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RESEARCH

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Mineral profile, carbohydrates fractionation, nitrogen compounds and in vitro gas production of elephant grass silages associated with cactus pear

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Abstract

Background: The high amount of soluble carbohydrates and the reduced dry matter content in cactus pear can cause excessive fermentation, resulting in nutrient losses, when it is preserved in the silage form. Thus, the association of cactus pear with elephant grass in the production of mixed silages may reduce nutritional losses during the ensiling process. Thus, the aim was to evaluate the mineral profile, carbohydrates fractionation, nitrogen compounds, and in vitro gas production of elephant grass silages associated with a cactus pear levels (0, 150, 300, and 450 g/kg on dry matter basis). The study was carried out in a completely randomized design, with 4 treatments and 5 replications, totaling 20 experimental silos.

Results: The increase in cactus pear levels in elephant grass silages composition provided an increase in the contents of K ($P=0.013$), Ca ($P<0.001$), Mg ($P<0.001$), Na ($P=0.001$), B ($P=0.044$) and Zn ($P=0.016$), reduced P ($P=0.039$) and promoted a quadratic effect for Fe ($P=0.045$) content. The addition of cactus pear levels in elephant grass silages increased the total carbohydrates ($P<0.001$) and $A+B1$ fraction ($P=0.002$) and promoted a quadratic effect for $B2$ fraction ($P=0.032$). For nitrogen compounds, the increase in cactus pear levels in elephant grass silages composition reduced the $B1+B2$ fraction ($P=0.002$) and increased the C fraction ($P=0.007$). There was no effect of cactus pear levels on the in vitro *gas* production of elephant grass silages ($P>0.05$).

Conclusions: Under the experimental conditions, the addition of cactus pear in elephant grass silage at levels up to 450 g/kg dry matter does not affect the in vitro gas production, however, it improves the mineral profile, contributes to the increase in the total carbohydrate content and the $A+B1$ fraction in the silages. Besides this, cactus pear addition reduces the $B1+B2$ protein fraction, increasing the fraction C content in the silages, suggesting the necessity to supply an additional soluble nitrogen source for good ruminal functioning.

Keywords: CNCPS, Forage conservation, Mixed silages, Semiarid zones

Background

In dryland regions, feed scarcity is considered a significant obstacle to animal production. Therefore, farmers use forage plants adapted to water deficit conditions. Cactus pear (*Opuntia stricta Haw*) is considered a plant resistant to edaphoclimatic conditions in arid and

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semiarid regions (Pereira et al. 2021). This adaptation to adverse environments determined the efficiency of cactus pear in converting water into biomass (241.8 t/ha of green matter and 12.5 t/ha of dry matter; Borges et al. 2019). Furthermore, due to its morphology and physiological mechanism, cactus pear is considered a valuable source of nutrients (water, energy, and non-fibrous carbohydrates) and is considered a valuable food in regions where other plants are unable to survive due to extreme environmental conditions (Vastolo et al. 2020).

An interesting characteristic of the cactus pear is the reduced effluent losses due to the formation of a gel (mucilage) in the grinding process of the material (Brito et al. 2020). This mucilage consists of natural hydrocolloids distributed throughout the plant with water absorption capacity (Vieira et al. 2021) due to its hydrophilic function, which minimizes water movement and increases the material's viscosity retaining moisture (Brito et al. 2020).

Although it is possible to preserve cactus pear in silage form, the high amount of soluble carbohydrates and the reduced dry matter content (Carvalho et al. 2020) can cause excessive fermentation, resulting in nutrient losses and reduced silages aerobic stability (Brito et al. 2020). Thus, there is an interest in associating cactus pear with tropical forages, available in semiarid regions, in the production of mixed silages, improving the silage's quality, in order to increase the dry matter and fiber content of the ensiled mass, in addition to reducing nutritional losses during the ensiling process (Macêdo et al. 2018; Santos et al. 2020; Silva et al. 2021).

Elephant grass (*Pennisetum purpureum* Schum) is a grass originated in subtropical Africa and arrived in Brazil in 1920, standing out mainly in the Brazilian semiarid region. The species is commonly used in various animal production systems (Oliveira et al. 2017). As elephant grass has a high biomass production (approximately 61.14 Mg/ha; Rocha et al. 2017), fast growth, low cost, good nutritional value (229.8 g/kg dry matter; 731.3 g/kg neutral detergent fiber, and 55% in vitro dry matter digestibility; Silveira et al. 2020), and good acceptance by animals, a possible strategy to take advantage of the surplus of forage production during the favorable season is the preservation of this forage species associated with cactus pear in the mixed silage form, ensuring the availability of food to be offered to the animals during the dry period (Santos et al. 2020).

To the best of our knowledge, the effect of including cactus pear in elephant grass silage has not yet been explored sufficiently. Santos et al. (2020), when evaluating the fermentative losses that occur in cactus pear silages with the inclusion of elephant grass, obtained the answer that the inclusion of up to 40% of elephant grass in cactus pear silages reduces moisture and fermentative losses, improving the fermentation profile of silages. However, there are no studies that evaluated the mineral profile, carbohydrates fractionation, nitrogen compounds, and in vitro gas production of cactus pear silages associated with elephant grass levels. Therefore, we hypothesized that the inclusion of levels up to 45% of cactus pear in elephant grass silages improves the mineral composition without affecting the silage's gas production. Thus, the aim was to evaluate the mineral profile, carbohydrates fractionation, nitrogen compounds, and in vitro gas production of elephant grass silages associated with a cactus pear levels.

Methods

The experiment was conducted at the Universidade Federal do Agreste de Pernambuco (UFAPE), Garanhuns, Pernambuco, Brazil (8° 53′ 25″ South latitude, 36° 29′ 34″ West longitude, 896 m altitude). The climate is classified as tropical type Aw' (Köppen and Geiger 1928), with an average annual temperature of 21.2 °C, and average annual rainfall is 897 mm, with hot and dry summers and mild and humid winters.

Four levels of cactus pear inclusion (0, 150, 300, and 450 g/kg on dry matter basis) were evaluated in elephant grass silage, in a completely randomized experimental design, with five experimental silos for treatments, totaling 20 experimental silos.

Elephant grass cv. IRI-381 used for making silages came from an experimental area established after 60 days of regrowth, cut at 10 cm from the ground. Cactus pear Mexican Elephant Ear (IPA-200016) was harvested at 12 months after regrowth. The harvest was carried out manually, and the collected material was processed in a stationary forage chopper (Nogueira Pecus 9004, Saltinho–SP, Brazil) to an average particle size of 2.0 cm. Samples of the material before ensiling (original material) were collected for chemical analysis (Table 1).

The material was mixed manually. After mixing, the material was ensiled in experimental silos (10 cm in diameter, 50 cm in height) made of polyvinyl chloride (PVC), equipped with a Bunsen valve to allow the escape of gases from fermentation. At the bottom of the experimental silos, 1 kg dry sand was deposited, protected by a cotton cloth, preventing the ensiled material from coming into contact with the sand, allowing the effluent to drain.

The material was compacted with a wooden plunger, aiming to reach a minimum density of 600 kg/m^3 natural material. Silos were weighed before and after filling. Once sealed, silos were kept in a covered shed for 90 days.

Silage samples were collected during the silos' opening, with the top layer (10 cm) of each silo being discarded.

Table 1 Chemical composition of cactus pear and elephant grass

Items	Cactus pear	Elephant grass
Dry matter (g/kg FM)	74.8	218.9
Mineral matter (g/kg DM)	152.7	71.8
Organic matter (g/kg DM)	847.3	928.2
Ether extract (g/kg DM)	19.6	18.0
Crude protein (g/kg DM)	74.3	36.2
Neutral detergent fiber (g/kg DM)	280.7	720.6
Acid detergent fiber (g/kg DM)	177.7	407.3
Total carbohydrates (g/kg DM)	795.2	874.0
Non-fibrous carbohydrates (g/kg DM)	545.5	154.0

FM fresh matter, *DM* dry matter

Samples were pre-dried in a forced ventilation oven at 55 °C for 72-h and individually processed in a knife mill (Wiley mill, Marconi, MA-580, Piracicaba, Brazil) at 3 mm mesh sieve to determinate the gas production and, at 1 mm mesh sieve to determine the mineral composition, carbohydrates and nitrogen fractionation.

Chemical analyses were performed for the determination of dry matter (DM; method 967.03), mineral matter (MM; method 942.05), ether extract (EE; method 920.29), crude protein (CP; method 981.10) and acid detergent fiber (ADF; method 973.18) contents (AOAC 2016), neutral detergent fiber (NDF; Van Soest et al. 1991).

The concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were determined according Nogueira and Souza (2005). Sodium and potassium levels were determined by flame photometry, whereas the concentrations of Ca and Mg were analyzed by titration, by determining the Ca contents, and subsequently, the Ca + Mg contents, and the Mg concentration was defined as the difference. Sulfur levels were determined indirectly, first by obtaining the concentrations of sulfates, and subsequently, by considering the atomic molecular weight, the concentration of S was determined. Phosphorus was determined using a molecular spectrophotometer while the determination of B, Cu, Fe, Mn, and Zn, was performed on an atomic absorption spectrophotometer (model Analyst 100, Perkin Elmer®, Sigma-Aldrich, Germany).

Total carbohydrates (TC) were measured according to Sniffen et al. (1992):

$$\mathrm{TC}\left(\mathrm{g/kg\ DM}\right) = 1000 - (\mathrm{CP} + \mathrm{EE} + \mathrm{MM})$$

Total carbohydrates were fractionated into $A+B1$, $B2$, and C, with the non-fibrous carbohydrates (NFC), corresponding to the $A+B1$ fractions, obtained by the difference between TC and neutral detergent fiber corrected for ash and protein (NDFap). The NDFap was determined according to Licitra et al. (1996) and Mertens (2002). The fraction C was obtained by the indigestible NDF after 288 h in situ incubation, in the rumen of fistulated goats, which were fed elephant grass and concentrate (Valente et al. 2011). After the end of the incubation, the material was washed, and the residual NDF was determined. Fraction $B2$ (available fraction of fiber) was obtained by the difference between NDFap and fraction C (indigestible fiber).

Protein fractionation was calculated by the CNCPS system (Sniffen et al. 1992). The protein was fractionated into A, $B1+B2$, $B3$, and C. The levels of non-protein nitrogen (NPN: fraction A), neutral detergent insoluble nitrogen (NDIN), and acid detergent insoluble nitrogen (ADIN) were determined according to Licitra et al. (1996). Fraction A was obtained by the difference between total nitrogen (Nt) and insoluble nitrogen (residual) in trichloroacetic acid (TCA 10%), with the equation:

$$A\,(\%\,\mathrm{Nt}) = Nt - N1\,/\,\mathrm{Nt} \times 100$$

where Nt = total nitrogen sample and $N1$ = content of insoluble nitrogen in trichloroacetic acid.

The B1 fraction (rapidly degraded true protein) was obtained by the difference between the borate phosphate buffer (BFB) insoluble N minus NPN, by the equation (Sniffen et al. 1992):

$$B1\,(\%\,Nt) = (N1 - N2/Nt) \times 100$$

where $N2$ = borate phosphate buffer insoluble nitrogen.

$B2$ and $B3$ fractions (insoluble protein with intermediate-slow degradation rate in the rumen) were determined by the difference between the insoluble N in borate phosphate borate buffer and the NDIN, the NDIN minus the ADIN, respectively. The B2 and B3 fractions values are achieved with (Sniffen et al. 1992):

$$B2(\%\,\mathrm{Nt}) = (N2 - \mathrm{NDIN/Nt}) \times 100$$

$$B3\,(\%Nt) = (\mathrm{NDIN} - \mathrm{ADIN}/Nt) \times 100$$

Fraction C (insoluble protein, indigestible in rumen and intestine) was determined by the residual N content of the sample after treated with acid detergent and expressed in percentage of Nt in the sample. Fraction $B1+B2$ was obtained by the equation (Sniffen et al. 1992):

$$B1 + B2 = 100 - (A + B3 + C)$$

The in vitro gas production was determined using the pressure transducer technique proposed by Theodorou et al. (1994), using a nutrient medium from Goering and Van Soest (1970).

The rumen fluid used as inoculum was obtained, jointly and homogenized, from three goats fistulated in the rumen (average body weight of 30.45 ± 108 kg), kept confined in stalls and fed with elephant grass (*Pennisetum purpureum*) cv. IRI-381 (216.68 g/kg dry matter, 38.27 g/kg crude protein, 19.75 g/kg ether extract, and 737,78 g/kg neutral detergent fiber) and concentrate based on corn meal (891.5 g/kg dry matter, and 101.6 g/kg crude protein) and soybean meal (892.5 g/kg dry matter, and 471.8 g/kg crude protein), in addition to mineral salt (Caprinofós, Tortura, Porto Alegre, Brazil), with water ad libitum.

Ruminal inoculum was collected through the cannula and stored in an anaerobic environment in a thermal bottle, and sent to the laboratory. Ruminal content was collected before morning feeding. The solid part was collected from the rumen through the cannula and manually pressed to separate the solid part from the liquid part. Ruminal inoculum was filtered through 4 layers of gauze, constantly injecting CO_2 to maintain the anaerobic environment and kept in a water bath (TECNAL, Piracicaba, SP, Brazil) at 39 °C.

One gram of the air-dried sample was directly inserted into bottles (160 mL). Fifteen bottles were used per treatment. Ninety mL (90 mL) of nutrient medium composed of buffer solution, pH indicator solution, macro- and micro-mineral solution, sodium hydroxide solution (1Molar) and reducing solution, prepared according Theodorou et al. (1994). Ten mL (10 mL) of ruminal inoculum was added to bottles under CO_2 spraying. The bottles were sealed with stoppers and rubber seals and placed on holders. After sealing the bottles, the gases generated inside each bottle were eliminated and later the bottles were placed in an oven at a constant temperature of 39 °C during the incubation period. The same procedure was applied to the blanks (bottles containing inoculum and medium, without samples). Four bottles were used as a blank. In total, 64 bottles were incubated.

The pressure (P; in psi), originated from gases accumulated in the upper part of the vials, was measured through a pressure transducer (Datalogger Universal Logger AG100) connected to a needle (0.6 mm). Pressure readings were taken more frequently during the initial fermentation period and subsequently reduced (2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 30, 36, 48, and 72 h of incubation). After quantifying the pressure through the transducer, the vials were manually shaken in circular motions for 15 s.

Pressure data were converted to gas volume (1 psi = 4.859 mL of gas) through equation:

$$\text{Gas Production (mL)} = 5.1612 * \text{PSI}-0.3017,\ R^2 = 0.9873$$

belonging when Laboratory of Gas Production located at Universidade Federal do Agreste de Pernambuco (UFAPE), Garanhuns, Pernambuco, Brazil (− 8° 90′ 77″ S, − 36° 49′ 49″ W, altitude of 844 m). From each pressure reading, the total produced by the vials without substrate (blank) was subtracted for each sample.

Cumulative gas production data were analyzed using the Gompertz two-compartment model, cited by Schofield et al. (1994), using the NLMIXED procedure of SAS (version 9.3, SAS Institute Inc., Cary, North Carolina, USA) (SAS, 2011):

$$\text{Vt} = \text{Vf}_1/1 + e^{(2-4\text{kd1}(t-\lambda))} + \text{Vf}_2/1 + e^{(2-4\text{kd2}(t-\lambda))}$$

where Vt = maximum total volume of gas produced; Vf_1 = maximum gas volume for the fast-digesting fraction (non-fiber carbohydrates; NFC); Vf_2 = maximum gas volume for the slow digesting fraction (fibrous carbohydrates; FC); kd1 (h) = specific growth rate for the rapid degradation fraction; kd2 (h) = specific growth rate for the slow degradation fraction; λ (Lag time) = duration of initial digestion events (latency phase), common to both phases; and t (h) = fermentation time.

The Gompertz two-compartment model was chosen, assuming that the gas production rate is proportional to the microbial activity, but the proportionality decreases with the incubation time, which can be attributed to the loss of efficiency in the fermentation rate with time (Magalhães et al. 2021).

Data were analyzed using the PROC GLM of the Software *Statistical Analysis System University* (SAS 2015) by analyzing variance and regression at 5% probability. The criteria for choosing the regression models were the parameters' significance estimated by the models and the values of the coefficients of determination. The PROC REG procedure of the Statistical Analysis System (SAS, version 9.1) estimated the regression equation between pressure and volume data. Cumulative gas production data were fitted using SAS University PROC NLMIXED and were estimated by the least-squares method using the iterative Gauss Newton process. The following statistical model was used:

$$Y = \mu + Tj + e_{ij}$$

where μ = overall average; Tj = effect of the inclusion level of cactus pear; e_{ij} = residual error.

Results

The increasing levels of cactus pear levels in the elephant grass silages composition promoted a linear increase in K ($P = 0.013$), Ca ($P < 0.001$), and Mg ($P < 0.001$) contents, in contrast, the P concentration

($P = 0.039$) were reduced (Table 2). The inclusion of cactus pear did not change the N content in the elephant grass silages ($P > 0.05$; Table 2).

The cactus pear levels increased Na ($P = 0.001$), B ($P = 0.044$), and Zn ($P = 0.016$) contents in elephant grass silages and promoted a quadratic effect for Fe content ($P = 0.045$) (Table 2). There was no effect of the combination cactus pear with elephant grass in the mixed silages form for S, Cu, and Mn contents ($P > 0.05$; Table 2).

The addition of cactus pear in elephant grass silages increased the TC content ($P < 0.001$) and the $A + B1$ fraction ($P = 0.002$) and promoted a quadratic effect for the $B2$ fraction ($P = 0.032$) (Table 3). There was no effect of the inclusion of cactus pear on the C fraction of the silages ($P > 0.05$; Table 3).

For nitrogen compounds, the increase in cactus pear levels in the elephant grass silages composition reduced the $B1 + B2$ fraction ($P = 0.002$) and increased the C fraction ($P = 0.007$) (Table 3). There was no effect of cactus pear levels on the CP content and the A and $B3$ fractions of elephant grass silages ($P > 0.05$; Table 3). There was no effect of cactus pear levels on the in vitro *gas* production of elephant grass silages ($P > 0.05$; Table 4).

Discussion

The dynamics of nutrients in plants vary according to species, age, phenological stage, edaphoclimatic conditions, management practices adopted, among others (Alves et al. 2017). Cactus pear is a succulent food, rich in water and mucilage, with significant mineral content, mainly potassium (47.8 g/kg DM), calcium (50 g/kg DM), and magnesium (14.2 g/kg DM) (Mayer and Cushman 2019). Although the fermentation process in silages generates mineral losses through percolation (Oladosu et al. 2016), the contents of K, Ca, and Mg are higher in silages with cactus pear, compared to the control treatment (0 g/kg cactus pear) due to the mucilage production, which provides greater water retention capacity and reduces mineral losses because of your hydrophilic properties. According to Santana et al. (2019), these minerals have a buffering activity, neutralizing the organic acids formed by fermentation, reducing the pH drop rate.

The results obtained for K, Ca, and Mg in all studied silages are above the requirements for cattle, according

Table 2 Mineral profile of elephant grass associated with different levels of cactus pear (n = 5)

Variables	Cactus pear levels (g/kg DM)				SEM	*P*-value	
	0	150	300	450		*L*	*Q*
g/kg dry matter							
Nitrogen	9.1	8.2	8.8	9.0	0.27	0.770	0.086
Phosphorus[a]	2.2	0.9	1.1	0.5	0.45	0.039	0.430
Potassium[b]	33.6	40.2	39.3	41.0	1.49	0.013	0.138
Calcium[c]	4.6	6.9	9.2	13.2	0.54	< 0.001	0.165
Magnesium[d]	1.9	2.2	2.7	3.9	0.23	< 0.001	0.067
mg/kg dry matter							
Sulfur	1.3	0.8	1.1	1.4	0.22	0.496	0.119
Sodium[e]	509.0	549.0	551.7	544.3	4.32	0.001	0.001
Boron[f]	8.5	1.6	9.7	15.9	2.85	0.044	0.052
Copper	15.6	11.5	11.4	12.1	1.56	0.160	0.159
Iron[g]	280.9	419.7	397.9	421.7	24.16	0.006	0.045
Manganese	37.1	32.9	34.2	34.9	1.24	0.531	0.205
Zinc[h]	26.1	31.2	34.2	35.6	1.87	0.016	0.345

SEM standard error of the mean, *L* linear effect, *Q* quadratic effect

Significance at 5% of probability

[a] $\hat{y} = 1.9600–0.0333x$, $R^2 = 0.76$

[b] $\hat{y} = 35.3650 + 0.1421x$, $R^2 = 0.67$

[c] $\hat{y} = 4.2630 + 0.1875x$, $R^2 = 0.97$

[d] $\hat{y} = 1.7153 + 0.0441x$, $R^2 = 0.89$

[e] $\hat{y} = 510.36667 + 3.0911x$, $R^2 = 0.96$

[f] $\hat{y} = 4.3713 + 0.2036x$, $R^2 = 0.45$

[g] $\hat{y} = 291.2623 + 8.4154x–0.1277x^2$, $R^2 = 0.84$

[h] $\hat{y} = 27.9330 + 0.1712x$, $R^2 = 0.61$

Table 3 Carbohydrate fractionation and nitrogen compounds of elephant grass associated with different levels of cactus pear ($n = 5$)

Variables	Cactus pear levels (g/kg DM)				SEM	*P*-value	
	0	150	300	450		*L*	*Q*
Carbohydrates fractionation							
TC (g/kg DM)[a]	709.3	825.3	832.3	841.8	3.44	< 0.001	0.370
$A + B1$ (g/kg TC)[b]	294.0	418.5	437.7	453.8	15.41	0.002	0.004
$B2$ (g/kg TC)[c]	371.8	271.1	260.1	258.9	20.90	0.001	0.032
C (g/kg TC)	334.2	222.6	202.2	195.1	17.73	0.084	0.674
Nitrogen compounds							
CP (g/kg DM)	56.8	51.5	55.0	56.4	1.72	0.763	0.085
A (g/kg CP)	17.7	16.5	18.1	17.9	0.48	0.297	0.320
$B1 + B2$ (g/kg CP)[d]	950.8	951.7	944.9	944.2	1.34	0.002	0.570
$B3$ (g/kg CP)	3.6	7.5	5.0	5.0	1.15	0.777	0.132
C (g/kg CP)[e]	27.9	24.3	32.0	32.9	1.40	0.007	0.021

DM dry matter, *TC* total carbohydrates, *CP* crude protein, *SEM* standard error of the mean, *L* linear effect, *Q* quadratic effect

Significance at 5% of probability

[a] $\hat{y} = 842.8533 + 0.6956x$, $R^2 = 0.96$

[b] $\hat{y} = 203.0490 + 34.9699x$, $R^2 = 0.86$

[c] $\hat{y} = 377.8999 – 2.3490x$, $R^2 = 0.85$

[d] $\hat{y} = 951.8833 – 0.1770x$, $R^2 = 0.78$

[e] $\hat{y} = 25.8773 + 0.1507x$, $R^2 = 0.54$

Table 4 In vitro gas production of elephant grass associated with different levels of cactus pear ($n = 5$)

Variables	Cactus pear levels (g/kg DM)				SEM	*P*-value	
	0	150	300	450		*L*	*Q*
Vt (mL/g DM)	249.3	246.2	255.8	234.1	8.72	0.383	0.291
Vf1 (mL/g DM)	147.7	152.0	148.7	138.5	4.65	0.176	0.156
kd1 (mL/g DM/h)	0.07	0.08	0.08	0.08	0.003	0.067	0.157
Vf2 (mL/g DM)	102.5	94.0	105.2	94.8	7.98	0.749	0.912
kd2 (mL/g DM/h)	0.02	0.02	0.02	0.02	0.001	0.059	0.375
λ (h)	6.5	7.4	7.0	8.2	0.51	0.063	0.768

Vt, maximum total volume of gas produced; Vf1, maximum gas volume for the fast-digesting fraction (non-fiber carbohydrates; NFC); Vf2, maximum gas volume for the slow digested fraction (fibrous carbohydrates; FC); kd1, specific growth rate for the rapid degradation fraction; kd2, specific growth rate for the slow degradation fraction; λ (Lag time)—duration of initial digestion events (latency phase), common to both phases; *SEM* standard error of the mean, *L* linear effect, *Q* quadratic effect

Significance at 5% of probability

to the NRC (2000), which recommends a daily intake of 3 to 4 g/kg of K, 1.54 g/kg of Ca and 1 g/kg Mg for cattle, according to body weight. High amounts of Ca, Mg in diets containing cactus pear *in natura* or as silage can cause diarrhea in ruminants, requiring supplementation with another forage plant to achieve an adequate balance between the nutrients offered to the animals (Silva et al. 2021).

The reduction in P^+ contents with the higher presence of cactus pear in elephant grass silages composition possibly occurred due to lower uptake of this component after the harvest of cactus pear, since P^+ is a crucial nutrient in the photosynthetic processes of CAM plants (Crassulaceae Acid Metabolism), acting in the recomposition of phosphoenolpyruvate and by participating in the transport and transduction of chemical energy (ATP and NADPH) (Alves et al. 2017), as well as Na. Therefore, with the reduction in P contents due to the increase in the proportion of cactus pear in the elephant grass silages, only the control silage (0 g/kg cactus pear) would meet the need for P in the cattle diet, whose dietary requirement is 1.6 g/kg of P for maintenance, according to NRC (2000).

Sodium is a micronutrient for plants with carbon fixation via CAM, such as cactus pear. Thus, the higher levels of this mineral are due to the increased levels of cactus pear in the silages, as the forage (0.40 g/kg DM; Mayer and Cushman 2019) has a higher content of this nutrient compared to elephant grass (0.23 g/kg DM; Sáez et al. 2018). According to Muhammad et al. (2021), Na in excess can cause a reduction in chloroplast pigment contents, which can promote a lighter color of the silages. The results obtained for Na in all silages are above the 0.6–0.8 g/kg of Na recommended by the NRC (2000) in cattle diets.

Boron is the main micronutrient for the cultivation of cactus pear, mainly related to plant growth. Boron forms compounds of the cis-diol complex with plasma membrane glycoproteins and glycolipids, thus maintaining its structure. It is involved in enzymatic reactions, transport of ions, metabolites, and hormones, in addition to the transport of sugars across the membrane, synthesis of lignin and flavonoids, and metabolism of auxins, nitrogen compounds, and phenols (Brdar-Jokanovic 2020). However, there is little research referring to boron contents in cactus pear, and there are no researches that analyzed the mineral composition of mixed-grass silages associated with cactus pear. In turn, the B content found for silages containing 450 g/kg cactus pear in its composition is higher than the 11.69 mg/kg of *B* observed by Ferraz et al. (2020) evaluating the mineral composition of cactus pear.

Iron is considered an abundant mineral in Brazilian soils, which causes adequate iron concentrations in plants to meet the requirements of animals (Fink et al. 2016; Schmidt et al. 2020). In ruminants, iron deficiency causes anemia, lethargy, reduced consumption, and reduced weight gain (Papachristodoulou et al. 2015). The Fe values observed in the silages containing cactus pear in its composition are above the 104.97 mg/kg DM found by Carvalho et al. (2020) when analyzing the concentration of this mineral in cactus pear silages of the Mexican Elephant Ear genotype. However, all the studied silages presented Fe concentrations within the limit recommended by the NRC (2000) for beef cattle (50–1000 mg/kg DM).

Zinc is an mineral relevant for the growth and development of higher plants. It involves protein synthesis, energy production, and maintenance of membrane integrity (Drissi et al. 2017). For ruminants, Zn is considered a micromineral related mainly to immunity, and its deficiency can cause mortality through the decrease in lymphocytes in the blood (Hilal et al. 2016; Goff 2018). The Zn contents found in the silages containing cactus pear were greater than the results obtained by Carvalho et al. (2020), who observed a Zn concentration of 12.42 mg/kg DM. Our improved results can be attributed to the higher content of this mineral in elephant grass (31.7 mg/kg DM; Maranhão et al. 2019) compared to cactus pear (17.67 mg/kg DM; Mayer and Cushman 2019).

Despite the importance that minerals play in the metabolic functions of ruminant animals, few studies assess the concentration of minerals in the forage that will be offered in the diet of animals in confinement, making it necessary to search for this information for adequate animal nutrition.

The cactus pear addition in elephant grass silages provided a beneficial effect for the carbohydrates fractionation, increasing the TC and the $A+B1$ fraction contents in relation to control silage (0 g/kg cactus pear). Higher values of the $A+B1$ fraction (consisting of soluble sugars, pectin, and starch) are desirable since silages with high contents of this fraction are considered good energy sources for developing microorganisms that use NFC as a substrate, since their efficiency of microbial growth requires energy. Furthermore, high $A+B1$ levels are connected to the time microorganisms remain in the rumen (Pessoa et al. 2020).

In this regard, the faster the passage of microorganisms, the less energy will be used for maintenance and the greater the efficiency of microbial synthesis (Santos et al. 2019). Although when the $A+B1$ fraction predominates among the diet's carbohydrates, it reduces ruminal microbial protein synthesis, probably due to the inefficiency of microorganisms, driving the ATP excess to carbohydrate maintenance and synthesis functions. Thus, it is necessary to include protein sources of rapid and medium degradation in the rumen to synchronize energy and nitrogen release (Negrão et al. 2020). This timing in the ruminal environment improves nitrogen ion capture and ATP usage efficiency and increases microbial protein synthesis (Harun and Sali 2019).

The decrease in the $B2$ fraction, corresponding to potentially degradable fibrous carbohydrates, coincided with the increase in $A+B1$ fractions, which reinforces the hypothesis of Sharma et al. (2020) of natural decomposition of the cell wall caused by fermentation inside the silo, which contributes to the increase in fractions of high availability. The reduction in the $B2$ fraction in silages with greater participation of cactus pear can be explained by the lower levels of NDF that cactus pear has (280.71 g/kg DM) compared to elephant grass (720.61 g/kg DM) (Table 1).

When evaluating the carbohydrates fractionation in cactus pear *in natura*, Pessoa et al. (2020) observed TC—812–836 g/kg DM; $A+B1$—626–819 g/kg TC; $B2$—125–257 g/kg TC and C—54.6–116 g/kg TC. Regarding the fractionation of carbohydrates in elephant grass silages, Ribeiro et al. (2014) obtained TC—857 g/kg DM; $A+B1$—74 g/kg TC; $B2$—556 g/kg TC and C—370 g/kg TC. Thus, we can observe in this study that the use of cactus pear in elephant grass silages is beneficial for the complementation of the analyzed fractions.

According to Epifanio et al. (2014) and Santos et al. (2019), grasses have a high content of the *B*2 fraction, thus, by reducing elephant grass in silages, the energy source of rapid availability is increasing, and this demands NPN for good ruminal functioning, since ruminal microorganisms, structural carbohydrate fermenters, use ammonia as a N source. However, high NPN levels can cause N losses in the absence of readily available carbon for microbial protein synthesis (Bach et al. 2005). Therefore, the characterization of the fractions that constitute the carbohydrates in feed represents an important tool for the adequacy of the nutrients to be offered to animals through the formulated diets, aiming at maximizing the ruminal microbial development and, consequently, the best prediction of animal performance, and rationalizes the use of resources in production systems (Gozho and Mutsvangwa 2008; Gómez et al. 2016; Santos et al. 2019).

Fractions *B*1 and *B*2 in the present study were considered as a single fraction (*B*1 + *B*2) and refer to true soluble and insoluble proteins with medium degradation speed. This fraction presented a reduction with the inclusion of cactus pear in the silages, thus decreasing the availability of nitrogen for the microbial population of the rumen, interfering in the efficiency of microbial protein production at the ruminal level.

The increase in fraction *C* (ADIN) in silages containing cactus pear can occur due to the formation of Maillard reaction products caused by undesirable fermentations resulting from the increase in temperature in silages with high moisture content (Ferreira et al. 2018). In this case, the increase in this fraction content can be attributed to the cactus pear levels, which presented in its composition, low dry matter content (74.8 g/kg natural matter) compared to elephant grass (218.9 g/kg natural matter) (Table 1).

The use of 300 and 450 g/kg cactus pear levels in elephant grass silage, although it resulted in an increase in the *C* fraction, promoted in the silages, in general, satisfactory values of fractions *B*1 + *B*2 and *B*3 which in a feeding system, should be considered in the formulation of rations, constituting, therefore, a food alternative for ruminants. Thus, we can infer that the nitrogen compounds fractionation in foods that will compose diets for ruminants allows us to estimate their respective levels, as well as the greater or lesser escape of ruminal nitrogen (Ahvenjärvi et al. 2018). With this information, it is possible to develop nutritional strategies to improve nitrogen use, both by ruminal microorganisms and by the ruminant, improving their product performance and reducing losses to the environment. Due to the absence of studies that evaluated the nitrogen compounds in elephant grass silages containing cactus pear in its composition, we can observe that the values obtained in this study are close to the findings by Magalhães et al. (2021) when evaluating the fractionation of nitrogen compounds from cactus pear *in natura*. The authors found values of *A*—90.9 g/kg CP; *B*1 + *B*2—53.4 g/kg CP; *B*3—173 g/kg CP and *C*—200 g/kg CP.

In general, forage plants rich in non-fibrous carbohydrates, such as cactus pear, have higher gas production (Magalhães et al. 2019). A fact observed by Magalhães et al. (2021) who, when evaluating the in vitro gas production of cactus pear genotypes observed that the cactus pear *Opuntia stricta* Haw presented a gas production of 193.2 mL/g of dry matter, while Souza (2019) when analyzing the in vitro gas production oof elephant grass genotypes of different sizes under irrigation, observed that elephant grass cv. Iri 381 presented a gas production of 120.92 mL/g of dry matter. However, in the present study, the association of elephant grass silage with cactus pear levels, up to 450 g/kg of dry matter, did not alter the in vitro gas production, with average values of Vt = 246.35 mL/g dry matter; Vf1 = 146.72 mL/g dry matter; kd1 = 0.077 mL/g dry matter/h); Vf2 = 99.12 mL/g dry matter; kd2 = 0.02 mL/g dry matter/h, and; λ 7.27 h. The absence of effect on in vitro gas production demonstrates that silages, despite differences in carbohydrate contents, do not change their gas production kinetics. Thus, it is suggested that more studies be carried out, testing cactus pear inclusion levels in elephant grass silages, above the 450 g/kg of dry matter used in this research. In this way, we can have a better estimate regarding what proportion of cactus pear should be added in elephant grass silages in order to have a significant result for the in vitro gas production.

Conclusions

Under the experimental conditions, the addition of cactus pear in elephant grass silage at levels up to 450 g/kg dry matter does not affect the in vitro gas production, however, it improves the mineral profile, contributes to the increase in the total carbohydrate content and the *A* + *B*1 fraction in the silages. Besides this, cactus pear addition reduces the *B*1 + *B*2 protein fraction, increasing the fraction *C* content in the silages, suggesting the necessity to supply an additional soluble nitrogen source for good ruminal functioning in a ruminant feeding system.

Abbreviations

ADF: Acid detergent fiber; ADIN: Acid detergent insoluble nitrogen; B: Boron; Ca: Calcium; Cu: Copper; CP: Crude protein; DM: Dry matter; λ: Duration of initial digestion events; EE: Ether extract; *t*: Fermentation time; Fe: Iron; Mg: Magnesium; Mn: Manganese; Vf_1: Maximum gas volume for the fast-digesting fraction; Vf_2: Maximum gas volume for the slow digested fraction; Vt: Maximum total volume of gas produced; MM: Mineral matter; NDF: Neutral detergent fiber; NDIN: Neutral detergent insoluble nitrogen; N: Nitrogen; NFC: Non-fibrous carbohydrates; NPN: Non-protein nitrogen; P: Phosphorus; PVC: Polyvinyl chloride; K: Potassium; Na: Sodium; kd1: Specific growth rate for the rapid degradation fraction; kd2: Specific growth rate for the slow degradation fraction; S: Sulfur; TC: Total carbohydrates; TCA: Trichloroacetic acid; Zn: Zinc.

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

Declarations

Ethics approval and consent to participate
The present study was appreciated and approved by the Ethics Committee on the Use of Animals (CEUA) of the Brazilian Agricultural Research Corporation (Embrapa Semiárido; Opinion no. 0004/2016).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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