RESEARCH ARTICLE



Forage cactus as a modulator of forage sorghum silage fermentation: An alternative for animal feed in drylands

Danillo Marte-Pereira¹, Juliana S. Oliveira², Francisco N. Sousa-Santos¹, Alberto J. Silva-Macêdo^{2*}, Paloma G. Batista-Gomes², Liliane Pereira-Santana³, Evandro S. Silva², Gabriel F. Lima-Cruz⁴, Alexandre Fernandes-Perazzo⁵, and Edson Mauro-Santos²

¹Universidade Federal do Maranhão, Departamento de Zootecnia, 65500-000, Chapadinha, Maranhão, Brasil. ²Universidade Federal da Paraíba, Departamento de Zootecnia, 58397-000, Areia, Paraíba, Brasil. ³Universidade Federal Rural do Pernambuco, Departamento de Zootecnia, 52171-900, Recife, Pernambuco, Brasil. ⁴Universidade Federal de Viçosa, Departamento de Zootecnia, 36570-900, Viçosa, Minas Gerais, Brasil. ⁵Universidade Federal do Piauí, Departamento de Zootecnia, 64049-550, Teresina, Piauí, Brasil. *Corresponding author (macedoajs@gmail.com).

Received: 26 May 2024; Accepted: 25 August 2024, doi:10.4067/S0718-58392025000100047

ABSTRACT

The rapid conversion of water-soluble carbohydrates in sorghum (*Sorghum bicolor* (L.) Moench) into lactic acid causes an abrupt decrease in silage pH, promoting alcoholic fermentation and aerobic deterioration. Despite the use of microbial and chemical additives, the results remain inconsistent. Mixing sorghum with cactus pear (*Nopalea cochenillifera* (L.) Salm-Dyck) can improve the stability and fermentative characteristics of silage. The aim of this study was to determine the ideal level of cactus pear that provides a good microbiological profile, as well as decreases losses during the ensiling process of mixed silages of cactus pear and sorghum. The treatments were composed of levels of cactus pear in the sorghum ensilage (0%, 25%, 50%, 75% and 100% cactus pear on a natural matter basis in the mixture), with four replicates each. It was found a quadratic effect (p < 0.05) on lactic acid bacteria counts, DM recovery and gas losses, with maximum estimated values of lactic acid bacteria and DM recovery in the levels of 51.8% and 33.5% of cactus pear, respectively, and minimum value of gas losses in the level of 37.9% of cactus pear in the silage. An increasing linear effect (p < 0.05) was observed on silage aerobic stability, contents of ether extract and non-fiber carbohydrates. When using cactus pear in the production of mixed silages with sorghum, it is recommended 50% cactus pear in the mixture, as it optimizes DM recovery and aerobic stability of the silages.

Key words: Aerobic stability, dry matter recovery, lactic acid bacteria, losses, yeasts.

INTRODUCTION

The quick conversion of water-soluble carbohydrates present in the sorghum *(Sorghum bicolor* (L.) Moench) by lactic bacteria into lactic acid causes abrupt decrease in the pH of the ensiled mass, favoring the growth of the yeast population, which is the main microorganism responsible for the alcoholic fermentation and aerobic deterioration of the silage when it is exposed to air (Tabacco et al., 2009; Schmidt and Kung Jr., 2010; Ferrero et al., 2019).

Based on that information, microbial and chemical additives have been developed to overcome problems during the fermentation process and after the silo (Tabacco et al., 2009; Lima et al., 2010; Thomas et al., 2013). However, the significant improvements in fermentative characteristics and aerobic stability of the evaluated silages have not been shown as a consistent result. Pyś et al. (2010), Khota et al. (2017), Santos et al. (2018), Ferrero et al. (2019), Rodrigues et al. (2020), and Diepersloot et al. (2021) and evaluated the effect of heterofermentative and chemical microbial inoculants on sorghum silage, and observed averages of 3.67 and 35.1, 96.5, 18.6, and 15 g kg⁻¹ for pH and residual water-soluble carbohydrates (WSCr), lactic acid, acetic acid,

and ethanol, respectively. Therefore, the effects of microbial and chemical additives in the sorghum silage remain variable and inconclusive.

The use of the mixed silage technique with cactus pear (*Nopalea cochenillifera* (L.) Salm-Dyck) would be an alternative to solve the problems related to the sorghum silage, since this technique aims to associate two or more crops to improve the nutritional and fermentative characteristics of silages.

The cactus pear arises as an option to compose mixed silage with sorghum, although it has unfavorable characteristics for silage, such as low DM content (< 200 g kg⁻¹) and high concentration of water-soluble carbohydrates (> 120 g kg⁻¹ DM) (Nogueira et al., 2019). On the other hand, the mucilage of the cactus pear is composed of highly hydrophilic polysaccharides (rhamnose, arabinose, galactose, and xylose) that minimize water activity and consequently the metabolism of spoilage microorganisms.

In addition, the cactus pear has buffering substances, such as: Oxalic, malic, citric, malonic, succinic and tartaric acids, which can prevent abrupt drop of the pH of the ensiled mass, as well as control the growth of the yeast population during the aerobic exposure of the silage (Brito et al., 2020). Nogueira et al. (2019) and Sá et al. (2020) evaluated cactus pear silages with additives, and observed that the silages without additive presented acetic acid values of 22.5 and 16.30 g kg⁻¹, respectively. Thus, the association between cactus pear and sorghum can decrease the alcoholic fermentation by controlling yeast, due to the amount of acetic acid from the fermentation of the cactus pear, reducing losses during the fermentation process and increasing the aerobic stability of the silage.

Therefore, the production of mixed silages becomes a promising technique, since the negative aspects of the sorghum for the ensiling process can be compensated by the cactus pear, showing the complementarity of these two crops. Thus, the present study aimed to identify the ideal level of cactus pear in a mixture that provides a good microbiological profile, as well as decreases losses during the ensiling process, and optimizes the DM recovery and aerobic stability of the silage.

MATERIAL AND METHODS

Experiment location

The experiment was carried out in the Forage Crops Production Sector at the Agriculture Sciences Center of the Universidade Federal da Paraíba (UFPB), in the town of Areia, PB, which is located in the micro-region of Brejo Paraibano (6°58'12" S, 35°42'15" W; 619 m a.s.l.) The climate of the region is classified as type As' hot and humid, according to the Köppen classification. According to data from the Meteorological Station of the Agriculture Sciences Center of the UFPB, the average annual rainfall in Areia is 1400 mm, the average annual temperature is 24.5 °C and the average air relative humidity is 80%. The rainiest 4 mo period is from April to July, and represent 62% of the total annual average.

Ensiling procedures

The sorghum (*Sorghum bicolor* (L.) Moench) came from a farm located in the town of Alagoinha (6°57'12" S, 35°32'19" W; 147 m a.s.l.), in the meso-region of Agreste Paraibano, and micro-region of Guarabira, which has a tropical rainy climate with dry summer (As'), according to the Köppen classification, with rainfall from February to August, and average annual rainfall and temperature around 1127 mm and 26.5 °C, respectively. The sorghum was sown on 6 June 2019 and harvested on 13 September 2019, when the grains were at the soft/hard dough stage.

The cactus pear (*Nopalea cochenillifera* (L.) Salm-Dyck) commonly known as miúda, with 1 yr of regrowth age, came from an orchard that was already implanted at the Semiarid National Institute (INSA), located in Campina Grande, PB, which is located in the meso-region of the Agreste Paraibano (7°16'47.24" S, 35°58'29.97" W; 494 m a.s.l.) The climate is classified as As' according to Köppen. The maximum temperature is 30 °C and the minimum temperature is 18 °C. The average annual rainfall is 876 mm.

The plants were harvested manually and processed in a stationary forage chopper machine (MC1001N, Laboremus, Campina Grande, Paraíba, Brazil), providing particles of approximately 2 cm. The material was immediately compacted with the aid of wooden sticks until reaching the approximate density of 600 kg m⁻³ of green mass (fresh matter (FM) basis) in each experimental silo.

The silages were made in 20 experimental polyvinyl chloride (PVC) silos, that measured 15 cm diameter × 30 cm height. Table 1 shows chemical composition of the material before ensiling. All experimental silos were adapted with a Bunsen valve to eliminate the gases resulting from fermentation. At the bottom of each silo it was added 1 kg dry sand, protected by non-woven fabric, preventing the mixture from coming into contact with the sand, thus allowing the drainage of the effluents. At the end of this process, the silos were closed, weighed and stored at room temperature in a covered, dry and ventilated place until the moment of opening (90 d after ensiling).

		Levels of cactus pear (%) ¹										
Variables	0	25	50	75	100							
DM, g kg ⁻¹ FM	365.51	252.87	200.06	187.18	84.50							
CP, g kg ⁻¹ DM	102.23	63.99	55.24	50.71	52.66							
EE, g kg ⁻¹ DM	14.56	12.07	8.01	12.49	22.58							
NDF, g kg ⁻¹ DM	715.88	677.69	665.74	539.69	353.16							
NFC, g kg ⁻¹ DM	167.33	246.25	371.01	497.11	571.60							
WSC, g kg ⁻¹ DM	139.81	180.97	177.81	198.94	162.54							

Table 1. Chemical composition of the material before ensiling. ¹Percentage on as fed basis. DM: Dry matter; FM: Fresh matter; CP: Crude protein; EE: ether extract; NDF: neutral detergent fiber; NFC: non-fiber carbohydrates; WSC: water-soluble carbohydrates.

Experimental design adopted

The experimental design adopted was the completely randomized design, which was composed of five treatments and four replicates, with the treatments characterized by the levels of cactus pear in the sorghum ensilage (0%, 25%, 50%, 75% and 100% cactus pear on a fresh matter basis in the mixture).

Microbial populations

The microbial populations were quantified when the experimental silos were opened, using selective culture media for each microbial group: MRS agar (Man, Rogosa, and Sharpe) containing 1.5 mL L⁻¹ acetic acid for lactic acid bacteria (LAB) and potato dextrose agar, containing 1% of 10% tartaric acid for molds and yeasts.

The quantification of the microbial groups was performed in 10 g sample of the replicates of each treatment, in which 90 mL sterile phosphate buffer solution were added and homogenized for 1 min, obtaining the dilution of 10¹. Then, successive dilutions were performed, aiming to obtain dilutions ranging from 10¹ to 10⁹, and the cultivation was performed in sterile disposable Petri dishes (González and Rodríguez, 2003).

The plates were incubated according to the specific incubation temperatures for each microbial group (Ávila et al., 2014; Santos et al., 2014), 37 °C for 48 h for LAB and 28 °C for 72 h for molds and yeasts. Plates with values between 30 and 300 CFU g⁻¹ silage were considered eligible for counting.

Fermentation profile of the silages

At the moment of opening, the pH and ammonia N (N-NH₃) values were determined following the methodology described by Bolsen et al. (1992). In order to determine the water-soluble carbohydrates (WSC) content, the concentrated sulfuric acid method described by DuBois et al. (1956) with adaptations by Corsato et al. (2008) was used.

Silage DM losses and recovery

Dry matter losses in the silages as effluents and gases were quantified by weight difference using the equations described by Zanine et al. (2010):

GL = (FWc - FWo)/(FMc × DMc) × 100

where, GL is gas losses (% DM), FWc is weight of silo filled at closure (kg), FWo is weight of silo filled at opening (kg), FMc is forage mass at silo closure (kg), and DMc is forage DM concentration at silo closure (%).

EL = [(EWo - Ew) - (EWc - Ew)]/FMc × 100

Where, EL is effluent losses (kg t⁻¹ as fed), EWo is weight of empty silo + sand at opening (kg), Ew is weight of empty silo (kg), EWc is weight of empty silo + sand at closure (kg), and FMc is forage mass at silo closure (kg).

The estimate of DM recovery (DMR) was obtained by the difference of DM weight before and after ensiling through the equation described by Zanine et al. (2010):

 $DMR = (FMo \times DMo)/(FMc \times DMc) \times 100$

where, DMR is DM recovery rate (%), FMo is forage mass at silo opening (kg), DMo is forage DM concentration at silo opening (%), FMc is forage mass at silo closure (kg), and DMc is forage DM concentration at silo closure (%).

Aerobic stability of the silages

The aerobic stability (AS) of the silages (expressed in h) was evaluated by monitoring the internal temperature of the silages exposed to air for a period of 120 h. The silage samples were placed with no compaction in experimental PVC silos without a lid and kept in a closed environment with controlled temperature (25 $^{\circ}$ C).

The maximum temperature (MT) of the silage, expressed in °C, and the time to reach the maximum temperature (MTH), expressed in h, were determined by monitoring the mass exposed to air every 30 min using digital immersion thermometers positioned in the center of the silage mass. The onset of deterioration was considered when the internal temperature of the silages reached 2 °C above the room temperature (Ranjit and Kung Jr., 2000).

Chemical composition of the silages

Plant samples before ensiling as well as silage samples were collected, with approximately 300 g each replicate. The samples were pre-dried in a forced ventilation oven for 72 h at 60 $^{\circ}$ C.

Those samples were then ground into 1 mm particles in a Wiley knife mill and analyzed for DM, organic matter (OM), mineral matter (MM), ether extract (EE) and crude protein (CP) contents according to the AOAC (2016) methods 934.01, 942.05, 924.05, 920.39, and 968.06, respectively.

The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) of the samples were determined through the methodology described by Mertens (2002). Non-fiber carbohydrates (NFC) were estimated by the following equation developed by Sniffen et al. (1992): NFC = 100 - (MM + CP + EE + NDF).

Statistical analysis

The data obtained were submitted to ANOVA and regression, according to the levels of cactus pear in the sorghum silage, using the statistical software SAS 9.4 (SAS Institute, Cary, North Carolina, USA). The regression equations were chosen based on the coefficient of determination (R^2) and the significance of the regression coefficients using the t test at $\alpha = 0.05$.

The following statistical model was used:

$$Y_i = \beta_0 + \beta_1 X_i + e_i$$

where Y_i is observed value for the dependent variable Y in the ith level of the independent variable X; β_0 is regression constant, represents the intercept of the line with the Y axis; β_1 is coefficient of regression, represents the variation of Y as a function of the variation of one unit of the variable X; X_i is ith level of the independent variable X (i = 1, 2, ..., n); e_i is the error associated to the distance between the observed value Y_i and the corresponding point in the curve of the proposed model, for the same level i of X.

RESULTS

Microbial count of the silages

It was found a quadratic effect (p < 0.05) on the LAB populations according to the levels of cactus pear in the mixed silage, with the maximum population value estimated by the regression model in the level of 51.8% cactus pear. Also, there was a linear effect (p < 0.05) on the mold population, which ranged from 3.85 to 1.50 log₁₀ CFU g⁻¹ silage. However, there was no effect (p > 0.05) on the yeast population, that averaged 1.32 log₁₀ CFU g⁻¹ silage (Table 2).

	Le	evels of	cactus	pear (%)1				
Variables	0	25	50	75	100	SEM	L	Q	R ²
LAB, CFU g ⁻¹ silage	4.42	5.68	6.32	6.23	4.50	0.199	0.282	< 0.001	96.64
Molds, CFU g ⁻¹ silage	3.85	4.85	1.82	1.50	1.50	0.701	0.002	0.794	67.89
Yeasts, CFU g ⁻¹ silage	1.78	1.25	0.75	1.50	1.50	0.798	0.076	0.789	96.93

Table 2. Microbial count according to the level of cactus pear in the mixed silages. ¹Percentage on as fed basis. SEM: Standard error of the mean; L: significance for linear effect; Q: significance for quadratic effect; R²: coefficient of determination; LAB: Lactic acid bacteria; CFU: colony forming units.

Fermentation profile of the silages

There was no effect of the levels of cactus pear (p > 0.05) on the variables pH and WSCr, which presented averages of 3.95 and 13.0 g kg⁻¹ DM, respectively. However, a linear effect was observed (p > 0.05) on N-NH₃, which ranged from 0.68% to 2.56% total N, according to the level of cactus pear in the mixed silage (Table 3).

Table 3. Fermentation profile according to the level of cactus pear in the mixed silages. ¹Percentage on as fed basis. SEM: Standard error of the mean; L: significance for linear effect; Q: significance for quadratic effect; R²: coefficient of determination; WSCr: residual water-soluble carbohydrates; N-NH₃: ammoniacal N based on total N percentage.

Levels of cactus pear (%) ¹									
Variables	0	25	50	75	100	SEM	L	Q	R ²
рН	3.86	3.86	3.83	3.93	4.30	0.146	0.072	0.146	93.98
WSCr, g kg ⁻¹ DM	8.66	10.26	13.46	15.03	17.96	0.455	0.136	0.953	98.99
N-NH₃, % TN	0.68	0.91	1.17	1.48	2.56	1.033	< 0.001	< 0.001	86.69

Losses during the ensiling process and DM recovery of the silages

Regarding the variables of losses during the ensiling process, it was found a linear effect (p < 0.05) on EL, which ranged from 8.6% to 19.8%. On the other hand, a quadratic effect (p < 0.05) was observed on GL, whose lowest value was estimated in the level of 37.9%, while, for DMR the highest value was estimated in the level of 33.5% cactus pear in the mixed silage (Table 4).

Table 4. Losses and recovery of DM according to the level of cactus pear in the mixed silages. ¹Percentage on as fed basis. SEM: Standard error of the mean; L: significance for linear effect; Q: significance for quadratic effect; R²: coefficient of determination.

	Levels of cactus pear (%) ¹						L	Q	R ²
Variables	0	25	50	75	100				
Gas losses, %DM	8.60	4.39	4.73	4.52	19.89	1.134	< 0.001	< 0.001	89.47
Effluent losses, kg t ⁻¹ (as fed)	6.79	14.16	24.43	52.84	124.19	2.083	< 0.001	< 0.001	81.60
DM recovery, g kg ⁻¹	907.10	941.90	928.20	902.00	676.90	1.174	< 0.001	< 0.001	94.31

Aerobic stability of the silages

A linear effect (p < 0.05) was found on MT, which reduced from 33.9 to 25.3 °C. However, there was also a linear effect (p < 0.05) on MTH and AS variables, that increased from 72.3 to 105.3 h and 55.5 to 120.0 h, respectively, according to the level of cactus pear in the mixed silages (Table 5).

Table 5. Variables of aerobic stability of the silages according to the level of cactus pear in the mixed silages. ¹Percentage on as fed basis. SEM: Standard error of the mean; L: significance for linear effect; Q: significance for quadratic effect; R²: coefficient of determination; MT: silage maximum temperature; MTH: time to reach maximum temperature; AS: aerobic stability.

		Level	s of cactus	pear (%)¹	_				
Variables	0	25	50	75	100	SEM	L	Q	R ²
MT, °C	33.96	30.26	26.73	26.43	25.33	0.337	< 0.001	< 0.001	98.39
MTH, h	72.33	81.16	96.00	88.66	105.33	6.882	0.007	0.806	82.53
AS, h	55.50	69.16	120.00	120.00	120.00	3.744	< 0.001	< 0.001	89.88

Chemical composition of the silages

It was observed a decreasing linear effect (p < 0.05) on the contents of DM, CP and NDF, which reduced from 344.0 to 57.4 g kg⁻¹, 94.5 to 60.8 g kg⁻¹ DM and 721.0 to 287.9 g kg⁻¹ DM, respectively. However, there was an increasing linear effect (p < 0.05) on EE and NFC contents according to the level of cactus pear in the sorghum silages, which increased from 18.2 to 27.8 g kg⁻¹ DM, and 166.3 to 638.9 g kg⁻¹ DM, respectively (Table 6).

Table 6. Chemical composition of the silages according to the level of cactus pear in the mixed silages. ¹Percentage on as fed basis. SEM: Standard error of the mean; L: significance for linear effect; Q: significance for quadratic effect; R²: coefficient of determination; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; NFC: non-fiber carbohydrates.

		Levels (of cactus p	_					
Variables	0	25	50	75	100	SEM	L	Q	R ²
DM, g kg ⁻¹	344.00	287.30	206.80	150.60	57.40	0.743	< 0.001	0.112	99.30
CP, g kg ⁻¹ DM	94.50	60.80	50.43	47.26	45.26	0.146	< 0.001	< 0.001	96.95
EE, g kg ⁻¹ DM	18.20	17.90	23.06	31.50	27.83	0.127	< 0.001	0.483	75.96
NDF, g kg ⁻¹ DM	721.00	680.34	624.03	591.16	287.96	3.238	< 0.001	0.002	93.12
NFC, g kg ⁻¹ DM	166.33	240.95	302.47	330.11	638.91	3.224	< 0.001	0.005	81.83

DISCUSSION

Microbial count of the silages

The levels of cactus pear promoted a significant increase in the lactic acid bacteria (LAB) population (\log_{10} CFU g⁻¹ silage) in the silages during the ensiling, with the maximum population estimated in the level of 51.8% cactus pear in the mixed silage (Table 2). Sá et al. (2020) evaluated the fermentation profile of the cactus pear silage, and observed an amount of LAB population similar to the one found in the present study (7.00 \log_{10} CFU g⁻¹ silage).

Pereira et al. (2019) selected strains of LAB isolated from the plant and silage of cactus pear, and observed a predominance of heterofermentative LABs (*Weissella cibaria*, *Weissella confusa* and *Weissella paramesenteroides*). Those microorganisms are able to produce antifungal compounds, mainly acetic acid, and allow the associated growth of populations of LAB producing other organic acids, such as propionic acid (Schmidt et al., 2011). This explains the reduction in mold and yeast populations (log₁₀ CFU g⁻¹ silage) as the levels of cactus pear increased in the mixed silages (Table 2).

The reduction in the population of those microorganisms may be related to buffering substances present in the cactus pear, such as: Oxalic, malic, citric, malonic, succinic and tartaric acids, which can prevent the abrupt drop in the pH of the ensiled mass, as well as control the growth of yeast populations during the aerobic exposure of the silage. This effect was also observed by Brito et al. (2020), who evaluated mixed silages of gliricidia (*Gliricidia sepium* (Jacq.) Kunth) and cactus pear, where the inclusion of the cactus pear controlled the mold and yeast populations.

Thus, the results of the microbiological profile of the mixed silage suggest that the levels of cactus pear in the silage optimized the development of the LAB population, with probable action of the heterofermentative ones, since there was an increase in the LAB population and reduction in the mold and yeast populations as the levels of cactus pear increased in the mixed silage (Table 2).

Tabacco et al. (2009) evaluated the effect of the inoculation with microbial additives in corn and sorghum silages, and observed an increased in the population of LAB (9.03 \log_{10} CFU g⁻¹ silage) and reduction in the population of yeasts (2.17 \log_{10} CFU g⁻¹) in the sorghum silage inoculated with *Lentilactobacillus buchneri*, however, the authors observed no difference between the counts of LAB (5.45 \log_{10} CFU g⁻¹) and yeasts (5.82 \log_{10} CFU g⁻¹) between the control silage and the one inoculated with *Lactiplantibacillus plantarum*. Therefore, the results observed by the authors in the sorghum silage inoculated with *L. buchneri* are similar to those observed in the present study as the levels of cactus pear in the mixed silage increased (Table 2). Thus, the use of cactus pear associated with sorghum may be a strategy to promote heterofermentative fermentation, which is similar to the effects of microbial inoculants.

Fermentation profile of the silages

The pH values of the ensiled mass are influenced by the conversion of water-soluble carbohydrates (WSC) into organic acids. In the present study, it was observed that the WSC from plants and mixtures (Table 1) were converted by lactic bacteria to lactic acid and possibly to acetic acid, as pointed out by the results observed in Table 2. However, increasing levels of cactus pear did not influence (p > 0.05) the silage pH values (Table 3). Although the inclusion of cactus pear in the mixed silage increased the amount of buffering substances and moisture in the ensiled mass, those increases did not prevent the reduction of silage pH, which is most likely due to the high contents of soluble carbohydrates before ensiling, regardless of the levels of cactus pear (Table 3).

Kung Jr. et al. (2018) reported that in general, plants with DM contents lower than 25% tend to present clostridium activity during their fermentation process, which results in high concentrations of N-NH₃. However, this was not observed in the present study, given the low N-NH₃ contents of the mixed silage of sorghum and cactus pear (Table 3), corroborating with the results observed by Sá et al. (2020), Santos et al. (2020), Brito et al. (2020) and Pereira et al. (2021), when they evaluated cactus pear silages and cactus pear-based silages. Therefore, the fermentative process of the silages of the present study was efficient in inhibiting the development of undesirable microorganisms, such as *Clostridium*, despite the DM values lower than 30%.

Losses during the ensiling process and DM recovery of the silages

In general, the inclusion of cactus pear in the mixed silage positively influenced the fermentative process and possibly provided a heterolactic fermentation profile, although the silages with 25%, 50% and 75% cactus pear showed values lower than 5.0% gas losses (GL) in the silage DM (Table 4).

The increase in effluent losses (EL) during the fermentative process is related to the higher moisture content of the cactus pear. Sá et al. (2020) evaluated the fermentative characteristics and chemical composition of the cactus pear silage, and found an EL value of 12 kg t⁻¹ FM. The authors associated this result to the amount of mucilage (gelatinous substance) increased as a function of the age of the cactus pear at the time of ensiling, which could have minimized effluent runoff. In their study, the cactus pear had a regrowth age of 2 yr. Therefore, the results found for EL were already expected, since the cactus pear used in this study was less than 2 yr old, which has in its composition less mucilage and consequently more free water.

Although the levels of cactus pear negatively affected EL, this negative effect was not observed on DM recovery (DMR), which showed values > 900.00 g kg⁻¹ in the levels 0%, 25%, 50% and 75% cactus pear, with maximum DMR (941.9 g kg⁻¹) estimated by the regression model in the level of 33.5% cactus pear in the mixed silage (Table 4). However, when the cactus pear was ensiled alone it showed DMR < 700.00 g kg⁻¹, and that result differs from those observed by Brito et al. (2020), Sá et al. (2020) and Santos et al. (2020), who evaluated cactus pear silages and cactus pear-based silages and observed a DMR > 900 g kg⁻¹, what can be explained by the age of the cactus pear orchard, which in the present study had 1 yr of regrowth.

However, when the sorghum is ensiled alone, that usually results in a DMR lower than 900.00 g kg⁻¹, as observed by Paiva et al. (2010), who evaluated sorghum cultivars grown in the semi-arid region of Paraiba, and found a DMR < 850.00 g kg⁻¹ for the cvs. BRS Ponta Negra and BRS 610. Thus, the use of cactus pear proved to be effective in overcoming the total losses in the sorghum silage, especially at intermediate inclusion levels.

Aerobic stability of the silages

Regarding the aerobic stability, sorghum silage is characterized by being unstable after the silo opening, generating costly losses, due to the need to discard silage with molds or with signs of deterioration. This low aerobic stability is related to the great population of yeast species in the ensiled mass at moment of silo opening.

The initial deterioration is due to an intense metabolic activity, that uses soluble compounds, such as residual water-soluble carbohydrates and lactic acid present in the silage as substrate for their growth (Pereira et al., 2021). Tabacco et al. (2009; 2011) observed in their researches that sorghum presents aerobic stability less than 45 h when ensiled alone, when they evaluated the effects of *L. buchneri* and *L. plantarum* in corn and sorghum silages. The results observed by these authors are similar to those observed in the present study, especially in silages with levels below 50% cactus pear FM.

The use of cactus pear in the production of the mixed silage promoted a reduction on the maximum temperature (MT) (°C) and increase in the time to reach the maximum temperature (MTH) (h). Regarding aerobic stability (AS), the influence of the cactus pear was only observed above the level of 25%, which provided an increase in the stability of the silage mass in 13.66 h. From the level of 50% cactus pear in the mixed silage, no break in aerobic stability (> 120 h) of the ensiled mass was observed (Table 5). Sá et al. (2020), Santos et al. (2020) and Brito et al. (2020) also did not observe aerobic stability break in their cactus pear-based silages, which is similar to the results observed in the present study.

This result may be related to the microbial characteristics of the silages (Table 2). Pereira et al. (2019) identified in their research a predominance of lactic bacteria of the genus *Weissella* in the cactus pear, which is classified as an obligatory heterofermentative LAB. The predominance of heterolactic fermentation has been attributed to the presence of buffering substances in the cactus pear, which promote a less sharp drop in pH, enabling the proliferation of heterolactic species, improve aerobic stability due to higher acetic acid production (Muck et al., 2018).

Chemical composition of the silages

The levels of cactus pear in the mixed silages reduced the contents of DM, CP and NDF and increased the contents of EE and NFC in the mixtures (Table 1) and consequently in the silages (Table 6). However, it can be said that there was an influence of the cactus pear on the fermentative and nutritional characteristics of the mixed silage when compared to the *in natura* material. The results of the chemical composition of silages observed by Brito et al. (2020) who evaluated silages of gliricidia and cactus pear, corroborate to those of the present study. These authors also found nonsignificant loss in the nutritional value of the mixed silages. These results may be related to the good fermentative process of mixed silages, which inhibited the activity of undesirable microorganisms, such as *Clostridium* and Enterobacteria, reducing proteolysis and DM losses (Kung Jr. et al., 2018).

The reduction in the NDF content of the silages during the fermentation process (Table 6) when compared to the fresh mixtures (Table 1) is related to acid hydrolysis through the organic acids produced during fermentation (Muck et al., 2018).

Evaluating the results of this study, the silage with 50% cactus pear presented the highest LAB count, as well as, from this level on, the lowest mold and yeast counts were observed in the mixed silages, directly influencing the AS of the silages (> 120 h).

Although the levels of cactus pear increased EL, a significant increase in DMR (> 940.00 g kg⁻¹) was observed in the level of 33.5% cactus pear in the mixed silage. However, in the level of 50% cactus pear, a DMR of > 920.0 g kg⁻¹ was observed without compromising the nutritive value of the mixed silages.

Therefore, based on the results observed during the research, the additive effect of the cactus pear in sorghum silages is evidenced, which normally presents a homolactic fermentative profile. Thus, those results will serve as a basis for the development of further studies involving this subject, as well as assist producers in making decisions about optimizing the use of forage resources in their properties.

CONCLUSIONS

When using cactus pear in the production of mixed silages with sorghum, a level of 50% cactus pear is recommended, as it optimizes dry matter recovery and aerobic stability.

Author contribution

Conceptualization: J.S.O., E.M.S. Methodology: D.M.P., F.N.S.S., A.J.S.M., L.P.S., E.S.S., G.F.L.C., A.F.P. Formal analysis: D.M.P., F.N.S.S., A.J.S.M., P.G.B.G., L.P.S., E.S.S., G.F.L.C. Investigation: D.M.P., F.N.S.S., P.G.B.G., L.P.S., E.S.S., G.F.L.C. Resources: E.M.S. Data curation: D.M.P. Writing-original draft: D.M.P., J.S.O. Writing-review & editing: J.S.O., A.J.S.M., A.F.P., E.M.S. Visualization: J.S.O., A.F.P. Supervision: E.M.S. Project administration: E.MS. Funding acquisition: E.MS. All co-authors reviewed the final version and approved the manuscript before submission.

References

- AOAC. 2016. Official methods of analysis of AOAC International. 20th ed. In Latimer Jr., G.W. (ed.) Association of Official Analytical Collaboration (AOAC) International, Rockville, Maryland, USA.
- Ávila, C.L.S., Carvalho, B.F., Pinto, J.C., Duarte, W.F., Schwan, R.F. 2014. The use of *Lactobacillus* species as starter cultures for enhancing the quality of sugar cane silage. Journal of Dairy Science 97:940-951. doi:10.3168/jds.2013-6987.
- Bolsen, K.K., Lin, C., Brent, B.E., Feyerherm, A.M., Urban, J.E., Aimutis, W.R. 1992. Effect of silage additives on the microbial succession and fermentation process of alfalfa and corn silages. Journal of Dairy Science 75(11):3066-3083. doi:10.3168/jds.S0022-0302(92)78070-9.
- Brito, G.S.M., Santos, E.M., Araújo, G.G.L., Oliveira, J.S., Zanine, A.M., Perazzo, A.F., et al. 2020. Mixed silages of cactus pear and gliricidia: Chemical composition, fermentation characteristics, microbial population and aerobic stability. Scientific Reports 10:6834. doi:10.1038/s41598-020-63905-9.
- Corsato, C.E., Scarpare Filho, J.A., Sales, E.C.J. 2008. Teores de carboidratos em órgãos lenhosos do caquizeiro em clima tropical. Revista Brasileira de Fruticultura 30:414-418. doi:10.1590/S0100-29452008000200025.
- Diepersloot, E.C., Pupo, M.R., Ghizzi, L.G., Gusmão, J.O., Heinzen Jr.C., McCary, C.L., et al. 2021. Effects of microbial inoculation and storage length on fermentation profile and nutrient composition of whole-plant sorghum silage of different varieties. Frontiers in Microbiology 12:660567. doi:10.3389/fmicb.2021.660567.
- DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28:350-356.
- Ferrero, F., Piano, S., Tabacco, E., Borreani, G. 2019. Effects of conservation period and *Lactobacillus hilgardii* inoculum on the fermentation profile and aerobic stability of whole corn and sorghum silages. Journal of the Science of Food and Agriculture 99:2530-2540. doi:10.1002/jsfa.9463.
- González, G., Rodríguez, A.A. 2003. Effect of storage method on fermentation characteristics, aerobic stability, and forage intake of tropical grasses ensiled in round bales. Journal of Dairy Science 86:926-933. doi:10.3168/jds.S0022-0302(03)73675-3.
- Khota, W., Pholsen, S., Higgs, D., Cai, Y. 2017. Fermentation quality and *in vitro* methane production of sorghum silage prepared with cellulase and lactic acid bacteria. Asian-Australasian Journal of Animal Sciences 30(11):1568-1574. doi:10.5713/ajas.16.0502.
- Kung Jr., L., Shaver, R.D., Grant, R.J., Schmidt, R.J. 2018. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages Journal of Dairy Science 101:4020-4033. doi:10.3168/jds.2017-13909.
- Lima, R., Lourenço, M., Diaz, R.F., Castro, A., Fievez, V. 2010. Effect of combined ensiling of sorghum and soybean with or without molasses and lactobacilli on silage quality and in vitro rumen fermentation. Animal Feed Science and Technology 155:122-131. doi:10.1016/j.anifeedsci.2009.10.008.
- Macêdo, A.J.S., Santos, E.M., Araújo, G.G.L., Edvan, R.L., Oliveira, J.S., Perazzo, A.F., et al. 2018. Silages in the form of diet based on spineless cactus and buffelgrass. African Journal of Range and Forage Science 35:121-129. doi:10.2989/10220119.2018.1473494.
- Mertens, D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. Journal of AOAC International 85:1217-1240.
- Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C., Kung Jr., L. 2018. Silage review: Recent advances and future uses of silage additives. Journal of dairy science 101:3980-4000. doi:10.3168/jds.2017-13839.
- Nogueira, M.S., Araújo, G.G.L., Santos, E.M., Gonzaga Neto, S., Oliveira, J.S., Perazzo, A.F., et al. 2019. Feed alternatives with cactus forage silage for animal nutrition. International Journal of Agriculture and Biology 22:1393-1398. doi:10.17957/IJAB/15.1213.

- Paiva, G.N., Gil, F.G., Freitas, P.M.D., Ramos, R.C.S., Aquino, M.M., Santos, E.M. 2010. Perdas na ensilagem de cinco cultivares de sorgo. In Anais de 4° Simpósio Internacional Sobre Caprinos e Ovinos de Corte, SINCORTE, João Pessoa, Paraíba. 16-20 Novembro 2009. Sociedade Brasileira de Zootecnia, Viçosa, Minas Gerais, Brasil.
- Pereira, G.A., Santos, E.M., Araújo, G.G.L., Oliveira, J.S., Pinho, R.M.A., Zanine, A.M., et al. 2019. Isolation and identification of lactic acid bacteria in fresh plants and in silage from *Opuntia* and their effects on the fermentation and aerobic stability of silage. The Journal of Agricultural Science 157:684-692. doi:10.1017/S0021859620000143.
- Pereira, D.M., Santos, E.M., Oliveira, J.S., Santos, F.N.S., Lopes, R.C., Santos, M.A.C., et al. 2021. Effect of cactus pear as a moistening additive in the production of rehydrated corn grain silage. Journal of Agricultural Science 159(9-10):731-742. doi:10.1017/S002185962100099X.
- Pyś, J.B., Karpowicz, A., Szałata, A. 2010. The effect of harvest date and additives on chemical composition and aerobic stability of sorghum silage. Slovak Journal of Animal Science 43:187-194.
- Ranjit, N.K., Kung Jr., L. 2000. The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. Journal of Dairy Science 83:526-535. doi:10.3168/jds.S0022-0302(00)74912-5.
- Rodrigues, P.H.M., Pinedo, L.A., Meyer, P.M., Silva, T.H., Guimarães, I.C. 2020. Sorghum silage quality as determined by chemical-nutritional factors. Grass and Forage Science 75:462-473. doi:10.1111/gfs.12495.
- Sá, W.C.C.S., Santos, E.M., Oliveira, J.S., Araujo, G.G.L., Perazzo, A.F., Silva, A.L., et al. 2020. Fermentative characteristics and chemical composition of cochineal nopal cactus silage containing chemical and microbial additives. Journal of Agricultural Science 158(7):574-582. doi:10.1017/S0021859620000829.
- Santos, E.M., Pereira, O.G., Garcia, R., Ferreira, C., Oliveira, J.S., Silva, T.C. 2014. Effect of regrowth interval and a microbial inoculant on the fermentation profile and dry matter recovery of guinea grass silages. Journal of Dairy Science 97:4423-4432. doi:10.3168/jds.2013-7634.
- Santos, F.N.S., Santos, E.M., Oliveira, J.S., Medeiros, G.R., Zanine, A.M., Araújo, G.G.L., et al. 2020. Fermentation profile, microbial populations, taxonomic diversity and aerobic stability of total mixed ration silages based on cactus and gliricidia. Journal of Agricultural Science 158(5):396-405. doi:10.1017/S0021859620000805.
- Santos, A.P.M., Santos, E.M., Oliveira, J.S., Ribeiro, O.L., Perazzo, A.F., Martins, A.P.R., et al. 2018. Effects of urea addition on the fermentation of sorghum *licolor*) silage. African Journal of Range & Forage Science 35:55-62. doi:10.2989/10220119.2018.1458751.
- Schmidt, R.J., Kung Jr., L. 2010. The effects of *Lactobacillus buchneri* with or without a homolactic bacterium on the fermentation and aerobic stability of corn silages made at different locations. Journal of Dairy Science 93:1616-1624. doi:10.3168/jds.2009-2555.
- Schmidt, P., Rossi Junior, P., Junges, D., Dias, L.T., Almeida, R., Mari, L.J. 2011. Novos aditivos microbianos na ensilagem da cana-de-açúcar: composição bromatológica, perdas fermentativas, componentes voláteis e estabilidade aeróbia. Revista Brasileira de Zootecnia 40:543-549. doi:10.1590/S1516-35982011000300011.
- Sniffen, C.J., O'Connor, J.D., van Soest, P.J., Fox, D.G., Russell, J.B. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. Journal of Animal Science 70:3562-3577.
- Tabacco, E., Piano, S., Cavallarin, L., Bernardes, T.F., Borreani, G. 2009. Clostridia spore formation during aerobic deterioration of maize and sorghum silages as influenced by *Lactobacillus buchneri* and *Lactobacillus plantarum* inoculants. Journal of Applied Microbiology 107:1632-1641. doi:10.1111/j.1365-2672.2009.04344.x.
- Tabacco, E., Piano, S., Revello-Chion, A., Borreani, G. 2011. Effect of *Lactobacillus buchneri* LN4637 and *Lactobacillus buchneri* LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions. Journal of Dairy Science 94:5589-5598. doi:10.3168/jds.2011-4286.
- Thomas, M.E., Foster, J.L., McCuistion, K.C., Redmon, L.A., Jessup, R.W. 2013. Nutritive value, fermentation characteristics, and in situ disappearance kinetics of sorghum silage treated with inoculants. Journal of Dairy Science 96:7120-7131. doi:10.3168/jds.2013-6635.
- Zanine, A.M., Santos, E.M., Dórea, J.R.R., Dantas, P.A.S., Silva, T.C., Pereira, O.G. 2010. Evaluation of elephant grass silage with the addition of cassava scrapings. Revista Brasileira de Zootecnia 39:2611-2616. doi:10.1590/S1516-35982010001200008.