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RESEARCH ARTICLE



## Buffel grass pre-dried as a modulator of the fermentation, nutritional and aerobic stability profile of cactus pear silage

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### ABSTRACT

This study aimed to evaluate fermentation losses and the fermentation profile of cactus pear silage with buffel grass pre-dried. Five levels of inclusion of buffel grass pre-dried in cactus pear silage (0%; 15%; 25%; 35% and 45% on a dry matter basis) were evaluated with 6 replications, in a completely randomised design. After 90 storage days, silos were opened and silages were analysed. The density, effluent losses, buffering capacity, flieg index, mineral matter, organic matter, crude protein, total carbohydrates and total digestible nutrients content were quadratically influenced ( $P < 0.05$ ) of the inclusion of buffel grass pre-dried in cactus pear silage. The inclusion of buffel grass pre-dried promoted a decreasing on gas losses, maximum pH, final pH during stability and CO<sub>2</sub> production at the three exposure times, and an increasing on dry matter recovery, temperature, pH, electrical conductivity, ammonia nitrogen, dry matter, neutral and acid detergent fibre, and hemicellulose contents in the cactus pear silages. The inclusion of up to 35% buffel grass pre-dried in cactus pear silage represents a viable strategy in the process of nutrient conservation and fermentation quality. In addition to presenting low carbon production during aerobic stability.

### ARTICLE HISTORY

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### KEYWORDS

Buffering capacity; carbon dioxide; chemical composition; dry matter; silage making; temperature

## Introduction

The search for efficient, low-cost alternatives that meet the needs of animals in the semi-arid region of Brazil has been one of the major obstacles to animal production, since in this region rainfall varies over the years. The use of preserved forages emerges as a strategy to ensure feeding and stability in different ruminant production systems in periods of forage shortage (Fluck et al. 2018).

Characteristics in the cactus pear composition favour silage making, such as non-structural carbohydrates (44% dry matter (DM)) and total digestible nutrients (66%–

74% DM) (Silva et al. 2022). However, the contents of DM (7%–16% fresh matter (FM)), neutral detergent fibre (25% DM) and crude protein (4%–7% DM) are found in low amounts (Cordova-Torres et al. 2022), which can result in excessive fermentation, leading to nutrient losses and reduced aerobic stability of silages, since excess sugars will allow yeast proliferation, resulting in alcoholic fermentation (Brito et al. 2020). Thus, the search for the combination of cactus pear with other tropical forages adapted to the semiarid region is necessary, aiming at achieving nutritional complementarity between foods and improving the fermentation of silages (Macêdo et al. 2017).

Buffel grass (*Cenchrus ciliaris* L.) stands out in arid and semiarid regions due to easy adaptation to climatic adversities, good forage production and productive capacity, producing up to 20 t/ha DM per year even after long dry periods. The chemical composition of this plant varies according to the regrowth age and environmental characteristics, with a dry matter content ranging from 19.4% to 31.0% FM, and neutral detergent fibre ranging from 64.2% to 65.5% DM (Macêdo et al. 2018). Nevertheless, despite very large cultivated areas, buffel grass is rarely exploited for silage production (Campos et al. 2017; Macêdo et al. 2018; Silva et al. 2021; Barros e Silva et al. 2022).

The nutritional value of silage (chemical composition, digestibility and digestion products) is associated with the fermentation pattern of the ensiled material, as well as with deterioration processes observed during the aerobic phase in the silo (Reis et al. 2008). Thus, when cactus pear is combined with tropical forages, such as buffel grass, as silage, a possible nutritional adjustment of this silage will occur, with reduced fermentation losses and high aerobic stability.

The aim was to evaluate the effects of the inclusion of increasing levels of buffel grass pre-dried on the fermentation characteristics of cactus pear silage.

## Material and methods

### Experiment location

The experiment was carried out at the Animal Science Sector I, Federal Institute of Education, Science and Technology of Bahia, Campus Senhor do Bonfim, state of Bahia, Brazil. The climate of the region is semiarid, with maximum and minimum temperature between 18°C and 32°C, respectively, relative humidity of 66% and annual rainfall of 768 mm.

### Silage making

Buffel grass pre-dried levels (0%, 15%, 25%, 35% and 45% on a dry matter basis) were added to cactus pear silages, following a 5 × 6 factorial arrangement, totalling 30 experimental units. Buffel grass cv. biloela (*Cenchrus ciliares* L.) was harvested after 122 regrowth days in already established grassland, cut 10 cm above the ground. Cactus pear (*Opuntia ficus indica*, cv. Gigante) came from a cactus plantation, with two years of planting. Harvesting was performed by hand. The pre-drying of Buffel grass was carried out in the field, where all the material was dehydrated for 7 days, being collected and stored in a dry place. Cactus pear and buffel grass pre-dried were ground in a

stationary forage machine (Nogueira Pecus 9004, Saltinho – SP, Brazil), to particles with an average size of 20 mm.

The material was mixed manually and ensiled in experimental silos (10 cm in diameter and 70 cm in height, with 0.55 cm<sup>3</sup>). To eliminate gases during the fermentation process, Bunsen valves were attached to the top of the silo. At the bottom of the experimental silos, 1.5 kg sterilised sand, protected with TNT, were deposited, preventing the ensiled material from coming into contact with the sand, allowing the effluent to drain (Pereira et al. 2005). Samples of non-ensiled material (original material) were collected for further laboratory analysis (Table 1).

### **Density, fermentation losses, fermentation profile and flieg index**

Silos were weighed empty after ensiling and weighed again after 90 days of ensiling, upon opening. A layer of 10 cm of silage at the top e bottom of the silos were discarded. The ensiled mass density (D), gas losses (GL), effluent losses (EL) and dry matter recovery (DMR) were determined according to Zanine et al. (2010).

To evaluate the fermentation profile, we measured the temperature (T, in °C), pH (Detmann et al. 2021), electrical conductivity (EC, dS/m), maximum electrical conductivity (MEC, dS/m); maximum time to reach maximum electrical conductivity (TEC; h); and final electrical conductivity (FEC; dS/m) (Jobim et al. 2007) of the ensiled mass upon silo opening. Buffering capacity (BC) was determined according to the methodology established by Mizubuti et al. (2009) and the ammonia nitrogen content (NH<sub>3</sub>-N, in % total N) was determined according to Bolsen et al. (1992).

The Flieg index was calculated using the equation described by Dong et al. (2017), with interpretation of the points through the following scores: very poor quality silages (scores <20.0); poor quality silages (scores between 21.0 and 40.0); reasonable quality silages (scores between 41.0 and 60.0); good quality silages (score between 61.0 and 80.0) and high quality silages (scores > 81.0).

### **Chemical composition**

The collected samples were pre-dried in a forced ventilation oven at 55°C for 72 h and ground to 1 mm particles (Wiley mill, Marconi, MA-580, Piracicaba, Brazil) for the determination of dry matter (DM, method: 967.03), mineral matter (MM, method:

**Table 1.** Chemical composition of cactus pear and buffel grass pre-dried before ensiling.

Items	Cactus pear	Buffel grass pre-dried
Dry matter (g.kg NM)	142.97	676.02
Mineral matter (g.kg DM)	93.07	205.51
Organic matter (g.kg DM)	906.93	794.49
Aether extract (g.kg DM)	10.90	11.14
Crude protein (g.kg DM)	7.33	106.2
Neutral detergent fibre (g.kg DM)	94.57	710.56
Acid detergent fibre (g.kg DM)	87.93	415.70
Hemicellulose (g.kg DM)	182.10	295.56
Total carbohydrates (g.kg DM)	888.70	772.73
Non fibre Carbohydrates (g.kg DM)	794.13	62.17

NM, Natural matter; DM, Dry matter.

942.05), crude protein (CP, method: 981.10) and aether extract (EE, method: 920.29), according to the AOAC methodology (2016). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were quantified according to Van Soest et al. (1991), with modifications by Senger et al. (2008) using an autoclave with a temperature of 110°C for 40 min. Total carbohydrates (TC) were estimated using the equation of Sniffen et al. (1992) and non-fibre carbohydrates (NFC), according to Hall (2003). Hemicellulose (HEM) was obtained by the difference between NDF and ADF values and total digestible nutrients (TDN) were estimated according to Capelle et al. (2001).

### ***Aerobic stability***

Aerobic stability (AE, expressed in hours) was evaluated using the methodology adapted from Kung Júnior (2000). The evaluation of the internal temperature followed intervals of 1 h, during a period of 96 h (Brito et al. 2020). During the stability test, the pH was monitored at 6-h intervals until 96 h of exposure to air (Araújo et al. 2020).

The maximum pH recorded after opening the silos (maximum pH); Time to reach maximum pH (maximum TpH, in hours); Time for the silage pH to show an upward trend (TEpH, in hours); Maximum temperature after silo opening (MT, in °C); Time to reach maximum temperature (TMT, in hours); Maximum difference between silage and ambient temperature (DTS, in °C); Sum of the maximum difference in silage temperature in relation to the environment ( $\sum$ DT, in °C); and the time for the silage temperature to show an upward trend (STUT, in hours) were analysed according to the methodology of Jobim et al. (2007).

### ***Carbon dioxide***

For the determination of carbon dioxide (CO<sub>2</sub>), silage was exposed to air in a system adapted to that described by Ahsbell et al. (1991), for 24, 48 and 96 h. A 5 × 3 factorial arrangement with 5 repetitions was adopted, totalling 120 experimental units.

The slightly compacted silages were added to the system, whose lower part had 100 mL 20% potassium hydroxide (KOH). To quantify the CO<sub>2</sub>, 10 mL potassium hydroxide that was at the bottom of the system was collected and, from this, the pH was measured (between 12.0 and 14.0) and, following, HCl titration (1n), until the pH was reduced (around 3). The volume of HCl spent was used for later determination of CO<sub>2</sub>. During air exposure, silage samples were collected to determine dry matter, crude protein and ammonia nitrogen.

### ***Statistical analysis***

Data were analysed using the PROC REG of the Statistical Analysis System University Software (SAS 2015) tested by analysis of variance and regression at the level of 5% probability for type I error. The significance of the parameters estimated by the models and the values of the coefficients of determination were used as criteria for choosing regression models. The following statistical model was used:  $Y = \mu + T_j + e_{ij}$ , where:  $\mu$  = overall mean;  $T_j$  = effect of the inclusion of cactus pear;  $e_{ij}$  = residual error.

For carbon dioxide, dry matter, crude protein and ammonia nitrogen, the statistical model was used:  $Y_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{ijk}$ , in which:  $Y_{ij}$  = Observed value of the variable;  $\mu$  = Overall mean;  $a_i$  = effect of inclusion of buffel grass pre-dried;  $b_j$  = effect of exposure time;  $ab_{ij}$  = effect of the interaction between a and b;  $e_{ijk}$  = Residual error.

The means of each variable were estimated and compared by Tukey's test with a probability of 5% for type I error. When there was an isolated effect for the variables opening time and inclusion levels of buffel grass pre-dried, breakdown and graphics were carried out using SigmaPlot version 10.0.

## Results

The silage density showed a quadratic effect ( $P < 0.001$ ). The model estimates a maximum point of 979.33 kg.m<sup>3</sup> with the inclusion of 8.68% buffel grass pre-dried (Table 2). The inclusion of buffel grass pre-dried promoted a decreasing in GL ( $P = 0.042$ ) with a reduction of 0.81% in GL for every 1% inclusion of buffel grass pre-dried. There was a quadratic effect ( $P < 0.001$ ) of the inclusion of buffel grass pre-dried on the EL, with a maximum point of 106.85 t/NM with the inclusion of 14.68% buffel grass pre-dried. The inclusion of buffel grass pre-dried promoted an increasing on DMR ( $P < 0.001$ ) with an increase of 33.02% for the inclusion of 45% buffel grass pre-dried (107.95%) when compared to cactus pear silage alone (81.15%) (Table 2).

The fermentation profile of cactus pear silage was altered by the inclusion of buffel grass pre-dried. There was increasing linear effect ( $P < 0.001$ ) for T, pH, EC and ammonia nitrogen with the inclusion of buffel grass pre-dried (Table 3). The inclusion of 15% promoted an elevation in the pH corresponding to 32.36% compared to the cactus pear silage alone. The silage BC presented quadratic model adjustment ( $P < 0.001$ ), the model demonstrates a minimum point of 62.95 E.mgNaOH/100 g DM with the inclusion of 40.75% buffel grass pre-dried (Table 3).

There was a quadratic effect ( $P < 0.05$ ) on the Flieg index. The equation derivation demonstrates a minimum point of 57.48 points on the Flieg scale with the inclusion of 15.32% buffel grass pre-dried (Figure 1).

During the stability test, MT had a quadratic effect ( $P = 0.007$ ) with the inclusion of buffel grass pre-dried, with a maximum point of 25.88°C with the inclusion of 24.5% buffel grass pre-dried (Table 4).

There was no effect of the inclusion of buffel grass pre-dried in cactus pear silages to on the TMT and AE ( $P > 0.05$ ). The inclusion of buffel grass pre-dried has influenced

**Table 2.** Density, fermentative losses and dry matter recovery of cactus pear silages associated with buffel grass pre-dried inclusion levels.

Variables	Buffel grass pre-dried levels (%)					SEM	P-value	
	0	15	25	35	45		L	Q
Dens (kg/m <sup>3</sup> ) <sup>a</sup>	945.80	1001.61	880.23	752.48	593.11	2.91	<0.001	<0.001
GL (% DM) <sup>b</sup>	51.60	11.48	10.73	10.12	11.01	13.18	0.042	0.119
EL (kg/t NM) <sup>c</sup>	69.55	127.72	104.87	9.87	8.57	5.29	<0.001	<0.001
DMR (% DM) <sup>d</sup>	81.15	78.29	93.76	109.28	107.95	3.25	<0.001	0.181

Dens, Density; GL, Gas losses; EL, Effluent losses; DMR, Dry matter recovery, DM, Dry matter; NM, Natural matter; SEM, Standard error of the mean; L, Linear; Q, Quadratic; Equations: <sup>a</sup> $\hat{y} = 956.507865 + 5.255198x - 0.302419x^2$ ,  $R^2 = 0.98$ ; <sup>b</sup> $\hat{y} = 38.437787 - 0.810199x$ ,  $R^2 = 0.60$ ; <sup>c</sup> $\hat{y} = 79.006132 + 3.793538x - 0.129183x^2$ ,  $R^2 = 0.74$ ; <sup>d</sup> $\hat{y} = 76.173880 + 0.746449x$ ,  $R^2 = 0.80$ .

**Table 3.** Fermentation profile of cactus pear silages associated with buffel grass pre-dried inclusion levels.

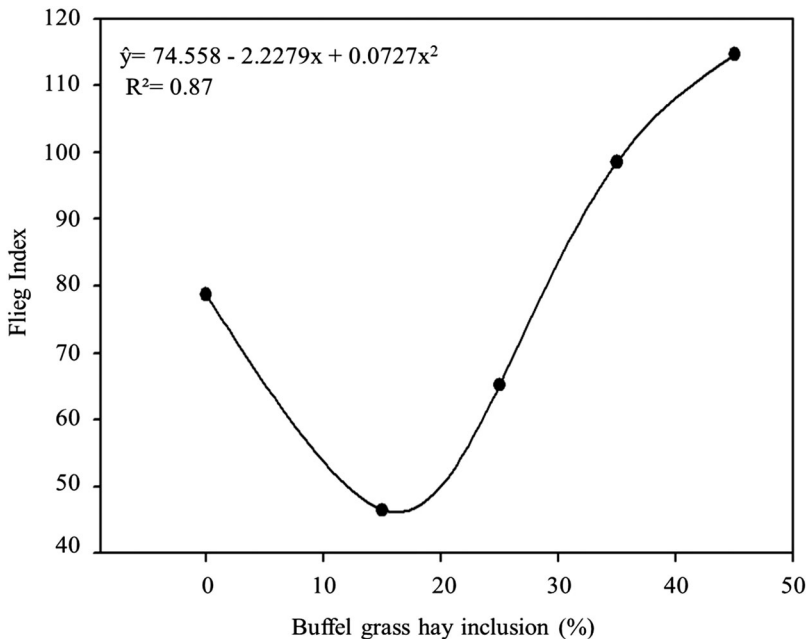
Variables	Buffel grass pre-dried levels (%)					SEM	P-value	
	0	15	25	35	45		L	Q
T (°C) <sup>a</sup>	25.50	26.33	26.83	27.00	27.33	0.17	<0.001	0.152
pH <sup>b</sup>	3.80	5.03	4.98	4.52	4.41	0.08	0.001	<0.001
EC (dS.m) <sup>c</sup>	2.24	2.22	2.32	2.63	3.04	0.07	<0.001	<0.001
BC (E.mgNaOH.100 g DM) <sup>d</sup>	109.19	98.12	63.69	54.79	69.54	2.33	<0.001	<0.001
NH <sub>3</sub> -N (g.kg total N) <sup>e</sup>	3.2	4.2	4.7	4.9	6.9	0.03	<0.001	0.075

T, Temperature; pH, hydrogenion potential; EC, Electrical conductivity; BC, Buffering capacity; N-NH<sub>3</sub>, Ammonia Nitrogen; DM, Dry matter; SEM, Standard error of the mean; L, Linear; Q, Quadratic; Equations: <sup>a</sup> $\hat{y} = 25.639344 + 0.040027x$ ,  $R^2 = 0.96$ ; <sup>b</sup> $\hat{y} = 3.888023 + 0.088100x - 0.001770x^2$ ,  $R^2 = 0.84$ ; <sup>c</sup> $\hat{y} = 2.078907 + 0.017351x$ ,  $R^2 = 0.76$ ; <sup>d</sup> $\hat{y} = 113.958089 - 2.502628x + 0.030702x^2$ ,  $R^2 = 0.81$ ; <sup>e</sup> $\hat{y} = 0.418142 + 0.05189x$ ,  $R^2 = 0.78$ .

quadratically ( $P < 0.001$ ) for DTS and  $\sum$  DT. The model estimates a maximum point of 27.11°C in  $\sum$  DT with the inclusion of 28.43% buffel grass pre-dried (Table 4; Figure 2A).

The maximum pH recorded and the final pH during stability was influenced in a decreasing linearly ( $P < 0.001$ ). The time for silage to reach maximum pH was quadratically influenced ( $P = 0.001$ ). From the differential equation, there was a time of 98.82 h with the inclusion of 13.63% buffel grass pre-dried for the silage to reach maximum pH (Table 4; Figure 2B).

Electrical conductivity during stability had a quadratic effect on maximum EC ( $P = 0.004$ ); Maximum TEC ( $P < 0.001$ ) and FEC ( $P = 0.017$ ). In the Equation derivation, a maximum point was found for the FEC of 4.78 dS/m with the inclusion of 43.46% buffel grass pre-dried (Table 4; Figure 2C).

**Figure 1.** Flieg index in cactus pear silages associated with buffel grass pre-dried inclusion levels.

**Table 4.** Aerobic stability of cactus pear silages associated with buffel grass pre-dried inclusion levels.

Variables	Buffel grass pre-dried levels (%)					SEM	P-value	
	0	15	25	35	45		L	Q
<i>Temperature – T</i>								
MT (°C) <sup>a</sup>	24.83	26.16	25.66	25.50	25.66	0.20	0.068	0.007
TMT (h)	1.00	1.16	16.16	1.00	11.83	8.38	0.415	0.846
DST (°C) <sup>b</sup>	0.30	2.06	1.83	1.96	1.80	0.13	<0.001	<0.001
∑DT (°C) <sup>c</sup>	-82.91	22.10	8.26	20.81	-7.00	6.64	<0.001	<0.001
AE (h)	96.00	86.00	95.66	92.00	96.00	2.53	0.610	0.068
<i>Hydrogenonic Potential – pH</i>								
Maximum pH <sup>d</sup>	7.01	6.38	5.40	5.24	4.77	0.27	<0.001	0.647
Maximum TpH (h) <sup>e</sup>	92.00	95.00	93.00	82.33	52.00	5.19	<0.001	0.001
Final pH <sup>f</sup>	6.82	6.37	5.23	5.00	4.42	0.29	<0.001	0.953
<i>Electrical conductivity – EC</i>								
MEC (dS/m) <sup>g</sup>	14.00	18.00	9.00	9.00	10.00	2.48	0.048	0.004
MTEC (h) <sup>h</sup>	26.00	91.00	91.00	90.00	95.00	5.08	<0.001	<0.001
FEC (dS/m) <sup>i</sup>	2.20	4.19	4.00	4.57	4.87	0.25	<0.001	0.017

MT, maximum temperature; TMT, Time to reach maximum silage temperature; DST, Maximum difference of silage temperature in relation to the environment; ∑DT, Sum of the maximum difference between the silage temperature and the environment; AE, Aerobic stability; Maximum TpH, Time to reach maximum pH; MEC, Maximum electrical conductivity; MTEC, Time to reach maximum electrical conductivity; FEC, Final electrical conductivity; SEM, Standard error of the mean; L, Linear; Q, Quadratic; Equations: <sup>a</sup> $\hat{y} = 24.983250 + 0.066606x - 0.001238x^2$ ,  $R^2 = 0.45$ ; <sup>b</sup> $\hat{y} = 0.407709 + 0.115008x - 0.001920x^2$ ,  $R^2 = 0.89$ ; <sup>c</sup> $\hat{y} = -77.973866 + 7.392574x - 0.130725x^2$ ,  $R^2 = 0.91$ ; <sup>d</sup> $\hat{y} = 6.995191 - 0.051272x$ ,  $R^2 = 0.96$ ; <sup>e</sup> $\hat{y} = 90.648677 + 1.201341x - 0.044651x^2$ ,  $R^2 = 0.96$ ; <sup>f</sup> $\hat{y} = 6.908989 - 0.055611x$ ,  $R^2 = 0.95$ ; <sup>g</sup> $\hat{y} = 15.332641 + 0.110846x - 0.000820x^2$ ,  $R^2 = 0.50$ ; <sup>h</sup> $\hat{y} = 29.993081 + 4.241541x - 0.0644866x^2$ ,  $R^2 = 0.91$ ; <sup>i</sup> $\hat{y} = 2.337628 + 0.113067x - 0.001317x^2$ ,  $R^2 = 0.91$ .

During exposure of silage to oxygen, increasing linear effect ( $P < 0.001$ ) on the DM content was observed during 24, 48 and 96 h. The inclusions of 15% and 25% buffel grass pre-dried reduced the DM content at 48 and 96 h of exposure when compared to 24 h (Table 5; Figure 3A).

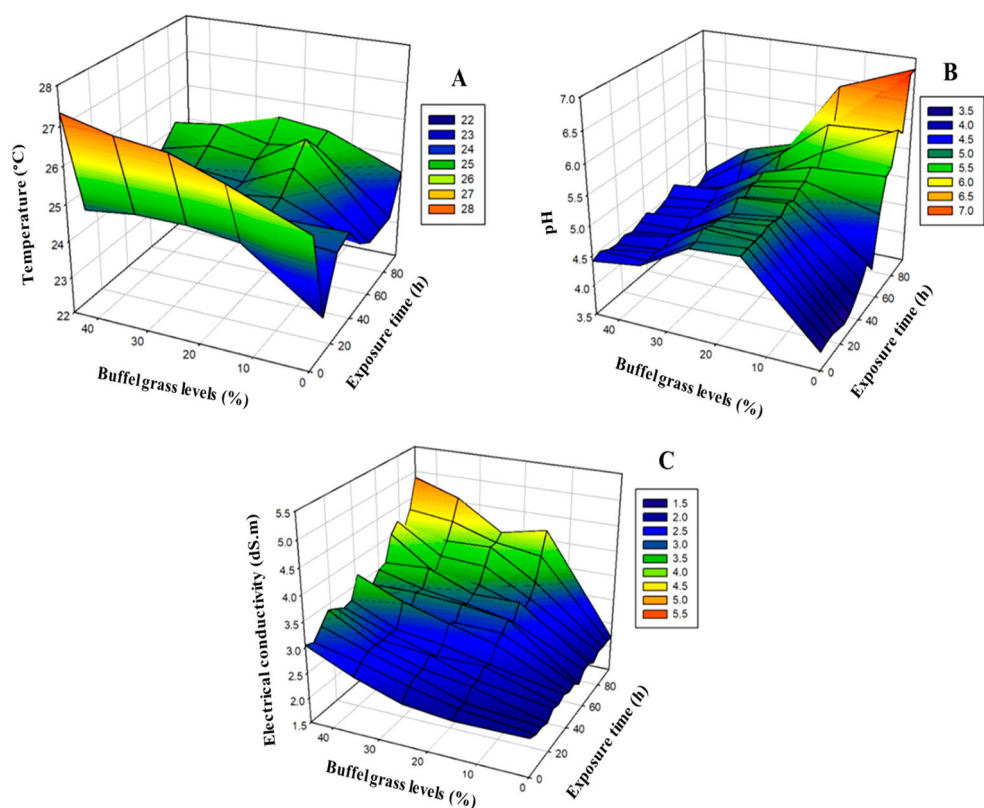
The inclusion of buffel grass pre-dried promoted an increasing linear effect ( $P = 0.002$ ) on CP content during the 24 h of exposure. There was a quadratic effect ( $P = 0.001$ ) of the inclusion of pre-dried on the CP content of the silage exposed for 48 h, the equation derived a maximum CP point at 106.40 g/kg DM with 40.67% inclusion of buffel grass pre-dried (Table 5). All levels of inclusion of buffel grass pre-dried increased the CP content at 96 h of exposure compared to 24 h (Figure 3B).

There was adjustment of the quadratic model of the inclusion of buffel grass pre-dried on the  $\text{NH}_3\text{-N}$  content exposed for 24 h ( $P = 0.019$ ) and 48 h ( $P = 0.012$ ) (Table 5). The inclusion of buffel grass pre-dried resulted in an increasing linear effect ( $P < 0.001$ ) on the content of  $\text{NH}_3\text{-N}$  exposed at 96 h. There was a reduction in the  $\text{NH}_3\text{-N}$  content with increasing exposure time of 0% and 15% buffel grass pre-dried inclusion, reducing at 96 h. The opposite effect was observed with 45% buffel grass pre-dried inclusion, with an increase in the  $\text{NH}_3\text{-N}$  content at 96 h of exposure (Table 5; Figure 3C).

There was a decreasing linear effect ( $P < 0.001$ ) on  $\text{CO}_2$  production with the inclusion of buffel grass pre-dried in the three exposure times (Table 5). There was an effect of exposure time ( $P < 0.001$ ) on  $\text{CO}_2$  production in silages with 15%, 25% and 35% buffel grass pre-dried, promoting a gradual increase in 96 h of exposure (Table 5; Figure 3D).

The inclusion of buffel grass pre-dried increased linearly ( $P < 0.001$ ) the contents of DM, NDF, ADF and HEM (Table 6). There was adjustment of quadratic model ( $P < 0.001$ ) on the contents of MM, OM, CP, TC and TDN. The inclusion of buffel grass





**Figure 2.** Dynamics of temperature **A**, pH **B** and electrical conductivity **C** in cactus pear silages associated with buffel grass pre-dried inclusion levels during oxygen exposure.

pre-dried promoted a decreasing linear effect on EE ( $P = 0.048$ ) and NFC ( $P < 0.001$ ) contents (Table 6).

#### 4. Discussion

Silage density is closely influenced by the DM content of the forage plant, particle size and the compaction process during its production (Krüger et al. 2020), so that the higher the compaction pressure, the greater the influence on silage fermentation (Tan and Dalmis 2019). Thus, cactus pear silage containing 45% buffel grass presented an average density value below the limit considered adequate for a well-compacted silage ( $600\text{--}800\text{ kg/m}^3$ ; Tomich et al. 2003). Silages with densities below  $600\text{ kg/m}^3$  result in a larger volume of residual air in the mass, which leads to a longer period of respiration (release of  $\text{CO}_2$  and loss of dry matter), greater consumption of soluble carbohydrates, decrease in the speed of production of organic acids and an increase in the final pH (McDonald et al. 1991).

Reduction in gas losses with the inclusion of buffel grass pre-dried can be justified by increasing DM content in the silage, reducing water activity inside the silo. This

**Table 5.** Dynamics of dry matter, crude protein, carbon dioxide and ammonia nitrogen in cactus pear silages associated with buffel grass pre-dried inclusion levels during oxygen exposure.

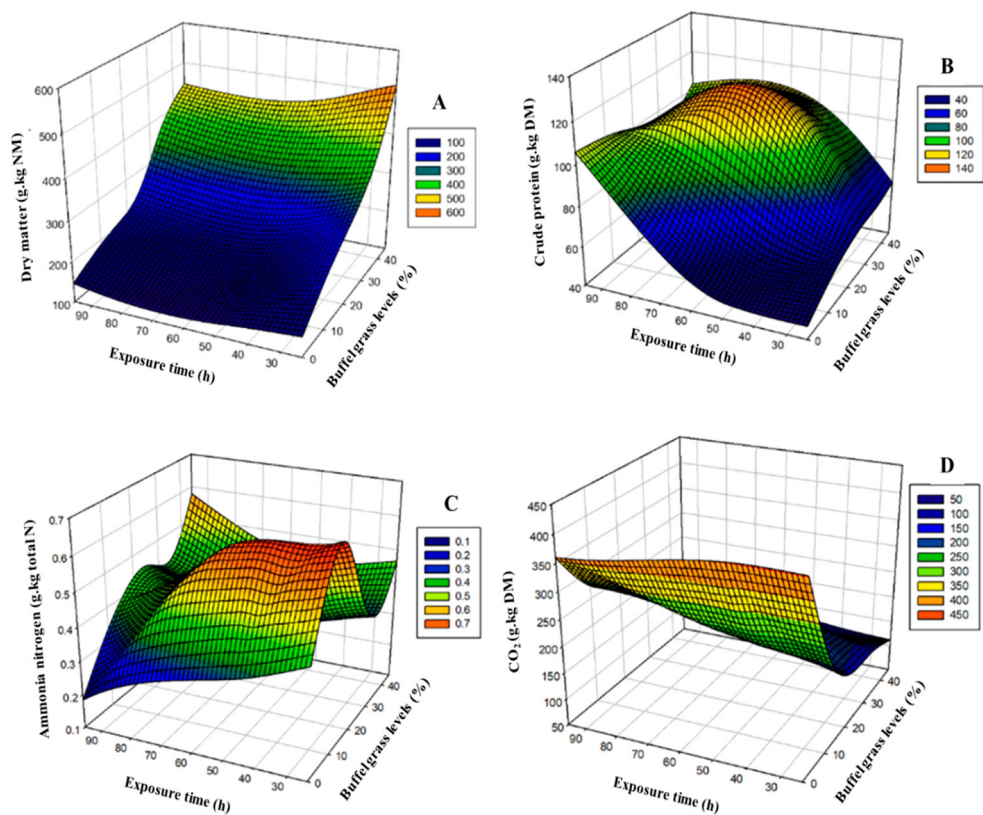
Variables	Buffel grass pre-dried levels (%)					SEM	P-value		
	0	15	25	35	45		L	Q	BHxT
<i>Dry Matter (g/kg NM)</i>									
24 h <sup>a</sup>	150.35	249.53	298.48	376.12	515.54	27.47	<0.001	0.203	0.022
48 h <sup>b</sup>	134.40	177.96	232.36	347.88	459.36	16.81	<0.001	<0.001	
96 h <sup>c</sup>	144.74	194.00	247.84	372.31	448.68	15.04	<0.001	0.001	
SEM	11.72	11.41	14.76	19.95	13.11	–	–	–	
P-value	0.610	0.020	0.043	0.070	0.158	–	–	–	
<i>Crude Protein (g/kg DM)</i>									
24 h <sup>d</sup>	47.30	59.26	64.03	64.09	67.57	4.39	0.002	0.273	<0.001
48 h <sup>e</sup>	49.17	68.14	108.05	108.19	102.38	494	<0.001	0.001	
96 h	105.45	101.42	94.18	99.86	101.69	10.19	0.749	0.555	
SEM	10.91	9.87	10.17	8.71	10.42	–	–	–	
P-value	<0.001	<0.001	0.024	0.030	0.014	–	–	–	
<i>Ammonia Nitrogen (g/kg total N)</i>									
24 h <sup>f</sup>	4.1	6.9	4.7	3.5	4.5	0.04	0.142	0.019	<0.001
48 h <sup>g</sup>	3.2	6.4	4.0	3.1	4.1	0.04	0.480	0.012	
96 h <sup>h</sup>	1.8	4.0	4.6	3.9	5.8	0.03	<0.001	0.282	
SEM	0.06	0.05	0.025	0.091	0.015	–	–	–	
P-value	<0.001	<0.001	0.401	0.120	<0.001	–	–	–	
<i>Carbon dioxide (g/kg DM)</i>									
24 h <sup>i</sup>	399.97	178.94	140.23	135.08	111.66	5.02	<0.001	<0.001	<0.001
48 h <sup>j</sup>	404.31	205.70	232.46	150.10	120.61	10.61	<0.001	<0.001	
96 h <sup>k</sup>	365.51	253.55	248.81	196.59	170.34	17.39	<0.001	0.152	
SEM	14.51	10.54	9.28	6.87	16.74	–	–	–	
P-value	0.151	<0.001	<0.001	<0.001	0.054	–	–	–	

DM, Dry matter; NM, natural matter; SEM, Standard error of the mean; L, Linear; Q, Quadratic; BH, buffel grass pre-dried; T, exposure time; BH x T, Interaction effect between buffel grass pre-dried levels and exposure time; a, b = means followed by distinct letters differ statistically by tukey test at 5% probability level for type I error. Equations: <sup>a</sup>y = 132.937567 + 7.711282x, R<sup>2</sup> = 0.96; <sup>b</sup>y = 95.742110 + 7.277301x, R<sup>2</sup> = 0.92; <sup>c</sup>y = 113.401041 + 7.004909x, R<sup>2</sup> = 0.93; <sup>d</sup>y = 50.237006 + 0.425794x, R<sup>2</sup> = 0.88; <sup>e</sup>y = 45.198225 + 3.019699x - 0.037167x<sup>2</sup>, R<sup>2</sup> = 0.88; <sup>f</sup>y = 0.0468718 + 0.007711x - 0.000212x<sup>2</sup>, R<sup>2</sup> = 0.22; <sup>g</sup>y = 0.384419 + 0.009210x - 0.000224x<sup>2</sup>, R<sup>2</sup> = 0.18; <sup>h</sup>y = 0.225886 + 0.007510x, R<sup>2</sup> = 0.80; <sup>i</sup>y = 335.581303 - 5.933421x, R<sup>2</sup> = 0.76; <sup>j</sup>y = 363.068602 - 5.851130x, R<sup>2</sup> = 0.85; <sup>k</sup>y = 346.621915 - 4.152350x, R<sup>2</sup> = 0.93.

**Table 6.** Chemical composition of cactus pear silages associated with buffel grass pre-dried inclusion levels.

Variables (g.kg DM)	Buffel grass pre-dried levels (%)					SEM	P value	
	0	15	25	35	45		L	Q
DM <sup>*a</sup>	128.89	214.33	297.00	372.05	431.37	10.43	<0.001	0.661
MM <sup>b</sup>	105.16	189.69	159.19	145.49	129.24	3.68	0.071	<0.001
OM <sup>c</sup>	894.83	810.30	840.80	854.50	870.75	3.68	0.071	<0.001
EE	20.14	14.11	15.57	14.80	15.35	1.51	0.048	0.060
CP <sup>d</sup>	52.25	81.18	92.91	82.77	73.67	3.14	<0.001	<0.001
NDF <sup>e</sup>	200.42	592.34	600.82	607.95	630.56	8.32	<0.001	<0.001
ADF <sup>f</sup>	153.98	419.61	420.45	419.94	401.94	9.14	<0.001	<0.001
HEM <sup>g</sup>	46.53	172.98	180.36	184.67	233.62	10.85	<0.001	0.001
TC <sup>h</sup>	822.40	714.99	732.40	756.92	781.72	5.67	0.002	<0.001
NFC <sup>i</sup>	621.97	122.65	131.58	148.96	151.15	9.69	<0.001	<0.001
TDN <sup>j</sup>	947.28	766.66	766.08	766.43	778.68	6.21	<0.001	<0.001

DM, Dry matter; MM, Mineral matter; OM, Organic matter; EE, Aether extract; CP, Crude protein; NDF, Neutral detergent fibre; ADF, Acid detergent fibre; TC, Total carbohydrates; NFC, Non-fibrous carbohydrates; HEM, Hemicellulose; TDN, Total digestible nutrients; \*in g.kg natural matter; SEM, Standard error of the mean; L, Linear; Q, Quadratic; Equations: <sup>a</sup>y = 122.973779 + 6.906580x, R<sup>2</sup> = 0.99; <sup>b</sup>y = 112.742230 + 5.173651x - 0.111159x<sup>2</sup>, R<sup>2</sup> = 0.73; <sup>c</sup>y = 887.257770 - 5.173651x + 0.111159x<sup>2</sup>, R<sup>2</sup> = 0.73; <sup>d</sup>y = 52.253774 + 2.781654x - 0.051794x<sup>2</sup>, R<sup>2</sup> = 0.97; <sup>e</sup>y = 322.051970 + 8.515425x, R<sup>2</sup> = 0.66; <sup>f</sup>y = 244.993279 + 4.924855x, R<sup>2</sup> = 0.53; <sup>g</sup>y = 76.210126 + 3.642764x, R<sup>2</sup> = 0.83; <sup>h</sup>y = 909.289423 - 120.31469x + 19.39567x<sup>2</sup>, R<sup>2</sup> = 0.75; <sup>i</sup>y = 509.86442 - 91.533971x, R<sup>2</sup> = 0.44; <sup>j</sup>y = 938.214409 - 12.650231x + 0.207832x<sup>2</sup>, R<sup>2</sup> = 0.94.



**Figure 3.** Dynamics of dry matter **A**, crude protein **B**, ammonia nitrogen **C** carbon dioxide **D** in cactus pear silages associated with buffel grass pre-dried inclusion levels during oxygen exposure.

resulted in a good fermentation pattern of the silage, which possibly reduced the development of yeasts that during fermentation consume soluble carbohydrates and produce ethanol, carbon dioxide, water and ATP, generating losses due to fermentation process, thus occurring an increase in the silage DMR (Araújo et al. 2022).

The increase in DMR with the inclusion of buffel grass pre-dried is also related to the low production of gases and effluents during fermentation of the ensiled mass. The DM content present in forages directly influence the GL and EL of silages, where DM content between 28% and 40% are considered ideal for ensiling the material. DM contents below 28% make the environment favourable to effluent losses and the proliferation of undesirable microorganisms. On the other hand, DM contents greater than 40% cause problems in compaction, leading air to enter the mass, causing qualitative and quantitative damage to the ensiled material (Jobim et al. 2007).

The increase in silage temperature is also influenced by the DM content of the material, and this increase reflects the exothermic reactions occurring in the ensiled material in the presence of O<sub>2</sub>, due to respiration and multiplication of microorganisms that are harmful to the quality of the silages (Araújo et al. 2020). As the DM content increases, the greater the upward trend of temperature, due to the need to produce

less heat to raise the temperature of silages with higher water contents in the composition (McDonald et al. 1991; Wilkinson and Davies 2012).

The highest pH was identified in the silage with 15% buffel grass pre-dried (5.03), showing an increase of 32.10% in relation to the control. The resistance to pH drop that some silages present may be related to the low DM content and the high buffering capacity of the forage plant. This promotes the stabilisation of the silage at a high pH value, also resulting in the loss of protein content (Ribeiro et al. 2017). Thus, the faster the pH is reduced to values below 4, the greater the preservation of the protein and carbohydrate content of the silage (Özyurt et al. 2016).

With the increase in EC according to the increase in the levels of buffel grass pre-dried in silage composition, it is assumed that, due to the higher content of mineral matter of the buffel grass pre-dried (Table 1), there is a high concentration of dissociated ions in its composition, due to the ability of buffel grass to accumulate more  $\text{Na}^+$  while the concentrations of  $\text{K}^+$  and  $\text{Ca}^{2+}$  decreased in environments with water deficit (Ghafar et al. 2021) reaching concentrations of Mg, P, Cl and Fe of 0.9%; 0.46%; 1.37% and 0.84%, respectively (Kumar et al. 2019) which directly affects EC. Castro et al. (2006) observed a significant increase in EC when wilting was promoted to raise the DM content from 2% to 65% in silages of Tifton (*Cynodon dactylon* spp.). The reduction in the moisture content increases the dry matter content, providing a higher concentration of nutrients, including the mineral matter of the silages (Campos et al. 2021).

The low  $\text{NH}_3\text{-N}$  content found in the evaluated silages is desirable and can be classified as excellent, as the  $\text{NH}_3\text{-N}$  values found were lower than 10% (McDonald et al. 1991). The low  $\text{NH}_3\text{-N}$  content is desirable in silage because its content is antagonistic to pH decline, and evidences the beneficial effect on the destination and use of mass nitrogen, resulting from the proteolytic metabolism of clostridium (Kung et al. 2018).

Forage BC can be influenced by soluble carbohydrate, nitrogen and mineral contents. Nogueira et al. (2019) points out that the high fermentation capacity of cactus pear is related to the low DM content and the high levels of soluble carbohydrates, combined with an intermediate buffering capacity of the cactus pear. The reduction in BC is attributed to the dilution of some mineral elements of cactus pear, such as Ca and K, which are present at high concentrations  $\text{Ca} = 40.0\text{--}80$  g/kg DM and  $\text{K} = 24.22\text{--}39.18$  g/kg DM (Silva et al. 2022), resulting in high BC. When comparing the silage of cactus pear alone to the silage of cactus pear with buffel grass pre-dried, all silages are superior to those of Pacheco et al. (2014), with 41.69 mg/100 g DM for grass silage without hay addition at 52.45 mg/100 g DM for silage with 40% gliricidia hay. In this sense, Melo et al. (2022) examined the addition of cactus pear to arboreal cotton silage, and reported a growing linear effect on the BC, up to 75 mg/100 g DM, an effect attributed to the metabolism related to carbon dioxide activity in cactus pear, due to the crassulacean acid metabolism (CAM).

The aerobic deterioration process is associated with the presence of fungi, such as yeasts, which use lactic acid as a substrate and deteriorate the silage (Moon 1983). Thus, silages containing lower carbon levels and lower pH values during long periods of exposure to  $\text{O}_2$  are considered stable silages (Weinberg et al. 2011).

The increase in  $\text{CO}_2$  flux associated with the rise in temperature demonstrates the beginning of the deterioration phase. In silages with higher moisture content, much of the  $\text{CO}_2$  present is dissolved (Shan et al. 2021), an effect that explains the amount of

CO<sub>2</sub> in silages with lower DM content in this study. This effect demonstrates that the increase in DM in silages promotes an increase in osmotic pressure, which reduces the activity of microorganisms that are harmful to the quality of the silage (McDonald et al. 1991). However, lower DM values result in higher H<sub>2</sub>O activity, requiring the silage to have a high heating rate to exceed the ambient temperature. In this sense, aerobic stability is determined as the time for raising the silage temperature (post-fermentation) by 2°C above ambient temperature (Driehuis and Wikselaar 2000), characterised by the resistance of the silage to deterioration after silo opening (Jobim et al. 2007). This increase in temperature that occurs after silo opening reflects the exothermic reactions in the ensiled mass in the presence of oxygen, such as respiration and multiplication of microorganisms harmful to the quality of the silage (Araújo et al. 2020).

In general, undesirable microorganisms are sensitive to pH below 5, but are sensitive to water availability in the medium, and are usually inactive in silages with more than 28% DM, so that, in silages with DM lower than 15%, the values of pH below 4 may not fully inhibit their growth (McDonald et al. 1991), particularly clostridia and enterobacteria. Increased EC demonstrates the loss of intracellular content, being soluble substances like pectin (Krüger et al. 2020), indicating that with increasing exposure of silage to aerobic environment and increasing inclusion of buffel grass pre-dried, there was a greater loss of intracellular content of the silages.

The CP content of the silages was favoured with the inclusion of buffel grass pre-dried, resulting in CP values above the minimum necessary (7% DM) to ensure an adequate rumen fermentation (Amorim et al. 2020). Differing from our findings, Macêdo et al. (2018) added buffel grass to cactus pear silage, and observed a reduction in the CP content of silages with increasing levels of buffel grass (18.1%–12.3% DM). Nevertheless, these authors used fresh buffel grass. Thus, the pre-drying of Buffel grass contributed to the increase in the content of this nutrient.

Silages of cactus pear alone presented lower content of the fibre fraction in relation to silages containing buffel grass pre-dried in their composition. On the other hand, the contents of TC, NFC and TDN were higher in these silages, but reduced with the presence of buffel grass pre-dried in silage composition. In this sense, it is notorious that the nutritional composition of a silage will depend on the concentrations of forage plant nutrients that will be used in the ensiling process. In this context, Gusha et al. (2013) expresses the need to study the inclusion of forage hay to increase the content of DM, CP and NDF in diets, as cactus pear contains rapidly digestible carbohydrates. Thus, by including buffel grass in cactus pear silage, Macêdo et al. (2018) observed a reduction in the degradability of the silage DM.

The inclusion of up to 35% buffel grass pre-dried in cactus pear silage represents a viable strategy in the process of nutrient conservation and fermentation quality. In addition to presenting low carbon production during aerobic stability.

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CA, and Gois GC: wrote the first draft of the paper; Gois GC: reviewed and commented on the first draft. All authors reviewed and approved the final manuscript.











## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Data availability statement

Further information on the data and methodologies will be made available by the author for correspondence, as requested.

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