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# Optimizing silage quality in drylands: Corn stover and forage cactus mixture on nutritive value, microbial activity, and aerobic stability

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ABSTRACT

The study aimed to determine the optimal ratio for the mixture of forage cactus and corn stover silage, emphasizing the evaluation of fermentative profile, microbial populations, dry matter losses, chemical composition, and aerobic stability. A completely randomized design with four treatments and five replications was employed in this study. Treatments included varying proportions of forage cactus in corn stover at ensiling (0%, 10%, 20%, and 40% of fresh matter). After a 100-day fermentation period, the assayed variables underwent regression analysis. Forage cactus inclusion led to linear dry matter and ether extract reductions, with crude protein showing a negative quadratic effect ($p < 0.05$). The pH and water-soluble carbohydrates exhibited negative quadratic effects with the increasing proportion of forage cactus ($p < 0.05$). Lactic and acetic acids exhibited quadratic effects, reaching their peaks at approximately 20% forage cactus ($p < 0.05$). Lactic acid bacteria demonstrated a linear decrease, while yeasts/molds displayed a negative quadratic effect ($p < 0.05$). Dry matter losses followed a quadratic pattern, with the minimum values observed at 20% forage cactus ($p < 0.05$). Additionally, the inclusion of forage cactus significantly enhanced aerobic stability ($p < 0.05$). The incorporation of 20% forage cactus into corn stover silage markedly improved its quality.

## 1. Introduction

Intensive livestock production systems in semiarid regions heavily rely on silage as a primary source of nutrition for ruminants. Corn (*Zea mays* L.) stands out as the most widely cultivated crop for silage production, a practice common in dryland areas across various countries (Mounce et al., 2016; Nilahyane et al., 2018). In Brazil, for instance, corn cultivation covers 19.6 million hectares, and the semiarid region contributes approximately 14% to this overall cultivation. However, corn yields average only 3.25 tons per hectare, a 30% reduction compared to the national average (IBGE, 2023). Consequently, total corn biomass production under these climatic conditions amounts to only 2.88 million tons of dry matter, significantly lower than the national average of 6.2 million tons of dry matter (IBGE, 2023).

Corn is cultivated during the rainy season, which lasts approximately three months, and receives an average rainfall of 400–700 mm (Alvares et al., 2013; Chimeli et al., 2008). However, constrained water availability hinders the crop's potential growth, leading to diminished production. When harvested at the optimal stage for silage, characterized by milky/dough-stage grain, it yields high-quality silage with well-known fermentation end-products (Kung et al., 2018).

Following grain harvest, substantial amounts of corn stover remain in the field, necessitating removal for the next crop cycle. This agricultural practice enhances soil quality, subsequently improving crop performance (Battaglia et al., 2021). The high yield of corn stover renders it an excellent roughage source for animal production systems.

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Nevertheless, any delay in the harvest can significantly decrease its nutritional value (Gao et al., 2019; Yan et al., 2019).

Gao et al. (2019) experimented to assess the impact of delaying grain harvest on corn stover quality over a period of 0–60 days. The authors reported that cutting the material for ensiling on the same day as grain harvest yielded significantly better nutritional value than at any other time. A 7-day delay led to increased dry matter (from 219 to 357 g $kg^{-1}$ DM), neutral detergent fiber (from 645 to 707 g $kg^{-1}$ DM), lignin (from 49 to 60 g $kg^{-1}$ DM), and a reduction in crude protein (from 90 to 59 g $kg^{-1}$ DM) and water-soluble carbohydrate contents (from 84 to 47 g $kg^{-1}$ DM). Ensiling this material revealed a good fermentative profile. However, it also led to significantly reduced metabolizable energy and dry matter digestibility (Gao et al., 2019), indicating potential negative impacts on animal performance (Åby et al., 2019).

In light of these constraints, the utilization of nutritional additives emerges as a primary solution. Nutritional additives, which can be forages or concentrates, are unsuitable for sole ensiling but enhance fermentation and nutritional value when mixed with other crops (Macêdo et al., 2018; Nogueira et al., 2019; Sá et al., 2020; Silva et al., 2023).

The forage cactus (*Opuntia stricta* Haw.) thrives in dryland regions due to the crassulacean acid metabolism (Edvan et al., 2020) and serves as a valuable additive for silage production (Macêdo et al., 2018; Santos et al., 2020). Its high moisture (150 g $kg^{-1}$ DM), non-fiber carbohydrates (590 g $kg^{-1}$ DM), and water-soluble carbohydrate contents (150 g $kg^{-1}$ DM) create ideal conditions for lactic acid production (Brito et al., 2020). In regions affected by water scarcity, employing forage cactus for ensiling offers the dual benefit of improving silage quality and providing high-quality water for animals. Some studies have reported a substantial decrease in water consumption by animals when forage cactus is included in their diets (Silva et al., 2021; Tegegne et al., 2007).

Furthermore, several reports have indicated that forage cactus increases the aerobic stability and dry matter recovery of the silages (Brito et al., 2020; Nogueira et al., 2019; Pereira et al., 2021). This attribute is advantageous for any silage, as it mitigates the risk of spoilage. Combining corn stover silage with forage cactus (*in natura*) has been explored to reduce dependence on corn grain as an energy source (Moraes et al., 2019).

We hypothesize that the addition of forage cactus to corn stover silage improves the fermentative profile, microbial populations, DM losses, chemical composition, and aerobic stability. The objective of this study was to establish the optimal ratio for mixed forage cactus-corn stover silage in terms of fermentative profile, microbial populations, DM losses, chemical composition, and aerobic stability.

## 2. Material and methods

### 2.1. Experimental site

The experiment was conducted at the Forage Crop Laboratory, located in Areia, Paraiba, at the Agricultural Science Center of Paraiba Federal University (06° 57′48″ S latitude, 35° 41′30″ W longitude, and 618 m of altitude). The climate in this region is classified as 'As,' denoting a sub-humid tropical climate with a rainy winter (Alvares et al., 2013). The annual precipitation is 1358.4 mm, with an average temperature of 24 °C (INMET, 2021).

The crops were cultivated on a private farm in São José dos Cordeiros, Paraiba (7°23′27″ S latitude, 36°48′28″W longitude, and 529 m of altitude). The climate in this region is classified as 'BSh,' indicative of a semiarid climate with hot winters and rainy summers, according to Alvares et al. (2013). The rainy season in this area has an annual precipitation of 551.7 mm and an average temperature of 24 °C (INMET, 2021).

### 2.2. Experimental design

The experiment was conducted in a completely randomized design comprising four treatments and five replications, yielding 20 experimental units. Treatments included varying proportions of forage cactus (FC) in corn stover (CS) at ensiling to produce the mixed silages, expressed on fresh matter (FM). These proportions were 0%, 10%, 20%, and 40% of FC.

### 2.3. Ensiling

The grain corn (*Zea mays* L.) cultivar BRS 2022 was sown on August 1, 2021, and harvested approximately 90 days later, on October 29, 2021. At the harvest, the corn was at the two-thirds milk line stage of grain development, representing approximately 75% humidity. The corn stover, containing non-viable ears that remained on the plants, was manually cut for ensiling one day after grain harvest. Forage cactus (*Opuntia stricta* Haw.) cv. Orelha de Elefante Mexicana was rainfed and harvested after two years of regrowth. During harvest, the mother cladode and one secondary cladode were left in the field to facilitate subsequent regrowth.

The forages were individually chopped using a forage machine (PP-35, Pinheiro Máquinas®, Itapira, SP, Brazil) to achieve a particle size of approximately 2 cm. The chemical composition of the forages before ensiling is detailed in Table 1. Subsequently, the forages were mixed according to their respective treatment proportions and ensiled using wooden sockets placed in polyvinyl chloride tubes measuring 30 cm in height and 15 cm in width. To facilitate effluent drainage, 0.5 kg of sand was positioned at the bottom of all silos, separated from the ensiled material by a non-woven fabric.

Each silo was covered with a lid equipped with an adapted Bunsen valve to release gases produced during fermentation. As a result of incorporating forage cactus with its elevated moisture content, the densities varied within the range of 500–700 kg $m^{-3}$. The fermentation process proceeded for 100 days.

### 2.4. Losses of dry matter

The gas losses (GL), effluent losses (EL), and dry matter losses (DM loss) were quantified by measuring the weight difference between the ensiling moment and the time of opening. The estimation of these variables was conducted using the equations proposed by Jobim et al. (2007):

**Table 1**
Mean values of chemical composition and microbial population of the forage cactus, corn stover and their mixture prior to ensiling.

| Item | Forage cactus | Forage cactus inclusion (% FM) | | | | SEM |
|---|---|---|---|---|---|---|
| | | 0 | 10 | 20 | 40 | |
| **Chemical composition (g $kg^{-1}$ DM)** | | | | | | |
| Dry matter | 100.3 | 284.7 | 296.5 | 283.8 | 226.3 | 16.9 |
| Organic matter | 842.8 | 901.5 | 911.6 | 876.9 | 852.1 | 7.2 |
| Crude protein | 57.0 | 56.8 | 58.3 | 46.7 | 50.8 | 1.3 |
| Ether extract | 16.1 | 25.4 | 33.1 | 26.7 | 22.0 | 2.3 |
| $NDF^{a}_{om}$ | 280.2 | 640.7 | 534.6 | 515.0 | 614.5 | 20.7 |
| $ADF^{b}_{om}$ | 98.7 | 292.1 | 297.3 | 261.9 | 295.3 | 36.7 |
| Total carbohydrates | 769.7 | 819.3 | 820.2 | 803.5 | 779.3 | 6.0 |
| Non-fibre carbohydrate | 489.5 | 178.6 | 285.6 | 288.5 | 164.8 | 18.4 |
| WSC (g $kg^{-1}$ DM)[c] | 150.6 | 199.4 | 252.6 | 257.8 | 252.7 | 13.2 |
| **Microbial population (log UFC $g^{-1}$)** | | | | | | |
| Lactic acid bacteria | 6.55 | 8.47 | 8.48 | 8.50 | 7.89 | 1.94 |
| Molds and yeasts | 5.25 | 8.48 | 5.34 | 5.47 | 4.27 | 1.25 |

FM: fresh matter basis; $NDF^{'}_{om}$ Neutral detergent fibre expressed exclusive of residual ash; ADF: Acid detergent fibre expressed exclusive of residual ash; WSC: Water-soluble carbohydrates; SEM: standard error of the mean.

$$GL\ (\%\ DM) = \frac{PCf - PCa}{(MFf \times MSf)} \times 10000 \quad \text{Eq. (A.1)}$$

Where PCf is the weight of the full silo at sealing (kg), PCa is the weight of the full silo at opening (kg), MFf is the forage mass at sealing (kg), and MSf is the forage DM concentration at closing.

Effluent losses were determined by assessing the weight difference before ensiling and after the fermentation period when the silo was emptied. To facilitate effluent drainage during the fermentative phase, approximately 0.5 kg of sand was positioned at the bottom of the silo.

$$EL\ \left(kg\ FM\ t^{-1}\right) = \frac{(PVf - Tb) - (PVi - Tb)}{(MFi \times 100)} \quad \text{Eq. (A.2)}$$

Where PVi is the weight of the empty silo + sand weight at sealing (kg), PVf is the weight empty silo + sand weight at opening (kg); Tb is the weight empty silo (kg), and MFi is the forage mass at sealing (kg).

The calculation of DM losses was conducted by evaluating the weight and DM content of the forage at ensiling and the resulting silage at the opening, determined using the formula:

$$DM\ loss\ \left(\ g\ kg^{-1}DM\right) = \frac{(MFa \times MSa)}{(MFf \times MSf)} \times 100 \quad \text{Eq. (A.3)}$$

Where MFf is the forage mass at sealing (kg), MSf is the forage DM concentration at sealing, MFa is the forage mass at the opening, and MSa is the forage DM concentration at the opening.

### 2.5. Chemical composition

Samples of approximately 0.3 kg were obtained both before the ensiling and after the fermentation period for subsequent analysis. Any spoiled portion on the silage surface was removed. The fresh material samples were then dried in a forced-air oven at 55 °C for 72 h, ground using a 1 mm screen in a Wiley Mill (Arthur H Thomas, Philadelphia, PA, USA), and subjected to analysis for dry matter (DM, Method 934.01), ash (Method 942.05), and total nitrogen (N, Method 968.06) contents, following the methods outlined by the Association of Official Analytical Chemists (AOAC, 2016). Ether extract content was analyzed according to the method of AOCS (2017). Crude protein was calculated by multiplying the total nitrogen by 6.25.

Neutral detergent fiber ($NDF_{om}$), which excludes residual ash, was measured according to the procedure described by Mertens (2002). Total carbohydrates (TC) were calculated using the equation proposed by Sniffen et al. (1992):

$$TC\ \left(g\ kg^{-1}\ DM\right) = 1000 - (CP + EE + Ash) \quad \text{Eq. (A.4)}$$

The non-fiber carbohydrates (NFC) were estimated using the equation of Hall (2003):

$$NFC\ \left(g\ kg^{-1}\ DM\right) = 1000 - (NDF + CP + EE + Ash) \quad \text{Eq. (A.5)}$$

### 2.6. Fermentative profile

Duplicated samples of varying weights were collected for each experimental replication to conduct the following analysis. A 25 g sample was immersed in 100 mL of distilled water for 2 h for pH determination, with readings conducted using a potentiometer (KASVI®, São José dos Pinhais, PR, Brazil) (Bolsen et al., 1992). A sample of 12 g was used for the extraction and quantification of the ammoniacal nitrogen content ($NH_3$–N) using the colorimetric method outlined by Chaney and Marbach (1962). To assess water-soluble carbohydrates (WSC), an air-dried sample with 0.05 g was weighted, and the extraction method from Corsato et al. (2008) was employed; measurements were conducted via colorimetry, following the methodology of Dubois et al. (1956).

For the analysis of lactic and acetic acids produced during fermentation, each silage sample (10 g) underwent centrifugation. To this, 1.0 mL of metaphosphoric acid and 0.10 mL of 50% sulfuric acid were added. A high-performance liquid chromatograph system (HPLC; Shimadzu®, model SPD-10A VP, Dallas, TX, USA) equipped with an Ultraviolet Detector was employed. The analysis utilized an Aminex HPX-87H column (BIO-RAD, Santa Clara, CA, USA), following the method outlined by Taylor and Kung (2002). The lactic-to-acetic ratio was calculated by dividing the concentration of the lactic acid by the acetic acid.

### 2.7. Microbial populations

Microbial populations were evaluated both before ensiling and at the opening time. Lactic acid bacteria (LAB), molds, and yeasts (MY) were isolated using selective media following the methodology described by Jobim et al. (2007). LAB was quantified using de Man, Rogosa, and Sharpe (MRS) agar (Difco®, Mansoura, Lebanon) acidified with 0.1% acetic acid. Molds and yeasts were quantified using Potato Dextrose Agar (PDA) (Difco®, Mansoura, Lebanon) acidified with 1% tartaric acid.

Serial dilutions ranging from $10^{-2}$ to $10^{-6}$ were prepared, followed by duplicate plating in Petri dishes. The plates were then incubated in a B.O.D. chamber at 35 °C, with LAB populations counted after 48 h and MY populations counted after 72 h. Petri dishes with colony counts ranging from 30 to 300 colony forming units (CFU) were considered, and the data were transformed to a logarithmic scale (log10).

### 2.8. Aerobic stability

The aerobic stability assay involved extracting approximately 1 kg of silage from the silos after sampling. This silage was then placed into clean and empty silos without compaction, allowing it to be exposed to air for a duration of 120 h. Temperature measurements were taken using digital thermometers (Incoterm®, model 9197, Porto Alegre, RS, Brazil) every 30 min at a constant room temperature of approximately 25 °C. The silages were considered to be undergoing aerobic deterioration when they reached and maintained a temperature of 2 °C above the room temperature, following the criteria outlined by Taylor and Kung (2002).

### 2.9. Statistical analysis

The data were subjected to analysis of variance (PROC ANOVA) and regression analysis (PROC GLM) using the Statistical Analysis System version 9.0 (SAS Institute, Cary, NC) software (SAS, 2004). A significance level of $\alpha < 0.05$ was applied. The mathematical model used for analysis is as follows:

$$Yij = \mu + \alpha i + \varepsilon ijk$$

Where: *Yij*: is the variable studied, $\mu$ is the overall mean, $\alpha i$ is the effect of the inclusion of forage cactus, and $\varepsilon ijk$: is the residual error.

## 3. Results

The chemical composition of the mixed CS-FC silages exhibited a linear decrease in DM ($p = 0.001$) and EE ($p = 0.006$) as the proportion of FC increased. The CP content exhibited a quadratic effect ($p = 0.002$), with the lowest concentration observed at 22.50% inclusion (Table 2). The variables NDF, ADF, NFC, and TC showed no significant effects ($p > 0.050$) (Table 2).

Regarding the fermentative profile, quadratic effects were observed for pH and WSC ($p < 0.050$) (Table 3). The pH reached its lowest values in the mixed silage containing 20% FC, while WSC levels were highest at the same inclusion level (Table 3). $NH_3$–N did not differ significantly among the treatments ($p > 0.050$), with an average of 0.06 g $kg^{-1}$ of

**Table 2**
Chemical composition of the mixed corn stover-forage cactus silages after 100 days of fermentation.

| Item (g kg$^{-1}$ DM) | Forage cactus inclusion (% FM) | | | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 40 | | L | Q |
| Dry matter[a] | 251.16 | 256.58 | 230.09 | 193.56 | 7.66 | 0.001 | 0.157 |
| Organic matter[b] | 860.46 | 871.46 | 867.76 | 840.30 | 8.27 | 0.208 | 0.001 |
| Crude protein[c] | 55.76 | 46.78 | 46.44 | 51.76 | 1.92 | 0.430 | 0.002 |
| Ether extract[d] | 26.88 | 32.10 | 29.12 | 14.96 | 3.23 | 0.006 | 0.026 |
| $NDF_{om}$ | 581.90 | 587.90 | 602.33 | 588.03 | 15.13 | 0.756 | 0.430 |
| $ADF_{om}$ | 296.00 | 307.13 | 315.67 | 315.10 | 10.95 | 0.257 | 0.459 |
| Non-fibre carbohydrates | 195.91 | 204.69 | 190.02 | 185.54 | 0.84 | 0.226 | 0.708 |
| Total carbohydrates | 777.81 | 792.59 | 792.22 | 773.54 | 0.84 | 0.503 | 0.080 |

DM: dry matter basis; FM: fresh matter basis; $NDF_{om}$ Neutral detergent fibre expressed exclusive of residual ash; ADF: Acid detergent fibre expressed exclusive of residual ash; SEM: standard error of the mean; L: linear effect; Q: quadratic effect.
[a] $\hat{Y} = -0.16 \cdot x + 26.06$ ($R^2 = 0.90$).
[b] $\hat{Y} = -0.004 \cdot x^2 + 0.13 \cdot x + 86.11$ ($R^2 = 0.99$).
[c] $\hat{Y} = 0.002 \cdot x^2 - 0.09 \cdot x + 5.52$ ($R^2 = 0.94$).
[d] $\hat{Y} = -0.002 \cdot x^2 + 0.06 \cdot x + 2.73$ ($R^2 = 0.98$).

**Table 3**
Fermentative profile, organic acids, and microbial populations of the mixed corn stover-forage cactus silages after 100 days of fermentation.

| Item | Forage cactus inclusion (% FM) | | | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 40 | | L | Q |
| **Fermentative profile** | | | | | | | |
| pH[a] | 4.32 | 4.17 | 4.06 | 4.68 | 0.07 | 0.017 | 0.005 |
| $NH_3$–N[b] (g kg$^{-1}$ total N) | 0.05 | 0.05 | 0.07 | 0.06 | 0.00 | 0.058 | 0.139 |
| WSC[c] (g kg$^{-1}$ DM) | 6.74 | 7.85 | 7.89 | 6.93 | 0.44 | 0.934 | 0.049 |
| **Organic acids** | | | | | | | |
| LA[d] (g kg$^{-1}$ DM) | 22.57 | 38.45 | 37.66 | 23.47 | 3.44 | 0.555 | 0.001 |
| AA[e] (g kg$^{-1}$ DM) | 15.37 | 25.16 | 29.67 | 27.86 | 1.27 | 0.001 | 0.001 |
| LA/AA[f] | 1.47 | 1.53 | 1.26 | 0.81 | 0.08 | 0.001 | 0.090 |
| **Microbial populations (log UFC g-1)** | | | | | | | |
| Lactic acid bacteria[g] | 8.39 | 8.30 | 8.21 | 7.88 | 0.15 | 0.030 | 0.696 |
| Molds and yeasts[h] | 5.95 | 3.56 | 3.78 | 3.70 | 0.11 | 0.001 | 0.001 |

DM: dry matter basis; FM: fresh matter basis; $NH_3$–N: ammoniacal nitrogen; WSC: water-soluble carbohydrates; LA: lactic acid; AA: acetic acid; LA/AA: lactic-to-acetic ratio; SEM: standard error of the mean; L: linear effect; Q: quadratic effect.
[a] $\hat{Y} = 0.001001 \cdot x^2 - 0.31 \cdot x + 4.33$ ($R^2 = 0.97$).
[b] $\hat{Y} = 0.001 \cdot x + 0.05$ ($R^2 = 0.63$).
[c] $\hat{Y} = -0.003 \cdot x^2 + 0.12 \cdot x + 6.80$ ($R^2 = 0.96$).
[d] $\hat{Y} = -0.041 \cdot x^2 + 1.62 \cdot x + 2.36$ ($R^2 = 0.94$).
[e] $\hat{Y} = -0.0207 \cdot x^2 + 1.14 \cdot x + 15.50$ ($R^2 = 0.99$).
[f] $\hat{Y} = -0.181 \cdot x + 15.84$ ($R^2 = 0.89$).
[g] $\hat{Y} = -0.013 \cdot x + 8.41$ ($R^2 = 0.97$).
[h] $\hat{Y} = 0.003 \cdot x^2 - 0.191 \cdot x + 5.72$ ($R^2 = 0.84$).

total N (Table 3). The organic acids in the mixed silages exhibited a quadratic effect on LA and AA ($p = 0.001$), with the maximum point for LA at 19.85% and for AA at 27.54%. The LA:AA ratio linearly decreased with higher proportions of FC (Table 3).

The microbial population showed a linear decrease for LAB ($p = 0.030$) according to the inclusion of FC (Table 3). A quadratic effect was observed for MY ($p = 0.001$), with the lowest counts recorded at 30.92% inclusion of FC (Table 3). Concerning losses in the mixed silages, GL exhibited a quadratic effect ($p = 0.035$), with the lowest values at 34% FC inclusion (Table 4). EL displayed a linear relationship with the inclusion of FC ($p = 0.017$) (Table 4). The DM loss exhibited a quadratic effect ($p = 0.005$), with the lowest values observed at 20% of FC inclusion (Table 4).

The aerobic stability increased linearly with the inclusion of FC ($p < 0.05$), and the mixed CS-FC silage with 40% inclusion did not show aerobic deterioration during the 120-h evaluation (Table 4). The control silage exhibited aerobic deterioration after 52 h of air exposure;

**Table 4**
Losses of dry matter, and aerobic stability of the mixed corn stover-forage cactus silages after 100 days of fermentation.

| Item | Forage cactus inclusion (% FM) | | | | SEM | *p*-value | |
|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 40 | | L | Q |
| **Losses of dry matter** | | | | | | | |
| GL[a] (g kg$^{-1}$ DM) | 156.0 | 99.0 | 79.3 | 119.4 | 22.8 | 0.410 | 0.035 |
| EL[b] (kg FM ton$^{-1}$) | 6.2 | 10.8 | 16.1 | 20.1 | 6.9 | 0.017 | 0.752 |
| $DM_{loss}$[c] (g kg$^{-1}$) | 159.0 | 104.4 | 87.2 | 139.5 | 27.3 | 0.800 | 0.005 |
| Silo density (kg m$^{-3}$) | 580.2 | 592.2 | 623.0 | 740.8 | 16.5 | 0.567 | 0.131 |
| **Aerobic stability** | | | | | | | |
| Hours[d] | 52.4 | 87.8 | 96.0 | 120.0 | 6.4 | 0.003 | 0.101 |
| Max. Temp[e] (°C) | 28.7 | 29.4 | 30.4 | 27.5 | 0.36 | 0.021 | 0.006 |

FM: fresh matter basis; DM: dry matter basis; GL: gas losses; EL: effluent losses; DMR: dry matter recovery; SEM: standard error of the mean; L: linear effect; Q: quadratic effect.
[a] $\hat{Y} = 0.01 \cdot x^2 - 0.68 \cdot x + 15.41$ ($R^2 = 0.99$).
[b] $\hat{Y} = 0.03 \cdot x + 3.73$ ($R^2 = 0.95$).
[c] $\hat{Y} = -0.016 \cdot x^2 + 0.67 \cdot x + 4.17$ ($R^2 = 0.99$).
[d] $\hat{Y} = 2.99 \cdot x + 54.83$ ($R^2 = 0.73$).
[e] $\hat{Y} = -0.005 \cdot x^2 + 0.18 \cdot x + 28.46$ ($R^2 = 0.45$).

however, the inclusion of only 20% of FC resulted in a 44-h increase in aerobic stability compared to the control silage (Table 4).

## 4. Discussion

The elevated moisture content in the FC contributed to a decrease in DM as its inclusion increased in the mixed silages (Gusha et al., 2013; Matias et al., 2020). Likewise, the EE content increased with the inclusion of FC, possibly attributed to the presence of a wax layer on the cladodes, serving as protection against water loss in dryland conditions (Oliveira et al., 2018).

The observed negative quadratic effect on organic matter content can be attributed to the ash content of the FC, as increased proportions of FC resulted in decreased organic matter content (*i.e.*, increased ash content). Naturally, FC is rich in minerals like calcium, potassium, and magnesium, which play a vital role in osmotic adjustments necessary for water absorption (Mayer and Cushman, 2019). Nevertheless, caution is essential when contemplating high inclusion levels. This is because while the mineral content may fulfill the needs of certain categories, careful monitoring is necessary to avoid disturbances in the calcium-to-phosphorus ratio, which could result in urolithiasis issues, particularly in confined sheep (Freeman et al., 2010).

Regarding CP content, it is notable that both CS and FC had low protein content since ensiling (Table 1), and this trend was also observed

in the silages (Table 2). The maximum CP value observed in the current study is insufficient (55 g $kg^{-1}$ DM), as a minimum of 70 g $kg^{-1}$ DM is required to maintain the ruminal activity (Van Soest, 1994). Consequently, these silages should not be offered exclusively to the animals, necessitating a proper dietary balance with protein and energy concentrates for each animal category.

The fibrous components of the silages did not exhibit significant changes with the inclusion of FC, which is a positive aspect of the current study. Elevated fibrous fractions can adversely impact digestibility and limit DM intake due to their filling effect (Chapman et al., 2014). Considering that the crops used are well-adapted to tropical conditions, and usually exhibit high contents of NDF and ADF, the values observed fall within the normal range reported in the literature compared to other tropical crops (Ribeiro et al., 2017).

The fermentative profile of the silages displayed favorable characteristics, including pH and $NH_3$–N, which were within the recommended values proposed by Kung et al. (2018). The silage with 40% FC exhibited a higher pH (Table 3), and this elevated pH is attributed to the distinctive properties of FC, particularly its mucilage. The mucilaginous molecules found in cladodes are long-chain hetero-polysaccharides with a negative charge. When dissolved, they increase viscosity, absorbing and retaining significant amounts of water. Moreover, they contribute to neutralizing the acids generated during fermentation, serving as a major source of cations in the medium (Du Toit et al., 2018, 2019). The presence of various organic acids in FC, a result of crassulacean acid metabolism (Petera et al., 2015), contributes to maintaining the silage pH within an optimal range for the growth of heterofermentative LAB (Santos et al., 2020). The $NH_3$–N content indicates low proteolysis within the silo, a well-described characteristic of corn silages (Kung et al., 2018). As FC stimulates fermentation, it appears to maintain this aspect of the fermentative pattern, effectively inhibiting bacteria involved in proteolysis (Nogueira et al., 2019).

The microbial population observed in the silages indicates a favorable fermentative pattern observed for all CS-FC mixed silages, promoting proper acidification of the medium through the production of LA (Table 3). MY counts also decreased linearly with FC inclusion, resulting in lower MY counts than in the control silage (Table 4). The partial inhibition of MY can be attributed to the high pentose content in FC (Pessoa et al., 2020), which is fermented by heterofermentative LAB (obligate or facultative) to produce LA and AA via the phosphoketolase pathway (Pahlow et al., 2003; Rooke and Hatfield, 2003). This observation aligns with the data presented in Table 3, where FC increased the AA contents, as reported in the study by Brito et al. (2020).

Furthermore, the increase in the pH values during the whole fermentative period provided ideal conditions for the growth of heterofermentative LAB, resulting in increased AA contents (Muck et al., 2018). This observation is noteworthy since corn silages typically exhibit low levels of AA, which is linked to poor aerobic stability (Kung et al., 2018). *Weissella*, the predominant LAB in FC, is recognized as a strong AA producer and exhibits antifungal properties, inhibiting mold and yeast growth in silages (Macêdo et al., 2018; Pereira et al., 2019).

The microbial and fermentative profiles are strongly related to DM loss (Borreani et al., 2018). Consequently, DM loss was more pronounced when FC was included at 20% (Table 4), attributed to the decline in GL, mainly linked to the diminished activity of MY and their generation of ethanol and $CO_2$ from the non-fermented WSC (Pahlow et al., 2003). However, EL increased linearly, reaching a maximum of 20 kg per ton of silage, probably due to the high moisture content of the FC (Table 1). Nevertheless, the recorded values for EL are relatively low, primarily attributed to the formation of mucilage in the FC. Mucilage, characterized as a hydrocolloid gum consisted of a heteropolysaccharide and protein matrix, acts to impede water leakage from the medium, stabilize emulsions, and reduces surface activity (Du Toit et al., 2018).

As discussed above, FC promotes heterolactic fermentation, which is strongly related to aerobic stability (Brito et al., 2020; Mayer and Cushman, 2019; Pessoa et al., 2020; Santos et al., 2020). However, the observed deterioration, even in the mixed CS-FC silages, can be attributed to the combination of a higher content of non-fermented WSC and lactic acid in these silages (Table 3). These are the main substrates prone to deterioration by various microorganisms (Borreani et al., 2018; Muck et al., 2018). Considering that yeasts and aerobic acetic bacteria are the main initiators of aerobic deterioration (Wilkinson and Davies, 2013) and that MY counts were reduced in the mixed CS-FC silages compared to the control silage (Table 3), it can be inferred that other microbial groups have initiated aerobic deterioration.

There are reports in the literature that *Acetobacter* predominates during the aerobic exposure of silages. *Acetobacter* can oxidize ethanol into acetic acid, along with converting lactic acid and acetic acid to $CO_2$ and water (Spoelstra et al., 1988). Due to the depletion of the organic acids in the feed-out phase, aerobic exposure favors the growth of other spoilage microorganisms (Jiang et al., 2020). According to Bai et al. (2021), who evaluated bacterial and fungal dynamics during five days of air exposure in corn silage with high (680 g $kg^{-1}$) and low moisture (620 g $kg^{-1}$), a significant increase was observed in the counts of aerobic bacterial and fungal communities from the opening time until the fifth day of air exposure. The main fungal genera found were *Candida*, *Monascus*, and *Kazachstania*, while the aerobic bacteria included *Acinetobacter*, *Bacillus*, and *Lactobacillus*. In this context, the microbial dynamics observed in the current study may be influenced by these factors, but additional research is required to enhance our comprehension of mixed CS-FC silages.

## 5. Conclusion

The inclusion of 20% FC in CS during ensiling improves the nutritive value, fermentative profile, microbial population, and DM preservation. The 90-h aerobic stability assessment indicates stable silage, making it suitable for on-farm use with cost-effectiveness. Further large-scale studies are required to comprehensively evaluate these variables.

## CRediT authorship contribution statement

**Gilberto de Carvalho Sobral:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Juliana Silva de Oliveira:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Edson Mauro Santos:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Gherman Garcia Leal de Araújo:** Resources, Methodology, Funding acquisition. **Francisco Naysson de Sousa Santos:** Validation, Investigation, Data curation. **Fleming Sena Campos:** Validation, Supervision. **Hactus Souto Cavalcanti:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Diego de Souza Vieira:** Investigation, Data curation. **Guilherme Medeiros Leite:** Investigation, Data curation. **Diego Francisco Oliveira Coelho:** Investigation, Data curation. **Liliane Pereira Santana:** Investigation, Data curation. **Paloma Gabriela Batista Gomes:** Investigation, Data curation. **Paulo da Cunha Torres Júnior:** Investigation, Data curation. **Maria Alyne Coutinho Santos:** Investigation, Data curation. **Nelquides Braz Viana:** Investigation, Data curation.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gilberto de Carvalho Sobral reports financial support was provided by Research Support Foundation of Paraíba State. Gilberto de Carvalho Sobral reports financial support was provided by Coordination of Higher Education Personnel Improvement.

## Data availability

Data will be made available on request.

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## References

Åby, B.A., Randby, Å.T., Bonesmo, H., Aass, L., 2019. Impact of grass silage quality on greenhouse gas emissions from dairy and beef production. Grass Forage Sci. 74, 525–534. https://doi.org/10.1111/gfs.12433.

Alvares, C.A., Stape, J.L., Sentelhas, P.C., de Moraes, G., Leonardo, J., Sparovek, G., 2013. Köppen's climate classification map for Brazil. Meteorol. Z. 22, 711–728. https://doi.org/10.1127/0941-2948/2013/0507.

AOAC, 2016. Official Methods of Analysis of AOAC International, twentieth ed. AOAC Int. Gaithersburg. https://doi.org/10.1093/9780197610145.001.0001. MD.

AOCS, 2017. Official Methods and Recommended Practices of the American Oil Chemist Society (AOCS), eighteenth ed. AOCS International Press, Champaign, IL.

Bai, C., Wang, C., Sun, L., Xu, H., Jiang, Y., Na, N., Yin, G., Liu, S., Xue, Y., 2021. Dynamics of bacterial and fungal communities and metabolites during aerobic exposure in whole-plant corn silages with two different moisture levels. Front. Microbiol. 12 https://doi.org/10.3389/fmicb.2021.663895.

Battaglia, M., Thomason, W., Fike, J.H., Evanylo, G.K., von Cossel, M., Babur, E., Iqbal, Y., Diatta, A.A., 2021. The broad impacts of corn stover and wheat straw removal for biofuel production on crop productivity, soil health, and greenhouse gas emissions: a review. GCB Bioenergy. https://doi.org/10.1111/gcbb.12774.

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. J. Dairy Sci. 75, 3066–3083. https://doi.org/10.3168/jds.s0022-0302(92)78070-9.

Borreani, G., Tabacco, E., Schmidt, R.J., Holmes, B.J., Muck, R.E., 2018. Silage review: factors affecting dry matter and quality losses in silages. J. Dairy Sci. 101, 3952–3979. https://doi.org/10.3168/jds.2017-13837.

Brito, G.S.M.S., Santos, E.M., Araújo, G.G.L., Oliveira, J.S., Zanine, A.M., Perazzo, A.F., Campos, F.S., Lima, A.G.V.O., Cavalcanti, H.S., 2020. Mixed silages of cactus pear and gliricidia: chemical composition, fermentation characteristics, microbial population, and aerobic stability. Sci. Rep. 10, 1–13. https://doi.org/10.1038/s41598-020-63905-9.

Chaney, A.L., Marbach, E.P., 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8, 130–132. https://doi.org/10.1093/clinchem/8.2.130.

Chapman, D.F., Lee, J.M., Waghorn, G.C., 2014. Interaction between plant physiology and pasture feeding value: a review. Crop Pasture Sci. 65, 721–734. https://doi.org/10.1071/CP13379.

Chimeli, A.B., Souza Filho, F.A., Holanda, M.C., Petterini, F.C., 2008. Forecasting the impacts of climate variability: lessons from the rainfed corn market in Ceará Brazil. Environ. Dev. Econ. 13, 201–227. https://doi.org/10.1017/S1355770X07004172.

Corsato, C.E., Scarpare Filho, J.A., Sales, E.C.J., 2008. Teores de carboidratos Em Órgãos Lenhosos do Caquizeiro. Rev. Bras. Frutic. 30, 414–418. https://doi.org/10.1590/S0100-29452008000200025.

Du Toit, A., De Wit, M., Hugo, A., 2018. Cultivar and harvest month influence the nutrient content of Opuntia spp. Cactus pear cladode mucilage extracts. Molecules 23. https://doi.org/10.3390/molecules23040916.

Du Toit, A., De Wit, M., Seroto, K.D., Fouché, H.J., Hugo, A., Venter, S.L., 2019. Rheological characterization of cactus pear mucilage for application in nutraceutical food products. Acta Hortic. 1247, 63–72. https://doi.org/10.17660/ActaHortic.2019.1247.9.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356. https://doi.org/10.1021/ac60111a017.

Edvan, R.L., Mota, R.R.M., Dias-Silva, T.P., Nascimento, R.R., Sousa, S.V., Silva, A.L., Araújo, M.J., Araújo, J.S., 2020. The resilience of cactus pear genotypes in a tropical semi-arid region subject to climatic cultivation restriction. Sci. Rep. 10, 1–10. https://doi.org/10.1038/s41598-020-66972-0.

Freeman, S.R., Poore, M.H., Young, G.A., Anderson, K.L., 2010. Influence of calcium (0.6 or 1.2%) and phosphorus (0.3 or 0.6%) content and ratio on the formation of urolithogenic compounds in the urine of Boer-cross goats fed high-concentrate diets. Small Rumin. Res. 93, 94–102. https://doi.org/10.1016/j.smallrumres.2010.05.007.

Gao, J.L., Wang, P., Zhou, C.H., Li, P., Tang, H.Y., Zhang, J.B., Cai, Y., 2019. Chemical composition and in vitro digestibility of corn stover during field exposure and the fermentation characteristics of silage prepared with microbial additives. Asian-Australas. J. Anim. Sci. 32, 1854–1863. https://doi.org/10.5713/ajas.18.0886.

Gusha, J., Katsande, S., Zvinorova, P.I., Ncube, S., 2013. The nutritional composition and acceptability of cacti (*Opuntia ficus-indica*)-legume mixed silage. Online J. Anim. Feed Res. 3, 116–120.

Hall, M.B., 2003. Challenges with non-fiber carbohydrate methods. J. Anim. Sci. 81, 3226–3232. https://doi.org/10.2527/2003.81123226x.

IBGE - Brazilian Institute of Geography and Statistics, 2023. SIDRA: database of statistical tables. Municipal agricultural production. URL. https://sidra.ibge.gov.br/tabela/839#resultado, 9.11.23.

INMET, 2021. Data of Automatic Stations. Brazilian National Meteorology Institute. URL. https://bdmep.inmet.gov.br/, 6.18.21.

Jiang, D., Niu, D., Zuo, S., Tian, P., Zheng, M., Xu, C., 2020. Yeast population dynamics on-air exposure in total mixed ration silage with sweet potato residue. Anim. Sci. J. 91, 1–9. https://doi.org/10.1111/asj.13397.

Jobim, C.C., Nussio, L.G., Reis, R.A., Schmidt, P., 2007. Avanços metodológicos na avaliação da qualidade da forragem conservada. Rev. Bras. Zootec. 36, 101–119. https://doi.org/10.1590/S1516-35982007001000013.

Kung, L., Shaver, R.D., Grant, R.J., Schmidt, R.J., 2018. Silage review: interpretation of chemical, microbial, and organoleptic components of silages. J. Dairy Sci. 101, 4020–4033. https://doi.org/10.3168/jds.2017-13909.

Macêdo, A.J.S., Santos, E.M., Araújo, G.G.L., Edvan, R.L., Oliveira, J.S., Perazzo, A.F., Sá, W.C.C.S., Pereira, D.M., 2018. Silages in the form of a diet based on spineless cactus and buffelgrass. Afr. J. Range Forage Sci. 35, 121–129. https://doi.org/10.2989/10220119.2018.1473494.

Matias, A.G.S., Araujo, G.G.L., Campos, F.S., Moraes, S.A., Gois, G.C., Silva, T.S., Emerenciano Neto, J.V., Voltolini, T.V., 2020. Fermentation profile and nutritional quality of silages composed of cactus pear and maniçoba for goat feeding. J. Agric. Sci. 158, 304–312. https://doi.org/10.1017/S0021859620000581.

Mayer, J.A., Cushman, J.C., 2019. Nutritional and mineral content of prickly pear cactus: a highly water-use efficient forage, fodder, and food species. J. Agron. Crop Sci. 205, 625–634. https://doi.org/10.1111/jac.12353.

Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85, 1217–1240. https://doi.org/10.1093/jaoac/85.6.1217.

Moraes, G.S.O., Guim, A., Tabosa, J.N., Chagas, J.C.C., Almeida, M.P., Ferreira, M.A., 2019. Cactus [Opuntia stricta (Haw.) Haw] cladodes and corn silage: how do we maximize the performance of lactating dairy cows reared in semiarid regions? Livest. Sci. 221, 133–138. https://doi.org/10.1016/j.livsci.2019.01.026.

Mounce, R.B., O'Shaughnessy, S.A., Blaser, B.C., Colaizzi, P.D., Evett, S.R., 2016. Crop response of drought-tolerant and conventional maize hybrids in a semiarid environment. Irrigat. Sci. 34, 231–244. https://doi.org/10.1007/s00271-016-0497-5.

Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C., Kung, L., 2018. Silage review: recent advances and future uses of silage additives. J. Dairy Sci. 101, 3980–4000. https://doi.org/10.3168/jds.2017-13839.

Nilahyane, A., Anowarul Islam, M., Mesbah, A.O., Garcia Y Garcia, A., 2018. Effect of irrigation and nitrogen fertilization strategies on silage corn grown in semi-arid conditions. Agronomy 8. https://doi.org/10.3390/agronomy8100208.

Nogueira, M.D.S., Araújo, G.G.L., Santos, E.M., Gonzaga Neto, S., Oliveira, J.S., Perazzo, A.F., Zanine, A.M., Pinho, R.M.A., Correa, Y.R., Pereira, D.M., 2019. Feed alternatives with cactus forage silage for animal nutrition. Int. J. Agric. Biol. 22, 1393–1398. https://doi.org/10.17957/IJAB/15.1213.

Oliveira, J.P.F., Ferreira, M.A., Alves, A.M.S.V., Melo, A.C.C., Andrade, I.B., Urbano, S.A., Suassuna, J.M.A., Barros, L.J.A., Barros Melo, T.T., 2018. Carcass characteristics of lambs fed spineless cactus as a replacement for sugarcane. Asian-Australas. J. Anim. Sci. 31, 529. https://doi.org/10.5713/2Fajas.17.0375.

Pahlow, Günter, Muck, R.E., Driehuis, F., Oude Elferink, S.J.W.H., Spoelstra, S.F., 2003. Microbiology of ensiling. In: Silage Science and Technology. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin, pp. 31–94. https://doi.org/10.2134/agronmonogr42.c2.

Pereira, D.M., Santos, E.M., Oliveira, J.S., Santos, F.N.S., Lopes, R.C., Santos, M.A.C., Correa, Y.R., Justino, E.S., Leite, G.M., Gomes, P.G.B., 2021. Effect of cactus pear as a moistening additive in the production of rehydrated corn grain silage. J. Agric. Sci. 159, 731–742. https://doi.org/10.1017/S002185962100099X.

Pereira, G.A., Santos, E.M., Araújo, G.G.L., Oliveira, J.S., Pinho, R.M.A., Zanine, A.D.M., Souza, A.F.N., Macêdo, A.J.S., Neto, J.M.C., Nascimento, T.V.C., 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. J. Agric. Sci. 157, 684–692. https://doi.org/10.1017/S0021859620000143.

Pessoa, D.V., Andrade, A.P., Magalhães, A.L.R., Teodoro, A.L., Santos, D.C., Araújo, G.G.L., Medeiros, A.N., Nascimento, D.B., Valença, R.L., Cardoso, D.B., 2020. Forage cactus of the genus Opuntia in different with the phenological phase: nutritional value. J. Arid Environ. 181 https://doi.org/10.1016/j.jaridenv.2020.104243.

Petera, B., Delattre, C., Pierre, G., Wadouachi, A., Elboutachfaiti, R., Engel, E., Poughon, L., Michaud, P., Fenoradosoa, T.A., 2015. Characterization of arabinogalactan-rich mucilage from Cereus triangularis cladodes. Carbohydr. Polym. 127, 372–380.

Ribeiro, J.S., Santos, L.L., Lima Júnior, D.M., Mariz, T.M.A., Ladeira, M.M., Azevedo, P.S., Lima, C.B., Silva, M.J.M.S., 2017. Spineless cactus associated with Tifton hay or sugarcane bagasse may replace corn silage in sheep diets. Trop. Anim. Health Prod. 49, 995–1000. https://doi.org/10.1007/s11250-017-1288-6.

Rooke, J.A., Hatfield, R.D., 2003. Biochemistry of ensiling. Agronomy 42, 95–139. https://doi.org/10.2134/agronmonogr42.c3.

Sá, W.C.C.S., Santos, E.M., De Oliveira, J.S., Araújo, G.G.L., Perazzo, A.F., Silva, A.L., Pereira, D.M., César Neto, J.M., Santos, F.N.S., Leite, G.M., 2020. Fermentative characteristics and chemical composition of cochineal nopal cactus silage containing chemical and microbial additives. J. Agric. Sci. https://doi.org/10.1017/S0021859620000829.

Santos, F.N.S., Santos, E.M., Oliveira, J.S., Medeiros, G.R., Zanine, A.M., Araújo, G.G.L., Perazzo, A.F., Lemos, M.L.P., Pereira, D.M., Cruz, G.F.L., Paulino, R.S., Oliveira, C.J.B., 2020. Fermentation profile, microbial populations, taxonomic diversity, and

aerobic stability of total mixed ration silages based on Cactus and Gliricidia. J. Agric. Sci. 158, 396–405. https://doi.org/10.1017/S0021859620000805.
SAS, 2004. SAS 9.0 User's Guide, vol. 4. SAS Institute, Cary, NC.
Silva, T.S., Araújo, G.G.L., Santos, E.M., de Oliveira, J.S., Campos, F.S., Godoi, P.F.A., Gois, G.C., Perazzo, A.F., Ribeiro, O.L., Turco, S.H.N., 2021. Water intake and ingestive behavior of sheep fed diets based on silages of cactus pear and tropical forages. Trop. Anim. Health Prod. 53 https://doi.org/10.1007/s11250-021-02686-3.
Silva, T.S., Araújo, G.G.L., Santos, E.M., Oliveira, J.S., Godoi, P.F.A., Gois, G.C., Perazzo, A.F., Ribeiro, O.L., Turco, S.H.N., Campos, F.S., 2023. Intake, digestibility, nitrogen balance, and performance in lamb-fed spineless cactus silage associated with forages adapted to the semiarid environment Spineless cactus silages in diets for lambs. Livest. Sci. 268, 105168 https://doi.org/10.1016/j.livsci.2023.105168.
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. J. Anim. Sci. 70, 3562–3577.
Spoelstra, S.F., Courtin, M.G., Van Beers, J.A.C., 1988. Acetic acid bacteria can initiate aerobic deterioration of whole crop maize silage. J. Agric. Sci. https://doi.org/10.1017/S0021859600082915.
Taylor, C.C., Kung, L., 2002. The effect of lactobacillus buchneri 40788 on the fermentation and aerobic stability of high moisture corn in laboratory silos. J. Dairy Sci. 85, 1526–1532. https://doi.org/10.3168/jds.s0022-0302(02)74222-7.
Tegegne, F., Kijora, C., Peters, K.J., 2007. Study on the optimal level of cactus pear (Opuntia ficus-indica) supplementation to sheep and its contribution as a source of water. Small Rumin. Res. 72, 157–164. https://doi.org/10.1016/j.smallrumres.2006.10.004.
Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, NY. https://doi.org/10.7591/9781501732355.
Wilkinson, J.M., Davies, D.R., 2013. The aerobic stability of silage: key findings and recent developments. Grass Forage Sci. 68, 1–19. https://doi.org/10.1111/j.1365-2494.2012.00891.x.
Yan, Y., Li, X., Guan, H., Huang, L., Ma, X., Peng, Y., Li, Z., Nie, G., Zhou, J., Yang, W., Cai, Y., Zhang, X., 2019. Microbial community and fermentation characteristic of Italian ryegrass silage prepared with corn stover and lactic acid bacteria. Bioresour. Technol. 279, 166–173. https://doi.org/10.1016/j.biortech.2019.01.107.