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Research Note

Nutritional properties and *in vitro* gas production in cactus pear (*Opuntia stricta*) and cassava (*Manihot esculenta*) shoot silages

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This study evaluated the effects of different inclusion levels of cactus pear (*Opuntia stricta*) (at 0%, 15%, 30% or 45% on fresh matter basis) ensiled with shoots of cassava (*Manihot esculenta*) on the mineral nutrients, carbohydrates fractionation, nitrogen compounds and *in vitro* gas production, using a completely randomised design consisting of four treatments and five replicates per treatment, totalling 20 experimental units. There were significant increases ($p < 0.05$) in the concentrations of Mg, B, Fe, total carbohydrates and fraction A+B1 (non-fibre carbohydrates) with increased cactus pear inclusion, whereas nitrogen and fractions B2 (available fibre) and C (insoluble protein, indigestible in rumen and intestine) significantly decreased ($p < 0.05$) with the inclusion. The inclusion of cactus pear significantly ($p < 0.05$) reduced crude protein content and the fractions A (non-protein nitrogen) and B3 (insoluble protein with a slow degradation rate in the rumen) in the silages. Fraction B1+B2 (rapidly degraded true protein + insoluble protein with an intermediate degradation rate in the rumen) significantly increased ($p < 0.05$) with increasing levels of cactus pear inclusion. Gas production parameters showed a quadratic effect for Ca, Mn, observed gas volume, estimated gas volume by the bicompartmental model, rate of degradation of fibre carbohydrates, and rate of degradation of non-fibre carbohydrates ($p < 0.05$). The use of cactus pear increased the content of soluble sugars in mixed silages made with the aerial part of cassava plants. However, gas production was low with the inclusion of 45% cactus pear. Based on the overall results, the combination of 45% cactus pear with 55% of the aerial part of cassava in mixed silages is recommended.

Keywords: carbohydrates, forage preservation, fractionation, *Manihot esculenta,* mineral nutrients, nitrogen, rumen degradability

The seasonality of forage production is intrinsic to cultivation environments, with different periods and intensities in each biome. Thus, in semiarid regions the use of silage is fundamental for the nutritional quality and productive efficiency of ruminant production systems (Gomes et al. 2022). In this scenario, cactus pear (*Opuntia* spp., family Cactaceae) have high efficiency in the use of soil and water resources as compared with grasses, for example, providing high production of phytomass and nutrients (Kumar et al. 2021).

The cactus pear (*Opuntia stricta*), also known as common prickly pear, is widely used in dryland regions as a component of agricultural production systems that aim at an efficient supply of forage for animal feeds (Alves et al. 2022). In South Africa, there are at least 42 cactus pear cultivars available and being researched to describe their morphological and nutritional attributes (du Toit et al. 2018; Novoa et al. 2019; Mabotja et al. 2021).

One factor limiting the production of cactus pear silage is its low dry matter content, which results in undesirable fermentation and effluent production (Araújo et al. 2020). However, cactus pear contains mucilage, which causes water retention, in addition to the availability of carbohydrates, a characteristic that provides substrates for fermentation (Pereira et al. 2020). Species like cassava (*Manihot esculenta*) are also grown in semiarid conditions because of their capacity to tolerate drought and low soil fertility. Furthermore, cassava is known to have high production potential under these marginal conditions while retaining high nutritional value (Bilong et al. 2022; Thanni et al. 2022). However, cassava was considered unsuitable for ensiling because of its low concentration of soluble carbohydrates combined with high crude protein content (Lima et al. 2022). These factors limit the availability of substrates and increase the buffering capacity of the ensiled mass.

The *in vitro* gas production technique simulates rumen fermentation and can be used to predict patterns of rumen fermentation. This technique simulates the enzymatic digestion and allows estimations of the digestibility of dry matter and organic matter, indicating the final products produced by fermentation, such as gases and short-chain fatty acids (Menezes et al. 2022).

In vitro gas production results almost entirely from the carbohydrates present in the incubated material and indicates degradation by ruminal microorganisms. Thus, the determination of carbohydrate fractions and nitrogen compounds and the kinetic parameters of ruminal degradation are extremely important for animal nutritionists. This information can be used in the formulation of diets for ruminants, maximising the synchronisation of the degradation of carbohydrates and nitrogenous compounds, minimising energy and nitrogen losses caused by ruminal fermentation and promoting greater efficiency of microbial synthesis (Magalhães et al. 2021).

In this context, the use of forage with high nutritional value and the complementary characteristics for silage-making are of great value, as long as the ideal proportions are known. Thus, the aim of this study was to evaluate the effects of different inclusion levels of the cactus pear ensiled with cassava shoots on the mineral content, carbohydrates fractionation, nitrogen compounds, and *in vitro* gas production.

The work was carried out at the experimental farm of the Federal University of Agreste de Pernambuco, located in the municipality of Garanhuns in mesoregion of Agreste Meridional of Pernambuco State, Brazil (8°53′25″ S, 36°29′34″ W; 96 m asl). The climate is classified as tropical type Aw, with an average annual temperature of 21.2 °C and average annual rainfall of 897 mm, with hot and dry summers and mild and humid winters (de Souza Cunha et al. 2022).

To prepare the silage, the shoots of cassava plants were manually harvested after 12 months of cultivation, taking advantage of the final third of the branch consisting of leaf, petiole and stem. The plants had an average height of 1.35 m, and the upper-third was cut approximately 45 cm from the ground. The collected material was chopped using a stationary forage machine (PP-35; Pinheiro Máquinas, Itapira, São Paulo, Brazil) and processed to particles of an average size of 2.0 cm. The cactus pear used was the clone IPA/200016 Orelha de Elefante Mexicana of species *Opuntia stricta* (Haw.) Haw., which was processed in a razor slicer (JK 500 2CV 60Hz; Rio de Janeiro, Brazil), allowing the cladodes to be cut to 2 × 2 cm cubes. The cactus pear was harvested preserving the basal cladode, in a cactus plantation of two years of establishment, without irrigation, and grown with the use of 40 tons ha^{-1} bovine manure. Samples of the material before ensiling (original material) were collected for further laboratory analysis (Table 1).

The forage was then mixed with levels of inclusion of cactus pear on a fresh matter basis, at 0%, 15%, 30% or 45%, with 5 replicates per treatment, totalling 20 experimental units, in a completely randomised design. The material was compacted with a wooden plunger, aiming to reach a density of 600 kg m^{-3} natural matter (NM). The material was ensiled in experimental silos (100 mm in diameter, 550 mm in height) made of polyvinyl chloride and equipped with a Bunsen valve to allow the escape of gases from fermentation. At the bottom of each experimental silo, 2 kg of dry sand was deposited, protected by a cotton cloth, preventing the ensiled material from coming into contact with the sand and allowing effluent to drain. The silos were weighed before and after filling, and then opened 90 days after sealing.

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

Initially, for the determination of minerals, the glassware was washed in running water, followed by immersion in nitric acid solution (5%) and abundant rinsing in ultrapure water (18.3 MΩ cm^{-1}). Tubes used for digestion that still showed some residue were washed in running water and immersed in a 5% neutral detergent solution, brushed until the complete elimination of residues, and finally washed again with running water. Next, the glass tubes were immersed in a plastic vat with a nitric acid solution (5%) for 48 h, followed by rinsing with ultrapure water (18.3 MΩ cm^{-1}). The glassware was later placed to dry in an oven (SolidSteel; Dubesser, Santo André, SP, Brazil) at 60 °C and stored in a protected place.

The Kjeldahl method was used to determine the total nitrogen (N) of the samples (AOAC 2016). Solubilisation (digestion) by wet route was used, through nitric perchloric solubilisation. The samples were solubilised with nitric (65%) and perchloric (70%) acids to determine the elements potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) (Malavolta et al. 1997). Potassium and sodium (Na) were determined by flame emission spectrometry (SP-500F/59; SP Labor, Presidente Prudente, SP, Brazil). The spectrophotometer was calibrated with the standards 0 and 50 mg l^{-1} for K, and 0 and 10 mg l^{-1} for Na, respectively, for readings 0 and 100. The reading of the analytical curve was subsequently done to obtain the reading of each sample. Phosphorus analysis was carried out by molecular spectrometry (K37-VIS; Kasvi, Nova Odessa, SP, Brazil) with the reading in the spectrophotometer at 420 nm, then building the analytical curve and estimating the concentration of P. Calcium and Mg were determined by atomic absorption spectrometry. Sulfur was analysed by the turbidimetry method, with readings of the sample made after 5 min in the turbidimeter. Determinations of B, Cu, Fe, Mn and Zn were performed with an atomic absorption spectrophotometer (Analyst 100; Perkin Elmer, Sigma-Aldrich, Germany). Chlorides (Cl) were determined by the argentometric method with visual detection of the equivalence point (Mohr's method) (Azmat et al. 2021)

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

$$TC = 100 - [CP + EE + MM] \quad (1)$$

Table 1: Chemical composition of the cactus pear and the aerial part cassava shoots before ensiling. SE = standard error; DM = dry matter; MM = mineral matter; OM = organic matter; EE = ether extract; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; TC = total carbohydrates; NFC = non-fibre carbohydrates; N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; Na = sodium; S = sulfur; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc

Variable	Cactus pear		Cassava	
	Mean ± SE	Range (minimum–maximum)	Mean ± SE	Range (minimum–maximum)
DM (g kg^{-1} NM)	74.80 ± 1.18	72.67–88.16	268.51 ± 2.95	268.17–270.64
MM (g kg^{-1} DM)	152.74 ± 2.14	143.47–162.93	58.05 ± 3.25	57.61–59.15
OM (g kg^{-1} DM)	847.26 ± 2.14	836.00–889.12	942.30 ± 3.25	940.64–948.12
EE (g kg^{-1} DM)	19.65 ± 0.83	19.35–20.18	39.01 ± 2.17	38.61–39.09
CP (g kg^{-1} DM)	74.29 ± 0.99	73.57–74.81	219.21 ± 0.65	218.94–220.41
NDF (g kg^{-1} DM)	280.71 ± 3.47	279.06–281.29	427.94 ± 0.97	426.12–428.94
ADF (g kg^{-1} DM)	177.73 ± 2.70	177.54–179.12	286.13 ± 4.34	285.32–289.83
TC (g kg^{-1} DM)	795.24 ± 10.68	794.36–796.97	683.82 ± 9.11	682.06–689.17
NFC (g kg^{-1} DM)	545.49 ± 4.39	544.12–546.07	255.90 ± 6.17	254.21–260.39
N (g kg^{-1})	11.88 ± 0.85	10.89–12.64	42.96 ± 0.92	40.16–43.21
P (g kg^{-1})	1.54 ± 5.78	1.24–1.72	1.24 ± 1.49	1.21–1.25
K (g kg^{-1})	45.65 ± 2.07	45.12–47.00	23.90 ± 1.09	23.00–24.14
Ca (g kg^{-1})	22.15 ± 0.90	21.69–23.64	11.10 ± 1.45	10.45–12.06
Mg (g kg^{-1})	6.89 ± 0.57	6.05–7.18	1.90 ± 3.02	1.87–1.93
Na (g kg^{-1})	330.00 ± 1.24	329.47–331.26	388.00 ± 0.78	387.02–389.46
S (mg kg^{-1})	2.08 ± 0.43	1.98–2.69	2.15 ± 0.64	2.14–2.16
B (mg kg^{-1})	59.52 ± 0.67	58.25–60.21	67.18 ± 0.98	66.39–68.03
Cu (mg kg^{-1})	17.09 ± 1.33	16.98–18.45	15.66 ± 1.0	15.00–16.80
Fe (mg kg^{-1})	115.04 ± 0.94	114.02–116.17	297.54 ± 2.01	295.15–299.75
Mn (mg kg^{-1})	38.30 ± 0.63	38.00–39.16	248.58 ± 1.45	247.01–249.16
Zn (mg kg^{-1})	22.39 ± 2.49	20.94–22.71	55.73 ± 4.12	52.16–58.42

and then divided into fractions A+B1, B2 and C. Non-fibre carbohydrates (NFC) correspond to fraction A+B1, through the difference between TC and the value of neutral detergent fibre corrected for ash and protein (NDFap). The NDFap value was determined according to Licitra et al. (1996) and Mertens (2002). The indigestible NDF corresponds to fraction C, obtained after 288 h of *in situ* incubation in rumen fistulated goats fed elephant grass and concentrate feed (Valente et al. 2011). After the end of incubation, material was washed and the residual NDF was determined. Fraction B2 corresponds to the available fibre, obtained by the difference between NDFap and fraction C.

Nitrogen compounds were fractionated into A, B1+B2, B3, and C (Sniffen et al. 1992). The contents of crude protein (CP, method 981.10) followed the method recommended by AOAC (2016). 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 10%, calculated as:

$$A\ (\%Nt) = Nt - N1 / Nt \times 100 \quad (2)$$

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, as follows (Sniffen et al. 1992):

$$B1\ (\%Nt) = (N1 - N2 / Nt) \times 100 \quad (3)$$

where N2 = borate phosphate buffer insoluble nitrogen. Fractions B2 and B3 (insoluble protein with an intermediate or slow degradation rate in the rumen, respectively) were determined by the differences between the insoluble N in borate phosphate borate buffer and the NDIN (fraction B2), and the NDIN minus the ADIN (fraction B3). Thus, values of the B2 and B3 fractions were achieved as follows (Sniffen et al. 1992):

$$B2\ (\%Nt) = (N2 - NDIN/Nt) \times 100 \quad (4)$$

$$B3\ (\%Nt) = (NDIN - ADIN/Nt) \times 100 \quad (5)$$

Fraction C (insoluble protein, indigestible in rumen and intestine) was determined by the residual N content of the sample after being treated with acid detergent and was expressed as the percentage of Nt in the sample.

Fraction B1+B2 was obtained by the following equation (Sniffen et al. 1992):

$$B1+B2 = 100 - [A + B3 + C] \quad (6)$$

Fraction B3 was obtained by the difference between NDIN and ADIN, and fraction C was considered as ADIN.

The *in vitro* gas production was carried out in duplicate according to the method of Theodorou et al. (1994). For this, 1 g of dry sample and 90 ml nutrient medium (buffer solution, pH indicator solution, macro and micro mineral solutions, 1M sodium hydroxide solution, and reducing solution) were added to glass flasks (160 ml). Next, 10 ml of ruminal inoculum (from three goats fistulated in the rumen) was added; the animals donating the ruminal inoculum were fed elephant grass and concentrate feed.

Ruminal inoculum was collected 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 four layers of gauze, constantly injecting CO_2 to maintain the anaerobic environment, and kept in a water bath (Tecnal Scientific Equipment, Piracicaba, SP, Brazil) at 39 °C.

The inclusion of the ruminal inoculum in glass flasks was carried out under a constant flow of CO_2, sealed and placed in an oven at a constant temperature of 39 ºC during the incubation period. The same procedure was applied to the blanks (glass flasks containing inoculum and nutrient medium, without samples).

Pressure originating from the gases accumulated in the upper part of the vials was measured using a pressure transducer (Datalogger Universal Logger AG100) connected to a needle (0.6 mm) recorded at 2, 4, 6, 8, 10, 12, 14, 24, 30, 48, 54 and 72 h of incubation. After quantifying the pressure through the transducer, the vials were manually shaken in circular motions for 15 sec. Pressure data were converted to gas volume (1 psi = 4.859 ml of gas) through the equation:

$$\text{Gas production (ml)} = 5.1612 \cdot \text{PSI} - 0.3017,\ R^2 = 0.9873 \quad (7)$$

at the Laboratory of Gas Production located at the Universidade Federal do Agreste de Pernambuco (UFAPE), Garanhuns, Pernambuco, Brazil. From each pressure reading, the total produced gas by the vials without substrate (blank) was subtracted for each sample.

The variables analysed in the *in vitro* test were: observed volume of gas (OVG, ml g^{-1} DM); estimated gas volume by the bicompartmental model (EGVB, ml g^{-1} DM); degradation of fibre carbohydrates (DFC, ml g^{-1} DM); rate of degradation of fibre carbohydrates (RDFC, ml h^{-1}); degradation of non-fibre carbohydrates (DNFC, ml g^{-1} DM); RDNFC: rate of degradation of non-fibre carbohydrates (RDNFC, ml h^{-1}), and colonisation time (*Lag time*, h).

Data were analysed using PROC GLM in the SAS system (SAS 2015) by analysis of variance and regression at 5% probability. As criteria for selecting regression models, the significance of parameters estimated by the models and the values of the coefficients of determination were adopted. 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:

$$Y = \mu + Tj + eij \quad (8)$$

where μ = overall mean; Tj = effect of the level of inclusion of cactus pear; and eij = residual error.

Nitrogen levels decreased ($p < 0.01$) with an increasing proportion of cactus pear in the silage (Table 2). The reduction in N is associated with the lower content of this element in cactus pear (11.88 g kg^{-1}) (Table 1), and thus decreased its density in the ensiled mass. The reduction in N contributed directly to the reduction in CP and fraction A (non-protein nitrogen) (Table 3). According to Campos et al. (2021), fermentation in silage alters the N content and N compounds as a result of the action of proteolytic enzymes and the activity of harmful microorganisms and the preservation of forage (*Clostridium* and enterobacteria) that act in the deamination and decarboxylation of proteins (He et al. 2020).

The Ca and Mg contents were higher in cactus pear cladodes (22.15 g kg^{-1} Ca; 6.85 g kg^{-1} Mg) compared with in cassava shoots (11.10 g kg^{-1} Ca; 1.90 g kg^{-1} Mg) (Table 1), and this may have provided an increase in Ca and Mg in the silage with an increase in cactus cladode inclusion rates in the current study ($p < 0.01$) (Table 2). High contents of Ca and Mg in cactus pear were also reported by Mabotja et al. (2021) when evaluating the nutritional variability in 42 cultivars of spineless cactus pear cladodes for crop improvement in South Africa. Those authors found values of 1 820 and 600.67 mg per 100 g dry weight of *Opuntia ficus-indica*, for Ca and Mg, respectively.

Although the fermentation process generates mineral losses through percolation (Mordenti et al. 2021), the Ca and Mg contents remained higher in silage with higher levels of cactus pear, even with greater water activity inside the silo, which can be explained by the hydrocolloid properties and mucilage production of cactus pear that provides greater water-holding capacity (Liguori et al. 2021) and consequently retains minerals because of its hydrophilic properties. The results obtained for Ca and Mg in all studied silages are above the daily intake requirements for cattle (Table 2) as determined by the NRC (2016).

Increased levels of cactus pear significantly increased ($p < 0.01$) the contents of B and Fe in the silage. The increase in B content is an important finding because it acts to improve the immune system and calcium metabolism in mammals (Sharma et al. 2020), in addition to a beneficial action on reproductive activity in males (testes) and direct action on the thyroid (Ibrahim et al. 2019). However, low or high levels of B deserve concern (Abdelnour et al. 2018) and adjustment in dietary supplementation. An increase in Fe content in silage allows for gains in animal performance. For instance, de Souza Cunha et al. (2022) state that iron deficiency in ruminants causes anaemia, lethargy, reduced intake and weight gain. However, excess iron in the diet can negatively affect the productive performance of ruminants, as the supply of iron-rich foods can affect the use of minerals such as Cu, P, Z and Mg (Kupczyński et al. 2017; Wysocka et al. 2020). The observed values of Fe in the silages are above the 104.97 mg kg^{-1} DM found by Carvalho et al. (2020) when analysing the Fe concentration in cactus pear silage. Thus, we can infer that cassava influenced the increase in Fe levels in the silages tested in this study. Also, according to Carvalho et al. (2020), the increase in Fe content in

Table 2: Macro and micro minerals from mixed silages of cassava shoots and cactus pear, compared between the studied silages and the daily intake requirements for cattle as determined by the National Research Council of the United States (NRC 2016) (N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; Na = sodium, S = sulfur; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc). SEM = standard error of the mean; significance at 5% probability

Variable		Inclusion levels of cactus pear				SEM	*p*-value		Nutrient requirements of beef cattle*		
		0%	15%	30%	45%		Linear effect	Quadratic effect	Feeding growing and finishing cattle	Feeding gestational cows	Maximum tolerable concentration
					Macro minerals (g kg^{-1})						
N [1]	Mean ± SE	36.54 ± 1.83	30.27 ± 1.22	27.04 ± 1.52	23.95 ± 1.34	0.86	<0.01	0.09	–	–	–
	Range (min–max)	97.71−102.19	95.35−106.94	99.65−105.78	104.06−110.20						
P	Mean ± SE	0.86 ± 0.13	0.87 ± 0.17	0.95 ± 0.04	0.60 ± 0.19	0.17	0.39	0.24	1.6	1.6−2.6	20.00
	Range (min–max)	60.16−84.54	52.56−87.86	29.56−119.75	7.33−59.38						
K	Mean ± SE	32.86 ± 1.08	33.42 ± 5.79	26.20 ± 0.59	26.31 ± 0.83	6.09	0.34	0.97	1.9−3.3	1.8−5.8	4.00
	Range (min–max)	97.43−116.62	86.48−95.97	85.05−89.86	77.25−83.25						
Ca [2]	Mean ± SE	13.21 ± 0.68	13.08 ± 0.77	14.52 ± 0.27	17.16 ± 0.12	0.47	<0.00	0.01	1.54	2.10	30.00
	Range (min–max)	109.25−123.78	95.74−112.42	99.47−104.58	98.79−118.62						
Mg [3]	Mean ± SE	2.11 ± 0.11	2.32 ± 0.13	2.81 ± 0.08	3.30 ± 0.17	0.07	<0.01	0.06	1.00	1.20	0.40
	Range (min–max)	102.39−117.78	80.56−93.16	81.18−86.83	76.94−87.17						
Na	Mean ± SE	370.50 ± 6.69	379.00 ± 2.00	299.97 ± 0.85	393.00 ± 0.27	44.26	0.95	0.35	0.60-0.80	0.60-0.80	–
	Range (min–max)	89.07−95.99	87.86−100.60	95.30−101.32	88.78−111.15						
					Micro minerals (mg kg^{-1})						
S	Mean ± SE	1.89 ± 0.46	1.74 ± 0.45	1.58 ± 015	2.17 ± 0.47	0.23	0.53	0.14	0.15	0.15	3.00−5.00
	Range (min–max)	52.56−84.18	57.69−78.91	58.42−79.96	74.02–93.12						
B [4]	Mean ± SE	31.93 ± 8.11	54.73 ± 6.24	55.33 ± 3.09	63.70 ± 7.40	3.75	<0.01	0.07	–	–	–
	Range (min–max)	32.92−59.09	65.59−93.99	77.65−84.94	78.35−111.24						
Cu	Mean ± SE	18.55 ± 0.65	15.62 ± 0.54	21.87 ± 0.28	15.76 ± 0.98	2.41	0.84	0.52	10.00	10.00	40.00
	Range (min–max)	112.30−122.65	88.07−104.66	93.72−117.59	82.82−100.82						
Fe [5]	Mean ± SE	279.25 ± 2.09	330.89 ± 2.94	377.21 ± 5.01	455.72 ± 3.02	33.15	<0.01	0.69	50.00	50.00	500.0
	Range (min–max)	83.31−99.79	82.94−109.87	124.78−183.32	181.41−234.24						
Mn [6]	Mean ± SE	225.79 ± 4.05	155.67 ± 3.39	153.82 ± 4.02	173.10 ± 7.44	19.94	0.09	0.04	20.00	40.00	1 000.0
	Range (min–max)	80.38−92.03	62.06−74.24	73.80−83.20	87.23−109.04						
Zn	Mean ± SE	49.75 ± 2.75	36.58 ± 1.08	42.45 ± 2.27	42.37 ± 8.32	5.39	0.51	0.24	30.00	30.00	500.0
	Range (min–max)	83.33−92.32	61.30−75.81	84.01−97.72	69.21−85.97						

*NRC (2016) – Macro minerals = daily requirements of macro minerals (g day^{-1}) according to live weight; Micro minerals = daily requirements of micro minerals (mg day^{-1}) according to live weight

Equations:

[1] $\hat{y} = 35.6 - 0.273x$, $R^2 = 0.966$

[2] $\hat{y} = 12.5 + 0.886x$, $R^2 = 0.821$

[3] $\hat{y} = 2.03 + 0.027x$, $R^2 = 0.972$

[4] $\hat{y} = 37.0 + 0.639x$, $R^2 = 0.825$

[5] $\hat{y} = 274.4 + 3.838x$, $R^2 = 0.985$

[6] $\hat{y} = 223.4 - 5.536x + 0.099x^2$, $R^2 = 0.967$

Table 3: Fractionation of carbohydrates, nitrogen compounds and *in vitro* gas production from mixed silages of cassava shoots and cactus pear. SEM = standard error of the mean; L = linear effect; Q = quadratic effect; significance at 5% probability

Variable		Inclusion levels of cactus pear				SEM	*p*-value	
		0%	15%	30%	45%		Linear effect	Quadratic effect
Carbohydrates								
TC (g kg^{-1} DM) [1]	Mean ± SE	676.00 ± 1.84	717.66 ± 2.18	731.00 ± 3.77	743.57 ± 3.30	6.51	<0.01	0.04
	Range (min–max)	664.01–694.20	703.52–728.57	713.73–746.96	732.55–759.82			
A+B1 (g kg^{-1} TC) [2]	Mean ± SE	355.27 ± 1.80	404.58 ± 2.48	427.82 ± 3.47	454.75 ± 6.44	17.38	<0.01	0.53
	Range (min–max)	343.46–379.20	373.19–425.76	378.46–482.26	417.12–486.09			
B2 (g kg^{-1} TC) [3]	Mean ± SE	353.28 ± 1.84	327.41 ± 6.48	313.04 ± 9.95	295.15 ± 7.10	20.34	0.04	0.84
	Range (min–max)	341.17–367.48	292.12–356.34	245.89–357.01	210.49–331.79			
C (g kg^{-1} TC) [4]	Mean ± SE	313.35 ± 4.59	268.00 ± 6.70	259.13 ± 2.84	250.11 ± 7.14	16.05	0.01	0.28
	Range (min–max)	277.04–376.50	217.90–295.53	241.73–271.85	213.14–271.48			
Nitrogen compounds								
CP (g kg^{-1} DM) [5]	Mean ± SE	228.42 ± 0.60	189.21 ± 8.78	169.03 ± 10.98	149.69 ± 9.67	5.39	<0.01	0.09
	Range (min–max)	209.67–240.33	179.16–197.34	161.00–184.87	138.84–157.90			
A (g kg^{-1} CP) [6]	Mean ± SE	32.96 ± 0.60	32.10 ± 0.61	28.88 ± 1.13	24.93 ± 1.06	0.84	<0.01	0.09
	Range (min–max)	A (g kg^{-1} CP)	32.08–33.37	31.21–32.57	27.62–30.34			
B1+B2 (g kg^{-1} CP) [7]	Mean ± SE	701.10 ± 1.28	696.49 ± 1.97	741.33 ± 1.79	771.78 ± 1.45	10.30	<0.01	0.11
	Range (min–max)	677.65–745.65	685.00–709.75	728.90–756.18	752.70–797.96			
B3 (g kg^{-1} CP) [8]	Mean ± SE	176.62 ± 2.09	155.14 ± 1.35	135.79 ± 2.82	112.40 ± 1.47	10.67	<0.01	0.93
	Range (min–max)	135.90–204.95	123.97−171.27	112.93−152.55	100.47−125.42			
C (g kg^{-1} CP)	Mean ± SE	89.31 ± 2.90	116.24 ± 8.85	93.99 ± 7.90	90.88 ± 9.48	10.52	0.71	0.17
	Range (min–max)	80.59−107.06	87.77−158.46	72.81−129.14	80.00−100.00			
In vitro gas production								
OVG (ml g^{-1} DM) [9]	Mean ± SE	200.80 ± 8.24	200.89 ± 8.06	202.49 ± 6.40	186.24 ± 8.50	3.92	0.03	0.05
	Range (min–max)	193.32–212.41	189.52–208.57	195.24–209.01	174.95–195.55			
EGVB (ml g^{-1} DM) [10]	Mean ± SE	195.70 ± 7.87	195.91 ± 8.16	197.75 ± 6.43	181.58 ± 8.02	3.82	0.03	0.05
	Range (min–max)	188.65–206.84	184.51–203.94	190.32–204.31	170.98–190.38			
DFC (ml g^{-1} DM)	Mean ± SE	101.53 ± 10.75	103.65 ± 6.62	97.64 ± 7.30	92.53 ± 7.02	4.05	0.09	0.39
	Range (min–max)	94.49–117.60	97.90–113.10	92.77–108.50	83.80–99.48			
RDFC (ml h^{-1}) [11]	Mean ± SE	0.026 ± 0.001	0.026 ± 0.001	0.023 ± 0.001	0.025 ± 0.001	0.004	0.01	0.02
	Range (min–max)	0.026–0.027	0.026–0.027	0.021–0.025	0.024–0.026			
DNFC (ml g^{-1} DM)	Mean ± SE	94.63 ± 3.85	92.79 ± 6.68	101.24 ± 5.15	89.64 ± 3.83	2.51	0.57	0.07
	Range (min–max)	89.66–99.05	84.30–100.20	96.63–108.30	85.43–94.02			
RDNFC (ml h^{-1}) [12]	Mean ± SE	0.100 ± 0.002	0.096 ± 0.003	0.090 ± 0.003	0.094 ± 0.004	0.001	<0.01	0.04
	Range (min–max)	0.09–0.10	0.09–0.09	0.08–0.09	0.08–0.09			
Lag time (h)	Mean ± SE	3.41 ± 0.12	3.29 ± 0.23	3.62 ± 0.27	3.65 ± 0.42	0.14	0.13	0.61
	Range (min–max)	3.26–3.54	2.98–3.52	3.24–3.85	3.14–4.03			

Fractionation of carbohydrates: TC = total carbohydrates; A+B1 = non-fibre carbohydrates; B2 = available fibre; C = indigestible fibre Nitrogen compounds: CP = crude protein; A = non-protein nitrogen; B1 = rapidly degraded true protein; B2 = insoluble protein with intermediate degradation rate in the rumen; B3 = insoluble protein with slow degradation rate in the rumen; C = insoluble protein, indigestible in rumen and intestine *In vitro* gas production: OVG = observed volume of gas; EGVB = estimated gas volume by the bicompartmental model; DFC = degradation of fibre carbohydrates; RDFC = rate of degradation of fibre carbohydrates; DNFC = degradation of non-fibre carbohydrates; RDNFC = rate of degradation of non-fibre carbohydrates; Lag time = colonisation time

Equations:

[1] $\hat{y} = 684.6 + 1.440x$, $R^2 = 0.903$

[2] $\hat{y} = 362.3 + 2.144x$, $R^2 = 0.968$

[3] $\hat{y} = 350.5 – 1.258x$, $R^2 = 0.985$

[4] $\hat{y} = 302.4 – 1.323x$, $R^2 = 0.832$

[5] $\hat{y} = 286.9 – 1.850x$, $R^2 = 0.996$

[6] $\hat{y} = 33.8 – 0.182x$, $R^2 = 0.936$

[7] $\hat{y} = 689.1 + 1.712x$, $R^2 = 0.865$

[8] $\hat{y} = 176.8 – 1.413x$, $R^2 = 0.998$

[9] $\hat{y} = 203.9 – 0.280x$, $R^2 = 0.508$

[10] $\hat{y} = 198.8 – 0.270x$, $R^2 = 0.487$

[11] $\hat{y} = 0.02 – 0.0001x + 0.000003x^2$, $R^2 = 0.563$

[12] $\hat{y} = 0.100 – 0.0005x + 0.000009x^2$, $R^2 = 0.901$

the ensiled material can be attributed to contamination of the phytomass after being chopped in a forage machine. Another point presented by Azevedo et al. (2018) is that high levels of Fe in the plants used to make silage would probably be related to the good availability of Fe in the soil, so it is pertinent to carry out an analysis of the soil composition in future studies. Therefore, all studied silages meet the iron requirements for cattle (Table 2), in line with NRC (2016).

A quadratic effect was observed for Mn concentration (p = 0.04) with increasing levels of cactus pear in the cassava shoot silage (Table 2). The increase in Mn may be associated with the epiphytic population of lactic acid bacteria that accumulate Mn as a defence mechanism against hydrogen peroxide (Si et al. 2017). As outlined by the NRC (2016), the studied silages meet the mineral requirements of Mn for cattle (Table 2). Thus, there is an increase of this mineral in the silage, thus favouring a source of Mn suitable for ruminants, promoting the activation of enzymes (pyruvate carboxylase), preventing the oxidation of lipids by free radicals, in addition to acting in the synthesis of the bone matrix, through cartilage synthesis (NRC 2007). However, the absorption of Mn by ruminants depends on several factors, such as age, physiological stage of the animal, and antagonistic minerals present in the diet (Oberson et al. 2019). Regarding antagonistic minerals, Fe shares a mutual absorption pathway with Mn, competing for binding sites on transferrin, which is the Fe transporting protein in plasma. With this, there is an inversely proportional relationship: when there is a reduction in the concentration of Fe in the body, there is an increase in transporters in the cell membrane, and consequently an increase in the absorption of Mn (Ye et al. 2017). Henry et al. (2000) report that excess Ca and P in the diet had an antagonistic effect on Mn absorption in dairy calves. Bueno et al. (2019) state that Mn may have an antagonistic interaction with Se, although few studies of this have been carried out. The inclusion of cactus pear did not significantly change (p > 0.05) the contents of P, K, Na, S, Cu and Zn in the silages.

The use of cactus pear in ensiling combined with the cassava shoots increased the content of TC (p < 0.01) and the fraction A+B1 (p < 0.01) (Table 3), providing greater availability of substrates for fermentation, in addition to being characterised as a good energy quality for ruminal microorganisms that use non-fibre carbohydrates (Assis et al. 2021). Antagonistic to the content of fraction A+B1, fractions B2 (p = 0.04) and C (p = 0.01) showed a reduction in their content with the inclusion of cactus pear (Table 3).

The inclusion of cactus pear resulted in a reduction (p < 0.01) in the CP content of the silage. However, the contents of crude protein in the silages are sufficient to ensure good fermentation at the rumen level, with values over 7%, which is considered the minimum protein to maintain good ruminal functioning (Van Soest 1994). A change in the dynamics of N compounds was observed, promoting the reduction in fraction A (p < 0.01) and fraction B3 (p < 0.01), but an increase in fraction B1+B2 (p < 0.01) (Table 3). Ensiling cactus pear with the aerial part of cassava plants did not change the indigestible fraction C (p > 0.05) (Table 3).

During fermentation, the interaction between the chemical characteristics of the plant and the population of microorganisms generates several metabolic pathways which confer the preservation or modification of the chemical constituents. Among the changes, protein hydrolysis is considered limiting in forage with high protein content, favouring proteases and an increase in free amino acids and peptides (He et al. 2020). Thus, the increase in non-protein nitrogen (fraction A) indicates an improvement in the nutritional characteristics of the silage.

The increase in the carbohydrate content is related to the increase in the proportion of the cactus pear as a consequence of its higher TC content. This effect enabled the increase of fraction A+B1, which corresponds to the carbohydrates that present rapid degradation rates, as in the case of sugars and their conjugates, such as glucose and disaccharides, in addition to carbohydrates that present values of intermediate degradation kinetics, such as starch, fruits and galactans, as well as pectin that is not a carbohydrate but is part of this group (Magalhães et al. 2019; Navarro et al. 2019; Villalba et al. 2021), and cactus pear notably presents a pectin content of 133.7–212.8 g kg^{-1} DM (Pessoa et al. 2020). Even though pectin is a constituent of the cell wall, it has high solubility and offers increased digestibility of DM (Villalba et al. 2021). Magalhães et al. (2021) suggest that higher concentrations of fractions A and B1 indicate that most carbohydrates are available for use by ruminal microorganisms, confirming that the silages studied have energy potential. In this study, the silages showed a reduction in fraction C with an increase in cactus pear levels in the silage, which may result in high digestibility of fibrous carbohydrates. Thus, the importance of carbohydrate fractions ingested by ruminants is based on the classification of ruminal bacteria regarding the use of carbohydrates that form the plant cell wall and of carbohydrates located in the cellular content without structural functions (Magalhães et al. 2019). Indeed, these factors were reflected in the gas production dynamics *in vitro*.

The inclusion of cactus pear resulted in a reduction in the CP content of silage and in fraction A, but an increase in fraction B1+B2 (true proteins). When a food has a high content in the fraction of rapid degradation, a source of carbohydrates (high degradation rate) is necessary for a synchronism between the degradation of carbohydrates and proteins for the microbial protein synthesis at an appropriate ruminal level. Fraction B3 (slow ruminal degradation) has a ruminal degradation rate of 0.02–1.0% h^{-1} (Santos et al. 2019).

The use of cactus pear resulted in a decrease in the OVG (p = 0.03) and in EGUB (p = 0.03) (Table 3). A quadratic effect was observed for RDFC (p = 0.02) and RDNFC (p = 0.04) with increasing levels of cactus pear in the cassava shoot silage (Table 3). Ensiling cactus pear with cassava shoot silage did not change the DFC, DNFC, and lag time of indigestible fraction C (p > 0.05) (Table 3).

The production of gases indicates the ability of fermentation and conversion of carbohydrates into potential energy for ruminants. Thus, stoichiometry of the fermentation of carbohydrates (hexoses) at the rumen level produces short-chain fatty acids; the propionate (C3) in its formation does not produce carbon dioxide, favouring the reduction of gas production (Silva de Oliveira et al. 2022). Therefore, diets or foods rich in non-fibre carbohydrates with high content of starch and pectin, for example, provide

greater propionate production. This affects the degradation of carbohydrates, because of the rise of pH that provides cellulolytic microorganisms with more suitable conditions in the rumen (Villalba et al. 2021).

The use of cactus pear when ensiling the aerial part of cassava plants improved the mineral profile, nitrogen and carbohydrate fractions, and it reduced *in vitro* gas production. Therefore, the combination of 45% cactus pear with 55% of the aerial part of cassava in mixed silages is indicated.

Owing to a high number of animals and high demands for water, in addition to energy and protein, the use of food and diets with high water content and considerable nutrient content has become an indispensable strategy and practice for animal production in arid and semiarid regions. However, additional studies are needed using mixed cactus pear and cassava shoots silages in animal feeding and performance trials to obtain more accurate results.

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