{Aa}		
Neutral detergent fibre (g kg^{-1} DM)							
7 days	177.2^{Ac}	230.7^{Ac}	293.2^{Ab}	350.0^{Aa}	323.2^{Aab}	0.93	0.0001
15 days	194.0^{Ab}	109.7^{Bc}	273.1^{ABa}	319.4^{Aba}	301.7^{Aa}		
60 days	172.4^{Ac}	204.8^{Abc}	232.1^{Bb}	282.1^{BCa}	280.3^{Aa}		
100 days	188.3^{Ad}	203.6^{Ac}	251.2^{ABb}	248.1^{Cb}	304.3^{Aa}		

†Means followed by the same letters, uppercase in the columns and lowercase letters in rows, do not differ by Tukey's test at the 5% probability level. DM, Dry matter. ‡Standard error of the mean.
§Not determined.

GG at 7 days after ensiling. At 15, 60 and 100 days after ensiling, the highest average was observed at 0% content and the lowest at 10, 20, 25 and 30% GG (Table 3).

Regarding the effect of days in relation to GG content on DM content, the highest averages were observed for 0% inclusion of GG in the TMRS on days 7, 15 and 60, which did not differ from each other, and the lowest at 100 days after ensiling. At 10% GG content, the highest average was observed at 7 days and the lowest at 15, 60 and 100 days after ensiling, which did not differ from each other. At 20% GG content, the highest average was found at 7 days and lowest at 100 days after ensiling. However, there was no effect of days on DM content at 25% GG content. At 30% GG content, the highest averages were observed at 7 and 15 days and the lowest at 60 and 100 days after ensiling, which did not differ from each other (Table 3).

There was an effect of GG content on CP of TMRS as the days after ensiling increased, with the highest measurements being observed at levels of 10, 20 and 25% GG, which did not differ from each other, and the lowest at 0 and 30% GG, which did not differ from each other, 7 days after ensiling. However, there was no effect on GG levels at 15 and 100 days after ensiling, with averages of 185.84 and 179.04 g kg^{-1} of CP, respectively. At 60 days after ensiling, the highest averages were observed at levels of 10, 20, 25 and 30%, which did not differ from each other, and the lowest at 0% GG content (Table 3).

Regarding the effect of days in relation to GG content on CP content, the highest averages were observed for 0% inclusion of GG in the TMRS on days 7, 15 and 60, which did not differ from each other, and the lowest at 100 days after ensiling. At 10% GG content, the highest averages were observed at 15 and 60 days, which did not differ from each other, and the lowest at 7 and 100 days after ensiling, which did not differ from each other. However, there was no effect of days on CP levels at 20 and 25% GG inclusion in the TMRS. At 30% GG content, the highest averages were observed at 15, 60 and 100 days, which did not differ from each other, and lowest at 7 days after ensiling (Table 3).

There was an effect of GG content on WSC of TMRS as the days after ensiling increased, with the highest average being observed at the level of 10% GG and the lowest at 0, 20, 25 and 30% GG, which did not differ from each other, at 15 and 60 days after ensiling. There was no effect on GG levels 100 days after ensiling, with an average of 130.6 g kg^{-1} DM. However, ND WSC values were affected at 7 days after ensiling (Table 3).

Regarding the effect of days in relation to GG content on WSC content, the highest average at 100 days and the lowest at 15 and 60 days after ensiling were observed for 0 and 20% GG inclusion in the TMRS, which differed from each other. However, there was no effect of days on WSC levels at 10, 25 and 30% GG inclusion in the TMRS, showing averages of 141.86, 115.56 and 101.10 g kg^{-1} DM, respectively (Table 3).

There was an effect of GG content on NDF of TMRS as the days after ensiling increased, with the highest average being observed at the level of 25% GG and the lowest at 0 and 10% GG, which did not differ from each other, at 7 days after ensiling. At 15 days after ensiling, the highest averages were observed at levels of 20, 25 and 30%, which did not differ from each other, and the lowest at the level of 10% of GG in TMRS. At 60 days after ensiling, the highest averages were observed at levels of 25 and 30%, which did not differ from each other, and the lowest at the level of 0% GG. At 100 days after ensiling, the highest average was observed at 30% and the lowest at the 10% GG level in the TMRS (Table 3).

Regarding the effect of days in relation to GG content on NDF content, there was no difference between days after ensiling for levels of 0 and 30% GG inclusion in the TMRS, presenting averages of 182.97 and 302.37 g kg^{-1} DM, respectively. At 10% GG content, the highest averages were observed at 7, 60 and 100 days, which did not differ from each other, and the lowest at 15 days after ensiling. At 20% GG content, the highest average was found at 7 days and the lowest at 60 days after ensiling. At the 25% GG level, the highest averages were observed at 7 and 15 days, which did not differ from each other, and the lowest at 100 days after ensiling (Table 3).

An increase in the growth of LAB was observed starting at 7 days of ensiling in all diets, ranging from 8.0–10.1 $\log_{10}$ cfu g^{-1} of silage. However, starting at 60 days, a decrease occurred in growth, with values close to 5.0 $\log_{10}$ cfu g^{-1} of silage observed (Figure 1).

The presence of MY was not detected at 7 and 15 days of ensiling but appeared after 60 days in all silages, except in the 10% GG TMRS at 100 days, with an average of 4.8 and 2.9 $\log_{10}$ cfu g^{-1} of silage at 60 and 100 days, respectively (Figure 1).

An effect of treatment and time was observed for the variables pH ($P < 0.0001$) and N-NH_3 ($P < 0.0001$; Table 3). The inclusion of 30% GG in the TMRS resulted in an increase in pH compared to the other diets. Regarding the storage time, a pH reduction was observed when the TMRS remained fermented for more than 60 days, when compared to 7 and 15 days of storage time (Table 4).

The concentration of NH_3-N did not exceed 0.1% of the total nitrogen and was significantly higher in the 30% GG TMRS. However, as the storage time increased, the concentration of NH_3-N increased and was highest at 100 days of ensiling (Table 4).

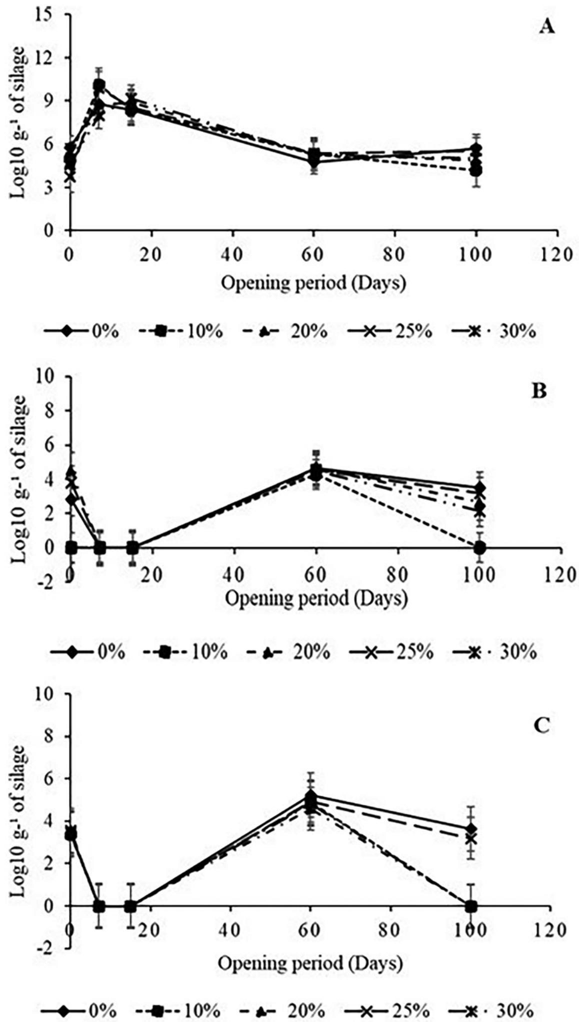

Figure 1. Quantification of lactic acid bacteria (A); moulds (B) and yeast (C) present in total mixed ration silages based on forage cactus and differing ratios of Guinea grass as a function to opening period (days). 0, 10, 20, 25 and 30% Guinea grass in total mixed ration silage.

Table 4. Means resulting from the effect of treatment and storage times for pH and ammoniacal nitrogen ($N\text{-}NH_3$) of silages total mixed ration silages based on forage cactus and different ratios of Guinea grass.

	Inclusion of guinea grass in TMR						
Item	0%	10%	20%	25%	30%	SEM[‡]	*P* value
pH	4.03[b]	3.97[b]	4.08[b]	4.11[b]	4.36[a]	0.04	<0.0001
$N\text{-}NH_3$ (% TN)	0.04[d]	0.05[cd]	0.06[bc]	0.08[b]	0.10[a]	0.01	<0.0001
	Opening period[†]						
	7 days	15 days	60 days	100 days			
pH	4.39[a]	4.33[a]	3.97[b]	4.07[b]		0.04	<0.0001
$N\text{-}NH_3$ (% TN)	0.04[c]	0.04[c]	0.07[b]	0.09[a]		0.01	<0.0001

[†]Means followed by the same letters in a row do not differ according to Tukey's test at the 5% probability level.
[‡]% TN, Percentage of total nitrogen. [§]Standard error of the mean.

There were no significant differences in diets (TMRS) for DMR ($P = 0.8133$), gas losses ($P = 0.7760$) and effluent losses ($P = 0.3095$) at 100 days of storage, with average values of 991.2 g kg^{-1}, 7.8 g kg^{-1} and 10.0 kg ton^{-1} of DM, respectively (Table 5).

An interaction effect between factors was observed for BC ($P = 0.0316$); lactic ($P < 0.0001$), acetic ($P < 0.0001$), propionic ($P < 0.0001$) and butyric acids ($P < 0.0001$) and ethanol ($P < 0.0001$; Table 6).

The aerobic stability test results for the surface layers of all silages showed no deterioration during the 96 h of evaluation (Figure 2). However, considering the silage mass

Table 5. Means resulting from the effect of treatment on dry matter recovery (DMR), gas losses (GL) and effluent losses (EL) from total mixed ration silages based on forage cactus and different ratios of Guinea grass at 100 days of storage.

	Inclusion of guinea grass in TMR						
Item	0%	10%	20%	25%	30%	SEM‡	*P* value
DMR (g kg^{-1} DM)	993.7	986.5	991.8	991.8	992.4	0.19	0.8133
GL (g kg^{-1} DM)	4.8	12.8	7.2	7.0	7.4	0.19	0.7760
EL (kg ton^{-1} DM)	14.4	7.6	10.4	11.9	5.8	1.38	0.3095

†Means followed by the same letters in a row do not differ according to Tukey's test at the 5% probability level. DM, Dry matter. §Standard error of the mean.

Table 6. Means resulting from the effect of the interaction treatment × storage time on lactic, acetic, propionic and butyric acids; buffering capacity and ethanol in total mixed ration silages based on forage cactus and different ratios of Guinea grass.

	Inclusion of guinea grass in TMR†						
Times	0%	10%	20%	25%	30%	SEM‡	*P* value
Buffering capacity (E.mg/100 g DM)							
7 days	70.0^{Ab}	70.0^{Bb}	70.0^{Bb}	80.0^{Bab}	90.0^{BCa}	0.00	0.0316
15 days	70.0^{Aa}	80.0^{ABa}	70.0^{Ba}	80.0^{Ba}	80.0^{Ca}		
60 days	80.0^{Ac}	80.0^{ABc}	90.0^{Abc}	90.0^{Bab}	100.0^{Ba}		
100 days	80.0^{Ac}	90.0^{Abc}	100.0^{Ab}	100.0^{Ab}	120.0^{Aa}		
Lactic acid (g kg^{-1} DM)							
7 days	53.4^{Ba}	44.2^{Ba}	48.4^{Ba}	43.9^{Ca}	57.5^{Ca}	0.67	<0.0001
15 days	88.2^{Aba}	65.2^{Bab}	45.1^{Bab}	59.7^{Cab}	32.6^{Cc}		
60 days	113.5^{Ab}	79.9^{Bb}	115.4^{Ab}	173.4^{Aa}	109.5^{Bb}		
100 days	99.9^{Ac}	133.4^{Abc}	149.6^{Ab}	111.6^{Bbc}	221.3^{Aa}		
Acetic acid (g kg^{-1} DM)							
7 days	11.8^{Aa}	7.8^{Ba}	5.5^{Ba}	4.5^{Ba}	6.1^{Ba}	0.11	<0.0001
15 days	12.3^{Aa}	12.7^{ABa}	5.7^{Ba}	11.3^{BCa}	5.8^{Ba}		
60 days	14.1^{Ac}	11.9^{ABc}	15.1^{Abc}	24.3^{Aab}	25.1^{Aa}		
100 days	14.5^{Ab}	17.0^{Ab}	21.7^{Ab}	13.8^{Bb}	33.4^{Aa}		
Propionic acid (g kg^{-1} DM)							
7 days	1.2^{Ab}	1.3^{Ab}	2.5^{Aa}	1.5^{Bab}	1.8^{ABab}	0.01	<0.0001
15 days	0.9^{Ab}	1.5^{Ab}	1.2^{Bb}	2.7^{Aa}	1.2^{Bb}		
60 days	1.8^{Aab}	1.2^{Ab}	1.4^{Bb}	1.2^{Bb}	2.7^{Aa}		
100 days	1.4^{Ab}	1.8^{Aab}	0.9^{Bb}	0.9^{Bb}	2.7^{Aa}		
Butyric acid (g kg^{-1} DM)							
7 days	1.9^{Aa}	1.4^{Bab}	1.1^{Bb}	0.9^{Bb}	1.1^{Bb}	0.01	<0.0001
15 days	1.4^{Ac}	1.9^{ABbc}	2.3^{Aab}	2.7^{Aa}	2.3^{Aab}		
60 days	1.7^{Ac}	2.4^{Aab}	1.5^{Bc}	2.7^{Aa}	2.0^{Abc}		
100 days	1.4^{Ab}	1.6^{Bb}	1.3^{Bb}	2.7^{Aa}	1.8^{Ab}		
Ethanol (g kg^{-1} DM)							
7 days	1.6^{Ca}	1.6^{Ca}	2.2^{Ca}	1.8^{Ca}	2.2^{Ba}	0.05	<0.0001
15 days	2.7^{Ca}	3.1^{BCa}	2.7^{Ca}	2.5^{BCa}	1.7^{Ba}		
60 days	5.0^{Bb}	4.8^{Bb}	5.1^{Bb}	4.5^{Bb}	10.8^{Aa}		
100 days	8.7^{Ab}	8.9^{Ab}	10.4^{Ab}	10.7^{Ab}	12.7^{Aa}		

†Means followed by the same letters, uppercase in the columns and lowercase letters in rows, do not differ by Tukey's test at the 5% probability level. DM, Dry matter. ‡Standard error of the mean.

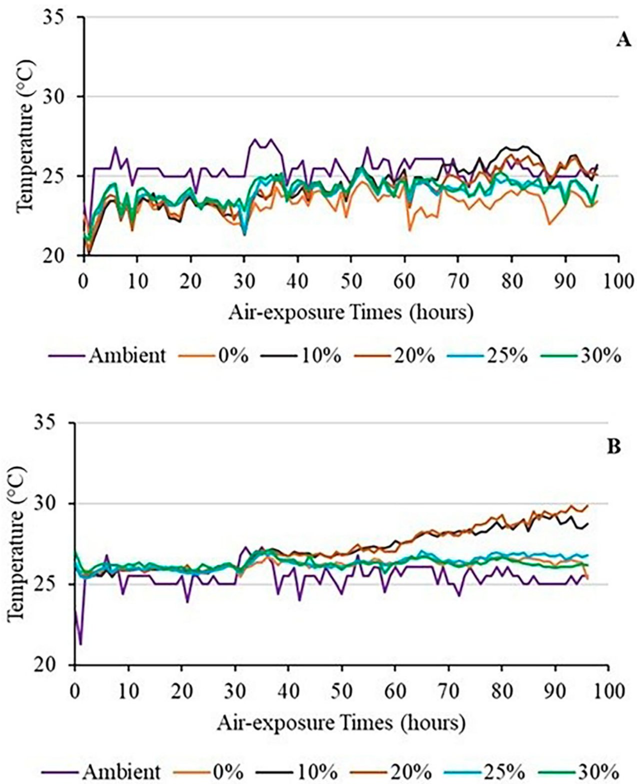

Figure 2. Surface temperatures (A) and internal temperatures (B) of total mixed ration silages based on forage cactus and differing ratios of Guinea grass as a function of air exposure time (hours). 0, 10, 20, 25 and 30% Guinea grass in total mixed ration silage.

internal layer of the different diets, aerobic deterioration started after 48 h of evaluation but only for TMRS with 20% and 10% GG (Figure 2).

An increase in the LAB count was observed after 24 h of exposure to air, but this population started to decrease in all TMRS evaluated as the length of the exposure period increased (Figure 3). There was no growth of moulds between 24 and 48 h of exposure to air in the TMRS, with the exception of the 20% GG TMRS diet, in which the mould concentrations increased, reaching counts of 5.5 and 9.6 log10 cfu g^{-1}, respectively, during this period. However, their mould counts decreased after 96 h of exposure to air, while in the other TMRS the mould content increased (except for the diet with 0% GG TMRS, which remained free of mould counts) (Figure 3).

Yeasts showed higher counts (6.7 log10 cfu g^{-1}) for the diet with 10% GG TMRS (during 24 h of exposure to air) but did not show counts at 48 h, nor did the diets containing 30, 20 and 0% GG TMRS. At 96 h of exposure to air, the counts increased again (Figure 3). The pH of all TMRS remained below 4.4, with some variations during the period of exposure to air, but without considerably increasing their average values.

An interaction effect occurred between the ratio of Guinea grass and hours of air exposure for acetic ($P < 0.0001$), propionic ($P < 0.0001$), lactic ($P < 0.0001$) and butyric

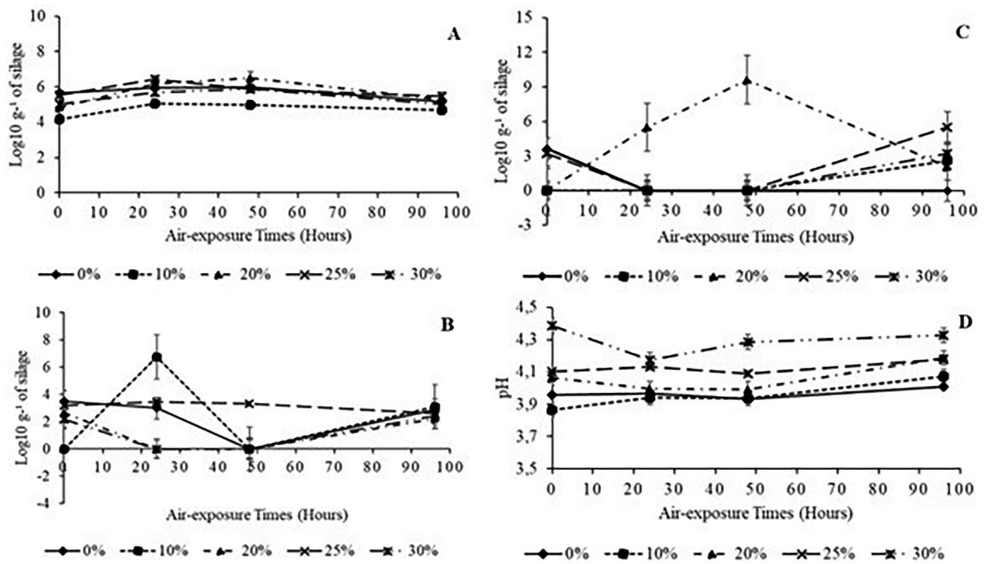

Figure 3. Quantification of lactic acid bacteria (A); moulds (B); yeast (C) and pH (D) in total mixed ration silages based on forage cactus and differing ratios of Guinea grass as a function to air-exposure times (hours). 0, 10, 20, 25 and 30% Guinea grass in total mixed ration silage.

acids ($P < 0.0001$); ethanol ($P < 0.0001$) and NH_3-N ($P < 0.0001$) in the aerobic stability test (Table 7).

Discussion

The increase in the DM content of the diets as the GG inclusion level decreased was expected, considering the increase in the ratio of wheat meal in place of grass. The NDF content was similarly diluted, as decreasing the GG levels in the TMRS and increasing the wheat meal contents reduced the fibrous content of the silages (Table 3).

The reductions in DM and NDF levels that occurred over the fermentation period were possibly due to acid hydrolysis of polysaccharides such as hemicellulose and pectin, causing the release of simpler compounds (McDonald et al. 2010), and by alkaline hydrolysis caused by ureolysis, since the urea added to the diets may have favoured the partial solubilisation of hemicellulose (Carvalho et al. 2018).

When initial DM levels in TMRs before ensiling (Table 1) were compared with those of silages, there was a significant DM increase, which is crucial for high DMR in silages (Table 4). Using TMRs minimises losses in forage cactus and guinea grass silages, thanks to forage cactus mucilage, retaining liquids, inhibiting fermentation losses, yielding high DMR (Monrroy et al. 2017), unlike isolated guinea GG silage.

Forage cactus is rich in complex polysaccharides, such as pectins, which comprise a variety of carbohydrates (Ribeiro et al. 2010). These pectins are esterified sugars rich in galactose, arabinose, xylose and fructose (Habibi et al. 2004). The adoption of forage cactus in all diets provided sufficient WSC for an adequate fermentation process.

The CP variation in silage, particularly in the 30% GG TMRS, may be due to some proteolysis, indicated by increased NH_3-N production (Table 4), which remained

Table 7. Means resulting from the effect of the interaction treatment × hours of air exposure on lactic, acetic, propionic and butyric acids; ethanol and ammoniacal nitrogen in total mixed ration silages based on cactus and different ratios of Guinea grass during aerobic stability.

	Inclusion of guinea grass in TMR†						
Times	0%	10%	20%	25%	30%	SEM‡	*P* value
Lactic acid (g kg^{-1} DM)							
0 h	99.9^{Bc}	133.4^{Abc}	149.6^{Ab}	111.6^{Bbc}	221.3^{Aa}	0.56	<0.0001
24 h	155.5^{Aab}	54.2^{Bc}	152.6^{Aab}	175.8^{Aa}	110.9^{Bb}		
48 h	132.8^{ABa}	94.9^{ABab}	140.1^{Aa}	74.1^{Bb}	105.0^{Bab}		
96 h	112.1^{Bc}	131.5^{Abc}	167.1^{Aab}	208.4^{Aa}	131.5^{Bbc}		
Acetic acid (g kg^{-1} DM)							
0 h	14.5^{Abc}	17.0^{Abc}	21.7^{Ab}	13.8^{BCc}	33.4^{Aa}	0.08	<0.0001
24 h	16.9^{Aab}	8.8^{Bc}	11.5^{BCbc}	20.1^{Aba}	20.6^{Ba}		
48 h	13.3^{Aa}	10.6^{ABa}	16.0^{ABa}	13.2^{Ca}	15.4^{Ba}		
96 h	18.9^{Aa}	8.3^{Bb}	6.6^{Cb}	20.7^{Aa}	19.0^{Ba}		
Propionic acid (g kg^{-1} DM)							
0 h	1.4^{Cb}	1.8^{Aab}	0.9^{Cb}	0.9^{Cb}	2.7^{Aa}	0.02	<0.0001
24 h	4.9^{Aa}	2.3^{Ab}	1.8^{BCbc}	1.9^{BCbc}	0.8^{Cc}		
48 h	2.7^{Ba}	2.3^{Aa}	2.6^{Ba}	2.2^{Ba}	2.5^{ABa}		
96 h	2.0^{BCb}	2.5^{Ab}	4.9^{Aa}	5.8^{Aa}	1.4^{BCb}		
Butyric acid (g kg^{-1} DM)							
0 h	1.4^{Bb}	1.6^{Bb}	1.3^{Bb}	2.7^{Aa}	1.8^{Ab}	0.01	<0.0001
24 h	2.4^{Aa}	1.9^{ABab}	1.8^{ABab}	1.7^{Bb}	1.4^{Ab}		
48 h	2.2^{Aa}	1.6^{Bb}	1.7^{ABab}	1.6^{Bb}	1.4^{Ab}		
96 h	2.0^{Aab}	2.4^{Aa}	2.2^{Aab}	2.3^{Aab}	1.8^{Ab}		
Ethanol (g kg^{-1} DM)							
0 h	8.7^{Ab}	8.9^{Ab}	10.4^{Ab}	10.7^{Aab}	2.7^{Aa}	0.05	<0.0001
24 h	6.2^{Bb}	6.2^{Bb}	6.5^{Bb}	6.0^{Bb}	11.6^{Aa}		
48 h	3.7^{Cb}	4.2^{Cab}	3.5^{Cb}	4.2^{BCab}	6.3^{Ba}		
96 h	3.9^{Cb}	3.4^{Cb}	2.3^{Cb}	2.3^{Cb}	13.4^{Aa}		
Ammoniacal nitrogen (% TN)							
0 h	0.05^{ABc}	0.07^{Bbc}	0.10^{ABab}	0.11^{Aab}	0.14^{Ba}	0.01	<0.0001
24 h	0.04^{Bc}	0.06^{Bbc}	0.06^{Bbc}	0.09^{Aab}	0.13^{Ba}		
48 h	0.09^{Ab}	0.12^{Aab}	0.08^{ABb}	0.09^{Aab}	0.13^{Ba}		
96 h	0.05^{ABc}	0.04^{Bc}	0.11^{Ab}	0.12^{Ab}	0.20^{Aa}		

†Means followed by the same letters, uppercase in the columns and lowercase letters in rows, do not differ by Tukey's test at the 5% probability level. DM, dry matter; % TN, percentage of total nitrogen.
‡Standard error of the mean.

below recommended levels (<100 g kg^{-1} of TN) (Costa et al. 2016). Rapid acidification during storage prevented the growth of undesirable microorganisms such as clostridia, minimising losses and leading to high DMR (Table 5). Additionally, propionic and butyric acid concentrations remained low (<3.0 g kg^{-1} DM) (Table 6), indicating a favourable fermentation process (Kung et al. 2018).

The effects on NH_3-N may also be related to the increase in DM, which resulted in decreased water activity, which is one of the main factors influencing the development of proteolytic microorganisms in silages (Charmley 2001). The higher urea content of this diet (30% GG) may also have influenced the production of NH_3-N, due to a certain amount of ureolysis occurring in the silo.

Nevertheless, the fermentation process that occurred in the TMRS evaluated is similar to that in other types of silages. These silages are of good quality and have adequate fermentation, with all silages reaching a satisfactory pH between 3.8 and 4.2 (McDonald et al. 1991), with the exception of the diet with 30% GG TMRS, which presented an average value of 4.36. This fact was attributed to the high BC of tropical grasses (Reis et al. 2013) because this diet had the highest Guinea grass inclusion level. Additionally, as previously mentioned, this diet had the highest production of NH_3-N, which also has a buffering action (Kung et al. 2018).

In addition, the cladodes of the forage cactus have buffering substances, such as oxalic, malic, citric and tartaric acids, resulting from crassulacean acid metabolism (Abidi et al. 2009; Petera et al. 2015; Isaac 2016), as well as considerable amounts of calcium, magnesium and potassium (Pereira et al. 2019), which contributed to the buffering of the ensiled TMRS.

As expected, MY counts were higher in the early period of ensiling, and then these populations decreased due to anaerobiosis and the intense lactic fermentation inside the silo. The first stage after ensiling of the material involves, among other things, the rapid depletion of oxygen, followed by the proliferation of LAB in the first days of ensiling (Muck et al. 2018), which started at 7 days of ensiling in this study. Thus, as the populations of LAB increased, the population of MY was inhibited (Figure 1).

On the other hand, during fermentation, increasing amounts of lactic acid were produced, promoting intense acidification of the silage environment (to approximately pH 4.0), which can reduce the LAB population (McDonald et al. 1991), with a decrease in its population observed starting at 60 days of ensiling, as also observed by Pereira et al. (2021). Therefore, due to the decrease in activity of LAB, an increase in the population of MY could then be observed (Figure 1). However, this population decreased at 100 days of ensiling, which was likely due to the increasing amounts of acetic acid produced, reaching >3% on a DM basis in the 30% GG TMRS (Table 6), moderately high concentrations with strong antifungal characteristics (Kung et al. 2018).

The likely explanation for the adequate levels of acetic acid in silages may be the occurrence of high populations of heterofermentative BAL due to the less pronounced reduction in pH throughout the fermentation period, due to the presence of buffering substances (Santos et al. 2020; Pereira et al. 2021; Santos et al. 2022), as previously cited for the 30% GG TMRS diet.

Probably, yeasts contributed significantly to the increase in the ethanol content (Table 6), especially in the 30% GG TMRS, likely due to the higher WSC content that this diet had before ensiling (Table 2), favouring yeast growth when LAB activity has stabilised at low pH, mostly up to 100 days, when the highest ethanol production was reached. In addition, it is important to note that heterofermentative LAB can promote ethanol production in the silages (Santos et al. 2014), which explains the appearance of this variable in all TMRS for up to 15 days of storage, when no yeast growth occurred. Thus, the heterofermentative LAB may have contributed to the increase in the ethanol concentration up to 100 days of storage.

When silage is exposed to oxygen, aerobic deterioration is inevitable. Oxygen can reactivate yeasts present in the silage, and many of these microorganisms use lactic acid as a source of energy in addition to WSC (Taylor and Kung 2002).

The analysis of the results obtained in the aerobic stability evaluation showed that the GG, when added in complete diets based on cactus pear, fulfilled its function of triggering lactic and acetic acid fermentation, thus reflecting in a better fermentative pattern. Similarly, the acetic acid produced during fermentation played an important role in ensuring aerobic stability in silages, mainly observed in the diet with 30% GG TMRS, which, as already mentioned, presented >3% of acetic acid on a DM basis.

However, because they had a low concentration of acetic acid after 24 h of exposure to air, and high counts of fungi and yeasts (Figure 3), diets with 20% and 10% GG TMRS showed aerobic deterioration after 48 h of exposure to air.

During aerobic deterioration of the silages, when lactic and acetic acids are degraded, enterobacteria have the opportunity to grow again (Kung et al. 2018). However, the presence of enterobacteria was not detected during the 96-h evaluation of silage aerobic stability, which may be attributed to the maintenance of the low pH of the silages (Figure 3).

The reason for the reactivation of LAB during air exposure is not known, as pH values remained low enough to inhibit the potential growth of these microorganisms, so a likely explanation would be the presence of oxygen because LAB are facultative anaerobes (Carr et al. 2002).

In all TMRS, the concentrations of propionic and butyric acids were below 1%, ranging from approximately 0.1% to 0.5% of DM, small values showing that the silages in this study were well fermented, as the growth of undesirable microorganisms was not favoured. Clearly, the increase or maintenance of lactic acid contents kept the pH values low enough to inhibit the growth of undesirable microorganisms, such as enterobacteria and clostridia.

Ethanol concentrations likely decreased with exposure time due to the high volatility of ethanol. All TMRS had a pH close to 4.0, despite an increasing trend until the end of the 96-h aerobic stability test, with the pH of TMRS 30% GG remaining higher. This result is associated with the heterolactic fermentation observed in the ensiled mass. According to Pereira et al. (2021), the use of cactus optimises heterolactic fermentation, explaining the results observed for the microbiological characteristics, DMR and chemical composition of the silage studied in the present study.

According to the data observed in the present study, the use of TMRS based on forage cactus and Guinea grass in the feeding of sheep on rural properties on drylands is promising, mainly from a nutritional and economic perspective. Furthermore, the use of these TMRS can be extended to other ruminant animals, provided that the diets are formulated taking into account the nutritional requirements of each species. However, there is a need to conduct experimental tests evaluating the consumption, digestibility, performance and ingestive behaviour of animals that consume the TMRS in the present study.

Conclusions

Guinea grass in combination with forage cactus can be added in total mixed ration silages up to a level of 30%, without compromising the chemical composition, silage losses and fermentative profile. However, considering aerobic stability, the addition of 10% and 20% guinea grass resulted in greater deterioration after 48 h of exposure to air.

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