

Impact of mixed forage silage with BRS Capiacu grass, ground corn, and varying forage palm levels on aerobic stability, fermentation profile, chemical composition, and digestibility

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ABSTRACT - This study aimed to evaluate different proportions of cactus pear in silage with BRS Capiacu grass, along with a 10% inclusion of ground corn grain, focusing on aerobic stability, fermentative profile, chemical composition, and digestibility. The treatments included cactus pear (*Opuntia* spp.) at increasing levels of 0, 15, 30, 45, and 60% of the natural matter in the silage with BRS Capiacu grass, plus 10% ground corn grain. Additionally, a control silage composed of BRS Capiacu grass without ground corn was used. A completely randomized design was employed with six treatments and eight replications. The BRS Capiacu grass was harvested after 100 days of regrowth. For ensiling, experimental PVC silos of known weights and of 50 cm long and 10 cm in diameter were used. The breakdown of aerobic stability of the silage without cactus pear (BRS Capiacu grass with 10% ground corn) occurred 144 h after opening the silo. The inclusion of cactus pear in the silage with BRS Capiacu grass and 10% ground corn grain resulted in a linear increase in pH value. Among the silages with cactus pear inclusion, there was no significant difference in gas losses ($P = 0.46$) and dry matter (DM) recovery ($P = 0.43$). There was no significant difference between the silages in terms of acetic acid content ($P = 0.91$). The content of DM and total digestible nutrients was lower in the control silage compared with the other silages. For each percentage unit of cactus pear inclusion, there was a reduction of 0.1195% in DM content. Additionally, for every 1% inclusion of cactus pear in silage with BRS Capiacu grass, there was an increase of 0.2007 percentage units in DM digestibility. The optimal proportion of cactus pear in silage with BRS Capiacu grass and 10% ground corn is up to 60% of the natural matter.

Keywords: ammonia nitrogen, digestibility, dry matter losses, organic acids, semi-arid

1. Introduction

In several regions of the world, silage production is essential for feeding animals and ensuring the production of milk, meat, leather, and wool for human consumption. Traditionally, the standard diet of ruminants in Brazil is based on forage plants (Monção et al., 2020a; Alencar et al., 2023; Cordeiro et al., 2023). However, forage production is not constant throughout the year, highlighting silage production as an important agricultural activity on farms (Alencar et al., 2023; Souza et al., 2023), especially in the semi-arid region of Brazil, which can experience up to 10 months without rain (Monção et al., 2019; Cordeiro et al., 2023; Hora et al., 2023).

In various parts of the world, elephant grass (*Pennisetum purpureum* Schum.) has been used to produce silage (Borges et al., 2019; Monção et al., 2020a; Jesus et al., 2021; Ribas et al., 2021). Elephant grass is a perennial grass of African origin known for its high green mass yield per unit area, making it a valuable feed source for ruminant animals. In Brazil, well-managed tall elephant grass genotypes can produce over 30 t ha⁻¹ of dry matter (DM) annually. The BRS Capiapu grass, a hybrid of elephant grass, has a production potential of up to 72 t ha⁻¹ of DM, with an average of 50 t ha⁻¹ of DM (Pereira et al., 2017; Monção et al., 2019; Monção et al., 2020b).

Despite its high mass productivity, BRS Capiapu grass has a low DM content (less than 250 g kg⁻¹ DM) and water-soluble carbohydrates, limiting silage production at harvest time (Valadares Filho et al., 2018). Several strategies can increase the DM content in BRS Capiapu grass, such as wilting (Ribas et al., 2021), the use of dehydrated industrial waste (Silva et al., 2021), and addition of cereals such as ground corn (Paula et al., 2020). Ground corn (1-2 mm) with 880 g kg⁻¹ DM is a moisture-sequestering additive that can enhance the DM content and fermentative quality of BRS Capiapu grass silage (Paula et al., 2020; Monção et al., 2024); however, it has a low water-soluble carbohydrate content (less than 200 g kg⁻¹ DM) (Valadares Filho et al., 2018). Water-soluble carbohydrates are essential substrates for lactic acid bacteria to produce lactic acid, which, due to its low pKa, quickly reduces the pH of the ensiled mass, conserving its nutrients (Kung Jr. et al., 2018).

Cactus pear (*Opuntia* or *Nopalea*) is rich in water-soluble carbohydrates, minerals, and vitamins and can improve the production of mixed BRS Capiapu grass silage (Borges et al., 2019; Monção et al., 2020a; Pastorelli et al., 2022; Alencar et al., 2023). According to Cordeiro et al. (2023), cactus pear contains 100 g kg⁻¹ DM, 50 g kg⁻¹ of crude protein (CP), 650 g kg⁻¹ of total digestible nutrients, and 350 g kg⁻¹ of water-soluble carbohydrates. The use of cactus pear for producing mixed silage is still novelty in the literature and rural areas. However, incorporating cactus pear into silage can enhance labor efficiency for harvesting, improving cactus productivity and sanitary management. The optimal level of cactus pear inclusion for ensiling with BRS Capiapu grass is yet to be defined, given its high moisture content (mean of 844 g kg⁻¹ DM) (Dubeux Jr. et al., 2021). Monção et al. (2020a) found that crushed cactus pear increased water exposure, leading to higher effluent losses. However, when cactus pear was sliced/chopped (using a slicer designed for cactus), Alencar et al. (2023) and Cordeiro et al. (2023) found that a 40% inclusion of cactus pear in silage with BRS Capiapu grass reduced effluent production, as the water remained trapped in the mucilage. Several factors on farms justify the ensiling of forage plants such as BRS Capiapu grass and cactus pear. In the semi-arid region of Brazil, the high productivity of these forages supports storing feed for animals during specific seasons like the dry period. Furthermore, cactus pear is rich in water-soluble carbohydrates, which are substrates used by bacteria for rapid acidification of the ensiled mass, making one of the great advantages of using this ingredient in mixed silage of BRS Capiapu and ground corn.

Based on these findings, we hypothesize that there is an optimal proportion of sliced cactus pear to mix with BRS Capiapu grass and ground corn during ensiling, which will enhance the fermentative profile and nutritional value of the produced silage. Therefore, the objective was to evaluate the inclusion level of cactus pear in silage with BRS Capiapu grass and 10% ground corn (of natural matter [NM]) on the fermentative profile and nutritional value of the silage.

2. Material and methods

2.1. Ethics statement

The care and handling procedures for the animals used in the experiment followed the guidelines of the Ethics Committee on the Use of Animals (CEUA) (protocol no. 222/2020).

2.2. Experiment location

The experiment was conducted in the municipality of Janaúba (15°52'38" S, 43°20'05" W), Minas Gerais, Brazil. According to Alvares et al. (2014), the region is classified as Aw type, with summer rains and well-defined dry periods in winter lasting up to 10 months each year. The average annual precipitation is less than 800 mm, and the average annual temperature is 27 °C. The climate is tropical mesothermal, almost megathermal due to the altitude, sub-humid, and semi-arid, with irregular rainfall leading to extended periods of drought.

2.3. Treatments, experimental design, and forage management

Experimental silos were used to produce BRS Capiacu grass silages (*Pennisetum purpureum* Schum.) with varying levels of cactus pear cv. Gigante (*Opuntia ficus indica* Mill.) inclusion at the time of ensiling. The experiment followed a completely randomized design, with six treatments (0, 15, 30, 45, and 60% cactus pear inclusion) and eight replications. For the treatments with cactus pear inclusion, 10% of ground corn grain (1-2 mm; of the NM of the forage mass) was added. Additionally, the control treatment consisted of BRS Capiacu grass silage without any additives. All roughage feeds were obtained from the experimental farm.

The BRS Capiacu grass was established in 2016 in a flat area measuring 50 × 100 m. After cutting to standardize the BRS Capiacu grass at 10 cm above the ground using manual pruning, maintenance fertilization was applied with 100 kg ha⁻¹ of nitrogen in the form of urea and 60 kg ha⁻¹ of potassium in the form of potassium chloride through sprinkler fertigation. The height of BRS Capiacu grass at harvest was approximately 3.9 m after 102 days of regrowth.

The cactus pear variety Gigante was planted in 2014 in a 250 m² area of eutrophic red-yellow latosol soil. Harvesting was performed manually without removing the primary cladode.

2.4. Silage production

Experimental PVC silos of known weight and of 50 cm long and 10 cm in diameter were used in the ensiling process. A layer of dry coarse sand (500 g/silo) was placed at the bottom of the silos and separated from the forage by a non-woven fabric (100 weight) to drain the effluents produced. The material from each treatment was deposited in the silos and compacted with a wooden plunger. For each treatment, the silage had an average density of 550 kg of natural material m⁻³ (Ruppel et al., 1995), approximately 4 kg of chopped material from each fresh forage. After filling, the silos were closed with PVC lids equipped with Bunsen valves, sealed with adhesive tape, and weighed. The silos were stored at room temperature and opened 113 days after ensiling. No microbial inoculant was used.

2.5. Aerobic stability

Aerobic stability was assessed by placing approximately 2.5 kg of silage in a mini-silo and incubating it in a temperature-controlled room at 25.0 ± 0.5 °C. The silage temperature was monitored hourly for nine days using a data logger positioned in the center of the material. A separate data logger placed near the mini-silos recorded the ambient temperature hourly throughout the incubation period. Aerobic stability was defined as the time (hours) that the silage temperature remained within 2 °C of the ambient temperature, following the method of Moran et al. (1996).

2.6. Assessment of pH, ammonia nitrogen, and organic acids

Silage samples were obtained from furrows using a 16-ton mechanical press. The samples were measured for pH using a potentiometer (Tecnal[ ], Piracicaba, S o Paulo, Brazil). Ammonia nitrogen (N-NH₃) concentration was determined following the method of Detmann et al. (2021). Organic acids were analyzed by high-performance liquid chromatography (HPLC) (Shimadzu[ ] Prominence System model 20A, Kyoto, Japan) equipped with a UV-Vis detector set at 210 nm. An autosampler injected a calibrated volume of 5  L of the sample onto a 300   7.8 mm RezexTM ROA-Acid Column (Phenomenex) maintained at 60  C in an oven. Analytes were eluted with 2.5 mM H₂SO₄ at a flow rate of 0.6 mL/min. External calibration was performed using standard solutions of organic acids.

2.7. Silage dry matter losses and recovery

Dry matter losses in silage as gas and effluent were determined by weight difference following Jobim et al. (2007). Effluent losses were calculated using Equation 1:

$$E = (Wop - SWen) / (GREM) \times 1000, \quad (1)$$

in which E is the effluent production (kg/t green mass), Wop is the set weight (full bucket + lid + wet sand + foam) at silo opening (kg), SWen is the set weight (full bucket + lid + dry sand + foam) at ensiling (kg), and GREM = green forage mass ensiled (kg).

Gas losses (% DM) were calculated using Equation 2:

$$G = [(Wen - SWen) \times DMen] - [(Wop - SWen) \times DMop] \times 100 / [(Wen - SWen) \times DMen], \quad (2)$$

in which G are gas losses (%DM), Wen is the weight of the full bucket at ensiling (kg), DMen is the forage DM content at ensiling, and DMop = forage DM content at silo opening. For each silo, DM recovery was calculated based on the initial and final weights and forage and silage DM contents according to Jobim et al. (2007).

2.8. Chemical composition

A subsample of the silages was pre-dried in a forced-air oven at 55  C. Subsequently, all samples were ground in a knife mill using a 2-mm sieve. A portion of the 2-mm material was further ground to 1 mm for laboratory analyses. The 2-mm fraction was used for *in situ* incubation. The samples were analyzed for DM content (INCT-CA G-001/1 and G-003/1), CP (INCT-CA N-001/2), ether extract (INCT-CA G-004/1), ash (INCT-CA M-001/2), neutral detergent fiber (NDF; INCT-CA F-001/2), and acid detergent fiber (INCT-CA F-003/2), with appropriate corrections for ash (INCT-CA M-002/2) and proteins (INCT-CA N-004/2). Additionally, indigestible neutral detergent fiber (iNDF; INCT-CA F-008/2), levels of nitrogenous compounds insoluble in neutral detergent (PIDN) and in acid detergent (PIDA), lignin (INCT-CA F-005/2), and non-fibrous carbohydrates were analyzed, following the recommendations by Detmann et al. (2021). Total carbohydrates were determined according to Sniffen et al. (1992). Total digestible nutrients (TDN) content was estimated according to NRC (2001). The chemical composition of BRS Capi   grass, cactus pear, and ground corn before ensiling is shown in Table 1.

2.9. *In vitro* digestibility

In vitro digestibility of DM and NDF was assessed using the method of Tilley and Terry (1963) with a modification. The Tecnal[ ] TE-150 *in vitro* incubator (Piracicaba, SP, Brazil) was employed. Non-woven fabric bags (100 g m⁻²) measuring 10.0   10.0 cm were used instead of the standard bags described in the original method. The incubation mimicked ruminal digestion for 48 h, followed by a further 48 h of digestion with pepsin and a weak acid solution (low pH).

Table 1 - Chemical composition of BRS capia   grass, cactus pear, and corn grain ground before ensiling

Item (g kg ⁻¹ as-fed basis)	BRS capia�� grass ¹	Cactus pear ²	Ground corn
Dry matter	226.0	100.2	908.0
Ash	107.8	143.0	17.0
Crude protein	78.0	51.0	68.0
Ether extract	11.0	19.1	45.0
Neutral detergent fiber	704.0	290.8	166.0
Acid detergent fiber	441.0	184.2	29.7
Lignin	83.0	22.5	27.7
Indigestible neutral detergent fiber	410.0	180.0	100.0
Total carbohydrates	803.2	786.9	870.0
Non-fibrous carbohydrates	89.6	496.1	704.0
Total digestible nutrients ³	500.0	630.0	861.0

¹ Harvest after 102 days of regrowth.² *Opuntia ficus indica* Mill harvested after 512 days of regrowth.³ Estimated according to NRC (2001).

2.10. Statistical analyses

Data on fermentative profile, organic acids, chemical composition, and *in vitro* digestibility were analyzed using analysis of variance (ANOVA) and regression techniques using the SISVAR[®] software, as follows:

$$Y_{ij} = \mu + t_i + e_{ij}$$

in which Y_{ij} = observed value for the variable i in relation to the treatment in the j repetition; μ = average of all experimental units for the variable under study; t_i = effect of treatment i on the observation value Y_{ij} ; e_{ij} = error associated with the independent Y_{ij} observation, which, based on the hypothesis, has a normal distribution with mean 0 and variance δ^2 .

Inclusion levels of spineless cactus into BRS Capi   grass silage were compared by decomposing the sum of squares into orthogonal linear contrasts and quadratic effects, at a 5% probability level, with subsequent adjustments of the regression equations. Mean values were considered different when $\alpha < 0.05$. For exploratory data analysis, principal component analysis (PCA) was performed using PAST[®] 4.03 software (Hammer et al., 2001).

3. Results

A significant interaction ($P < 0.01$) was observed between silage type and time after opening the silos on temperature values (Figure 1). The temperatures of the silages at 0, 24, 48, 72, and 96 h, regardless of cactus inclusion, were similar, averaging 25.26, 25.26, 25.79, 25.73, and 25.86   C, respectively. At 120, 144, and 168 h, the control silage had a higher temperature (mean of 26.67   C) compared with the others (average of 25.72   C). Aerobic stability of the silage without cactus pear (BRS Capi   grass with 10% ground corn) broke down 144 h after opening the silo. Silages with BRS Capi   grass, 10% ground corn, and varying levels of cactus lost stability 192 h after opening.

After 240 h of aerobic exposure, there was a difference in the pH of the silage. For each 1% increase in cactus pear inclusion in the silage with BRS Capi   grass, the pH of the ensiled mass increased by 0.012 units (Figure 2).

There was no difference between the silages within the aerobic exposure times (Figure 3). As the aerobic exposure times increased, the pH values changed. For every hour after opening the silo, the pH value increased by 0.04 units.

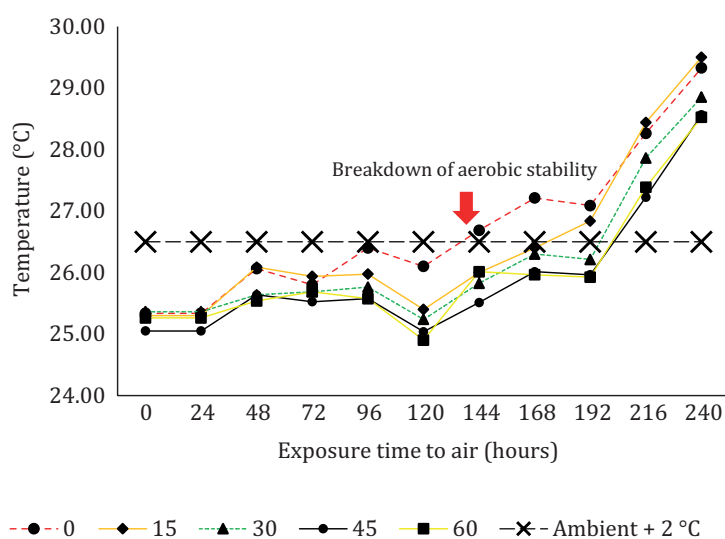


Figure 1 - Average temperatures during aerobic stability of BRS Capi   grass silage with 10% ground corn grain and increasing levels of cactus pear after various times of silo opening (interaction between treatments and times after opening; $P < 0.01$).

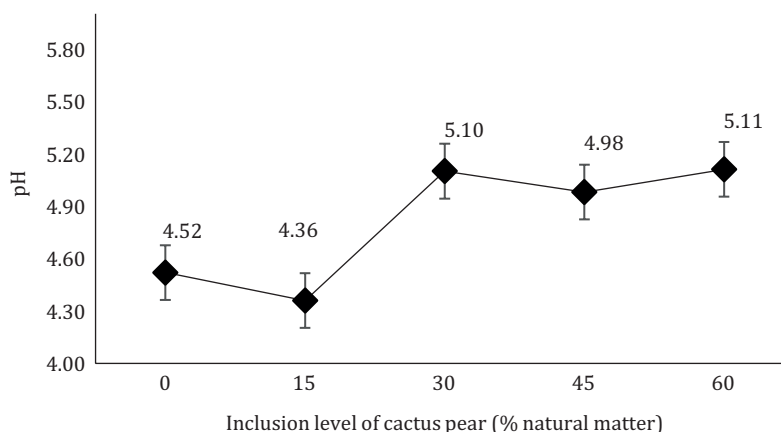


Figure 2 - Average pH values after 240 h of aerobic exposure of BRS Capi   grass silages with 10% ground corn and increasing levels of cactus pear ($\hat{Y} = 4.4 + 0.012X$; $R^2 = 0.67$).

No significant difference in pH values ($P > 0.05$) or gas losses ($P > 0.05$) was observed between BRS Capi   grass silage (control, without additives) and BRS Capi   grass silage with 10% ground corn and various levels of cactus pear. However, the inclusion of cactus pear in the silage with BRS Capi   grass and 10% ground corn resulted in a linear increase in pH value ($P < 0.01$). Additionally, there was an increase in ammonia nitrogen content ($P < 0.01$) and effluent losses ($P < 0.01$; Table 2). For each percentage unit of cactus pear inclusion, pH increased by 0.02 units, ammonia nitrogen by 0.27%, and effluent losses by $1.39 \text{ kg t}^{-1} \text{ NM}$. The BRS Capi   grass silages with 30, 45, and 60% cactus pear showed greater ammonia nitrogen losses compared with the control silage ($P < 0.05$). Among the silages with cactus pear inclusion, there was no difference in gas losses ($P = 0.46$; mean of 8.76% of DM) or DM recovery ($P = 0.43$; average of 97.70%).

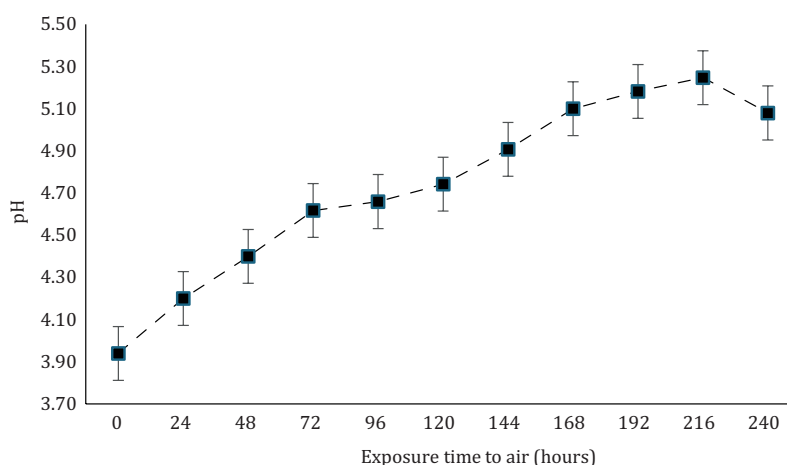


Figure 3 - pH values of BRS Capiaçú grass silage with 10% ground corn and increasing levels of cactus pear after different silo opening times ($\hat{Y} = 4.3 + 0.004X$; $R^2 = 0.74$).

Table 2 - Fermentation profile of BRS Capiaçú grass silages with 10% ground corn grain and increasing levels of cactus pear (*Opuntia* spp.)

Item	Control	Cactus pear inclusion level (% NM)					SEM	P-value	
		0	15	30	45	60		L	Q
pH ^{1,2}	4.25	3.23	3.48	4.17	4.37	4.42	0.18	<0.01	0.47
Ammoniacal nitrogen (%TN) ³	7.12	10.23	7.45	15.78*	20.64*	24.57*	2.47	<0.01	0.29
Gas losses (%) DM	10.03	7.83	7.16	10.71	8.99	9.11	1.34	0.31	0.46
Effluent losses (kg t ⁻¹ NM) ⁴	63.29	17.19*	21.87*	53.00	83.70*	90.80*	5.60	<0.01	0.83
DM recovery (%)	91.97	98.06 *	98.29*	97.12 *	97.07*	97.96*	0.57	0.43	0.26

TN - total nitrogen; DM - dry matter; NM - natural matter; SEM - standard error of the mean.
 * Means differ from the control silage (BRS Capiaçú grass silage without ground corn) by Dunnet's test (P<0.05).
¹ pH values at the opening of silos.
² $Y = 3.28 + 0.0218X$, $R^2 = 0.9033$.
³ $Y = 7.35 + 0.2793X$, $R^2 = 0.8720$.
⁴ $Y = 11.50 + 1.3936X$, $R^2 = 0.9455$.

The control silage had a higher concentration of lactic acid compared with treatments with 30, 45, and 60% cactus pear inclusion. Including cactus pear in the silage with BRS Capiaçú grass altered the concentration of organic acids in DM (Table 3). For every 1% increase in cactus pear inclusion, the lactic acid content decreased by 0.12 percentage units (P<0.01). There was no difference in acetic acid content between the silages (P = 0.91; average of 2.22% of DM). The butyric acid content (P = 0.02) followed a quadratic regression model, peaking at 43.35% cactus inclusion. The lactic:acetic acid ratio decreased linearly (P<0.01) by 0.0562% for each percentage of cactus pear inclusion. The ethanol content (P = 0.01) in the silages showed a quadratic regression behavior (maximum point at level 55.16%) with the inclusion of cactus.

The contents of DM, non-fibrous carbohydrates (NFC), total digestible nutrients (TDN), *in vitro* DM digestibility, and *in vitro* neutral detergent fiber digestibility were lower in the control silage compared with the other silages (Table 4). There was a linear effect for all analyzed variables regarding chemical composition. The contents of ash (P<0.01), NFC (P = 0.04), and TDN (P<0.01) showed an increasing linear effect, with increases of 0.0416, 0.2255, and 0.2353%, respectively, for each percentage unit of cactus pear inclusion in the silage with BRS Capiaçú grass. For each percentage unit of cactus pear inclusion, there was a reduction of 0.1195% in DM content, 0.0081% in CP, 0.2591% in NDF content, 0.2725% in NDF corrected for ash and protein, 0.1963% in acid detergent fiber content, and 0.0337% in total carbohydrates.

Table 3 - Organic acid contents of BRS capiaçu grass silage with increasing levels of cactus (*Opuntia* spp.)

Item (% DM)	Control	Cactus pear inclusion level (% NM)					SEM	P-value	
		0	15	30	45	60		L	Q
Lactic acid ¹	7.56	7.68	9.49	4.34*	3.44*	1.56*	0.87	<0.01	0.34
Acetic acid ²	1.67	2.19	2.45	1.85	2.58	2.07	0.27	<0.01	<0.01
Butyric acid ³	2.59	1.04*	0.94*	2.42	2.07	1.72*	0.26	<0.01	0.02
Lactic-acetic ratio ⁴	4.60	3.66	3.85	2.06*	1.41*	0.65*	0.33	<0.01	0.54
Ethanol ⁵	0.62	2.43	1.71	5.90*	5.28*	5.02*	0.75	0.06	<0.01

DM - dry matter; NM - natural matter; SEM - standard error of the mean.

* Means differ from the control silage (BRS Capiçu grass silage without ground corn) by Dunnet's test (P<0.05).

¹ Y = 8.96 - 0.1219X, R² = 0.8042.² Y = 0.81 + 0.0607X - 0.0007X², R² = 0.6123.³ Y = 4.01 - 0.0562X, R² = 0.9147.⁴ Y = 2.32 + 0.0585X, R² = 0.5482.⁵ Y = 1.7623 + 0.1324X - 0.0012X², R² = 0.6252.**Table 4 - Chemical composition and nutrient digestibility of BRS Capiçu grass silage with increasing levels of cactus (*Opuntia* spp.)**

Item	Control	Cactus pear inclusion level (% NM)					SEM	P-value		
		0	15	30	45	60		L	Q	
Chemical composition (%)										
Dry matter ¹	22.66	29.57*	28.09*	24.73*	23.75*	22.78	0.61	<0.01	0.14	
Ash ²	11.17	7.27*	5.90*	8.58*	9.06*	8.81*	0.72	<0.01	0.45	
Crude protein ³	7.46	8.39	8.22	8.00	8.18	7.80	0.15	0.02	0.96	
Neutral detergent fiber ⁴	71.35	49.83*	45.20*	43.31*	39.33*	33.33*	1.61	<0.01	0.17	
NDFap ⁵	61.24	39.72*	36.82*	32.86*	28.55*	23.42*	1.51	<0.01	0.41	
Acid detergent fiber ⁶	45.63	30.51*	28.31*	26.99*	22.59*	18.65*	1.14	<0.01	0.13	
Hemicellulose ⁷	25.71	19.32*	16.88*	16.31*	16.74*	14.68*	0.82	0.01	0.57	
Total carbohydrates ⁸	80.27	81.59	83.11*	80.66	79.99	80.63	0.75	0.04	0.99	
Non-fibrous carbohydrates ⁹	8.92	31.74*	37.91*	37.34*	40.65*	47.29*	1.69	<0.01	0.45	
Total digestible nutrients ¹⁰	56.67	62.38*	65.66*	64.91*	71.86*	76.93*	2.28	<0.01	<0.01	
In vitro digestibility (%)										
IVDMD ¹¹	55.20	60.06*	62.86*	62.22*	68.15*	72.47*	1.94	<0.01	0.19	
IVNDFD	53.61	58.48*	61.76*	59.88*	65.81*	65.61*	2.05	0.41	0.68	

MN - natural matter; NDFap - neutral detergent fiber corrected for ash and protein; IVDMD - in vitro dry matter digestibility; IVNDFD - in vitro neutral detergent fiber digestibility; SEM - standard error of the mean.

* Means differ from the control silage (BRS Capiçu grass silage without ground corn) by Dunnet's test (P<0.05).

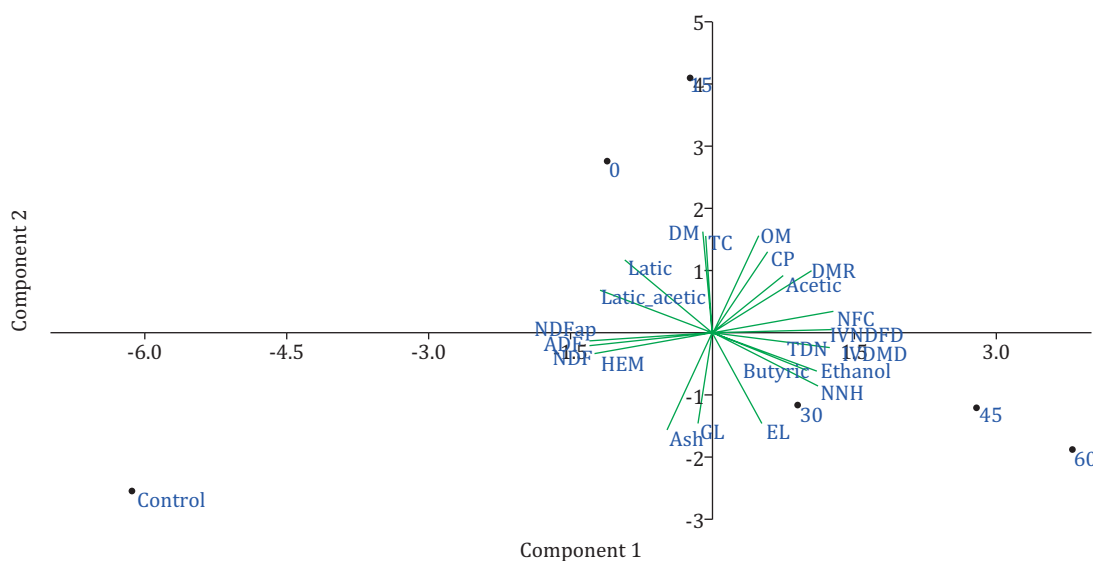
¹ Y = 29.368 - 0.1195X, R² = 0.9466.² Y = 6.68 + 0.0416X, R² = 0.5553.³ Y = 8.362 - 0.0081X, R² = 0.7322.⁴ Y = 49.98 - 0.2591X, R² = 0.9724.⁵ Y = 40.458 - 0.2725X, R² = 0.9901.⁶ Y = 31.304 - 0.1963X, R² = 0.9567.⁷ Y = 18.674 - 0.0627X, R² = 0.8018.⁸ Y = 82.208 - 0.0337X, R² = 0.4309.⁹ Y = 32.224 + 0.2255X, R² = 0.8942.¹⁰ Y = 61.296 + 0.2353X, R² = 0.8855.¹¹ Y = 59.138 + 0.2007X, R² = 0.8855.

The means for *in vitro* DM digestibility (P<0.01) fit an increasing linear regression model. For every 1% inclusion of cactus pear in the silage with BRS Capiçu grass, DM digestibility increased by 0.2007 percentage units. There was no difference among the silages regarding the *in vitro* NDF digestibility (P = 0.68; average of 62.30%).

Regarding the principal component analysis (PCA), the Scree plot indicated that among the 22 principal components (PC) generated, five had variances above 1%. The two most important PC explained 87.11% of the total variation in the results. The highest eigenvalues from the correlation matrix were observed in PC1 (56.43%) and PC2 (33.65%). The dependent variables DM recovery, CP, and acetic acid content had higher positive coefficients within the PC1 and PC2 sets (Figure 4). BRS Capiçu grass silage with 45 and 60% cactus showed higher eigenvalues for variables ammonia nitrogen (0.2406),

effluent losses (0.1121), *in vitro* DM digestibility (0.2673), and *in vitro* NDF digestibility (0.2714). There was a negative correlation between DM content and both effluent losses and ammonia nitrogen content. Additionally, there was a negative correlation between NDF content and both *in vitro* DM digestibility and TDN content.

In the cluster analysis, three groups with greater Euclidean distance were identified. Silages with 45 and 60% cactus inclusion exhibited similar behavior to each other, as did silages with 0, 15, and 30% cactus inclusion (Figure 5).



DMR - dry matter recovery; GL - gas losses; EL - effluent losses; NNH - ammoniacal nitrogen; DM - dry matter; OM - organic matter; CP - crude protein; NDF - neutral detergent fiber; NDFap - neutral detergent fiber corrected for ash and protein; ADF - acid detergent fiber; HEM - hemicellulose; TC - total carbohydrates; NFC - non-fibrous carbohydrates; TDN - total digestible nutrients; IVDMD - *in vitro* dry matter digestibility; IVNDFD - *in vitro* neutral detergent fiber digestibility; lactic - lactic acid; acetic - acetic acid; butyric - butyric acid; Lactic_acetic - lactic:acetic acid ratio.

Figure 4 - Schematic representation of the first (PC1; 56.43%) and second (PC2; 33.65%) principal components for the evaluated characteristics.

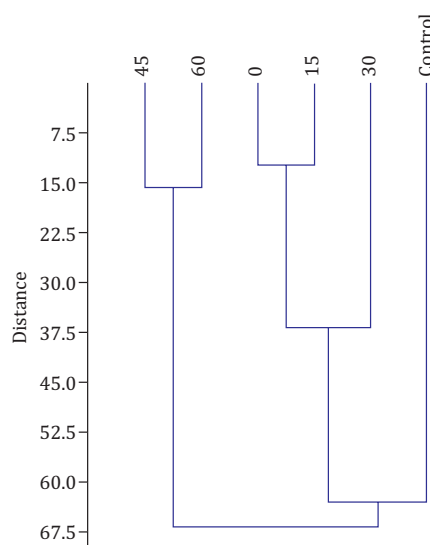


Figure 5 - Cluster analysis of BRS Capi   grass silages without and with 10% ground corn grain and increasing levels of cactus pear (*Opuntia* spp.).

4. Discussion

Ensiling BRS Capiaçú grass with cactus pear concentrates labor during the harvest, standardizes regrowth, and increases mass productivity. It also reduces the need for cutting and supplying cactus throughout the dry period (Borges et al., 2019; Brito et al., 2020; Cordeiro et al., 2023). The harvesting of BRS Capiaçú grass is mechanized, which simplifies cutting management. However, the appropriate proportion of these forages in mixed silage must be evaluated, as an excess or lack of cactus in silage with tropical grasses can result in nutrient losses and reduced aerobic stability (Bruto et al., 2020; Monção et al., 2020b; Alencar et al., 2023).

In our study, adding cactus pear in silage with BRS Capiaçú grass increased the time before aerobic stability of silage broke down. This longer time for breakdown can be attributed to the concentration of acetic acid and the lower lactic:acetic acid ratio due to the heterolactic fermentation caused by the increases in pH with the inclusion of cactus pear, which influenced aerobic stability. We found no difference in acetic acid content among the silages (mean of 21 g acetic acid/kg silage DM). According to Gerlach et al (2021), a certain amount of acetic acid is desirable in silage to minimize growth of yeasts and molds during aerobic exposure such that target values of 10–30 g acetic acid/kg silage DM are often recommended (Kung et al., 2018). Thus, factors such as the concentration of propionic acid (Bruto et al., 2020), antimicrobial substances present in cactus mucilage (Ortiz-Rodríguez et al., 2016), and lactic:acetic acid ratio may also explain the longer aerobic stability and increased pH in silages with higher proportions of cactus.

A highlight finding in this study is that the inclusion of 10% ground corn in the BRS Capiaçú grass silage increased DM recovery compared with the control silage. This greater recovery may be due to the moisture-sequestering effect of the ground corn, which has a higher DM content. Paula et al. (2020) also observed improved DM recovery in BRS Capiaçú grass silage with the inclusion of ground corn, also due to the high DM content.

The inclusion of cactus pear in the silage with BRS Capiaçú grass increased the pH at the time of the silo opening. Cactus pear is rich in minerals, which in ionizable form (K^+ , Ca^{2+} , and Mg^{2+}) have a high buffering capacity, preventing significant pH variation in the ensiled mass (Levic et al., 2005). This result is especially evident when observing the ash content. Normally, the pH of forage plants at the time of ensiling is close to neutral, and it is expected to drop to values between 3.8 and 4.2 during the anaerobic phase (Kung Jr. et al., 2018). In cactus pear silage, the cations (K^+ , Ca^{2+} , and Mg^{2+}) interact with the organic acids formed by fermentation, neutralizing them and preventing a pH drop (Bruto et al., 2020; Dubeux Jr. et al., 2021). However, the observed pH values (3.23 to 4.42) are considered adequate for well-fermented silages (McDonald et al., 1991).

In addition to pH, the ammonia nitrogen ($N-NH_3$) content of the silage is an indicator of the fermentation process. The $N-NH_3$ content increased linearly with the inclusion of cactus, indicating nitrogen losses from the ensiled mass. These losses occur via secondary fermentations. According to McDonald et al. (1991), high concentrations of total $N-NH_3$ in silages are values above 10%, which indicate poorly fermented silages due to a slow pH drop during the anaerobic phase. This condition was observed in silage with cactus inclusion above 30%, in which proteolytic bacteria decompose amino acids from the silage protein, releasing $N-NH_3$ into the effluents. The ammonia nitrogen content is an indicator of proteolytic and clostridial activity, contributing to an increased pH (Bruto et al., 2020). Despite the high ammonia nitrogen losses in silages with more than 30% cactus inclusion, the CP content did not reduce markedly, and the silage with 60% cactus pear had a lower CP in value.

There were no differences in gas losses (average of 8.76% of DM), but silages with more than 45% cactus pear inclusion showed more than 83.70 kg t^{-1} NM of effluent losses. The DM losses in silage are not desirable; however, in tropical grass silages, values close up to 80 kg t^{-1} NM are common (Santos et al., 2013; Brito et al., 2020; Monção et al., 2020a; Ribas et al., 2021; Alencar et al., 2023). Alencar et al. (2023) reported effluent losses of 78.70 kg t^{-1} NM in BRS Capiaçú grass silage with 40% forage cactus, while Monção et al. (2020a) observed losses of 61.24 kg t^{-1} NM in BRS Capiaçú grass silage with 20%

crushed cactus pear. High effluent losses indicate bacterial activity from the *Clostridium* genus, which ferments nutrients into butyric acid. The high values of butyric acid in the control silage may be due to the low DM content (22.66%).

Regarding the concentration of organic acids in silages, there was a linear reduction in lactic acid and the lactic:acetic acid ratio. According to Kung Jr. et al. (2018), forage plant silages should have lactic acid levels between 6 and 10% of DM. In this research, the highest concentrations of lactic acid were observed in silage with 15% cactus inclusion, while the lowest values were found in silage with 60% inclusion. After closing the silo, lactic acid synthesis should ideally reach close to 10% of DM to quickly reduce the pH of the ensiled mass. These characteristics indicate that the forage cactus, with its high NFC content (around 60% of DM), provided energy for the lactic acid bacteria to produce lactic acid (pKa value of 3.86), favoring the rapid reduction of pH, which is important for nutrient conservation in the ensiled mass (Kung Jr. et al., 2018). It was also found that NFC was used for ethanol synthesis in silages with more than 30% cactus pear inclusion. Epiphytic yeasts primarily follow the pyruvate decarboxylase and acetaldehyde pathway for ethanol synthesis. According to McDonald et al. (1991), ethanol production represents DM and energy losses in silage. Although ethanol can serve as an energy substrate for ruminants by converting to acetate in the rumen (Chalupa et al., 1964), some ethanol is lost during storage (Alli et al., 1982) and unloading from the silo.

The inclusion of 10% ground corn (1-2 mm) increased the DM content by 13.10% compared with control silage versus silage with 0, 15, 30, and 45% cactus pear inclusion. The DM content of the ensiled mass is one of the most crucial factors in fermentative capacity. BRS Capiapu grass at harvest for silage has a DM content between 18 and 22%. However, high DM losses in the form of effluents are observed, especially when storage time exceeds 120 days. Including 10% ground corn was crucial for ensiling BRS Capiapu grass, increasing the DM content to 29.57%. With 45% cactus pear inclusion, the DM content was 23.75%. For Kung Jr et al. (2018), in tropical grasses, the ideal DM content at the time of ensiling is at least 25% to avoid greater DM losses. According to Ribas et al. (2021), wilting BRS Capiapu grass for 30 h in the sun during manual harvesting is an additional option to reduce moisture in the harvested material. However, it is possible to produce BRS Capiapu grass silage with DM content above 23% with an adequate fermentation profile (Brito et al., 2020; Alencar et al., 2023). Thus, the inclusion of up to 60% cactus in the silage with BRS Capiapu grass and 10% ground corn met the DM content requirement and improved the TDN content of the produced silage. The ash, NFC, and TDN contents and digestibility increased linearly with the inclusion of cactus pear. This is justified because cactus is rich in minerals and NFC (Dubeux Jr. et al., 2021; Cunha et al., 2022).

Based on the exploratory analysis of the data, several dependent variables were identified as having greater relevance in decision-making regarding the best proportion of cactus inclusion in silage with BRS Capiapu grass. Mixed BRS Capiapu grass silage with 10% ground corn and up to 45% cactus inclusion showed higher coefficients in decision-making. Furthermore, BRS Capiapu grass silage with 45% cactus exhibited better DM and NDF digestibility, which are key factors in the exploratory data analysis. The cluster analysis revealed that silage with 45 and 60% cactus inclusion had a smaller Euclidean distance, indicating similar characteristics of the dependent variables. Although BRS Capiapu grass silage with 60% cactus pear presented greater DM losses in the form of effluents, it had the best DM and nutrient digestibility results. Furthermore, no difference was observed in DM recovery compared with the other silages, the most important thing. The multivariate analysis also showed a similarity between treatments with 45 and 60% cactus pear inclusion, which justifies the use of up to 60% cactus pear in BRS capiapu grass silage.

5. Conclusions

The optimal proportion of cactus pear in silage with BRS Capiapu grass and 10% ground corn is up to 60% of the NM. At this proportion, fermentative losses are acceptable, and the inclusion of cactus enhances the chemical composition and digestibility of the silage produced. The use of 10% ground corn in BRS Capiapu grass silage improves the fermentative profile, chemical composition, and digestibility of dry matter and neutral detergent fiber.

Data availability

The data supporting this study will be shared upon reasonable request to the corresponding author.

Author contributions

Conceptualization: Monção, F. P. and Rocha Júnior, V. R. **Formal analysis:** Monção, F. P.; Rocha Júnior, V. R.; Leal, D. B.; Rigueira, J. P. S.; Silva, R. K. O. J.; Antunes, A. B.; Caldeira, L. A.; Carvalho, C. C. S. and Santos, A. S. **Funding acquisition:** Monção, F. P. and Rocha Júnior, V. R. **Investigation:** Leal, D. B.; Silva, R. K. O. J. and Antunes, A. B. **Methodology:** Monção, F. P.; Rocha Júnior, V. R. Leal, D. B.; Rigueira, J. P. S.; Silva, R. K. O. J.; Antunes, A. B.; Caldeira, L. A.; Carvalho, C. C. S. and Santos, A. S. **Project administration:** Monção, F. P. **Resources:** Monção, F. P.; Rocha Júnior, V. R.; Caldeira, L. A.; Carvalho, C. C. S. and D'Angelo M. F. S. V. **Visualization:** Monção, F. P.; Rocha Júnior, V. R. and Santos, A. S. **Writing – original draft:** Monção, F. P.; Rocha Júnior, V. R.; Leal, D. B. and Rigueira, J. P. S. **Writing – review & editing:** Monção, F. P. and Rocha Júnior, V. R.

Conflict of interest

The authors declare no conflict of interest.

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