FULL ARTICLE

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Fermentation profile, chemical composition, and aerobic stability of cassava shoots silages with cactus pear

Perfil fermentativo, composição química e estabilidade aeróbia de silagens de brotos de mandioca com palma forrageira

Cleyton de Almeida Araújo[1]; Getúlio Figueiredo de Oliveira[1]; Marcelo de Siqueira Pinto[2]; Diego de Sousa Cunha[3]; Raquel da Silva Lima[2]; Claudenilde de Jesus Pinheiro Costa[2]; Daniel Anderson de Souza Melo[2]; Fleming Sena Campos[4]; André Luiz Rodrigues Magalhães[2]; Glayciane Costa Gois[4]

[1] Universidade Federal do Vale do São Francisco, Programa de Pós-graduação em Ciência Animal, Petrolina – PE, Brazil
[2] Universidade Federal do Agreste de Pernambuco, Programa de Pós-graduação em Ciência Animal e Pastagens, Garanhuns – PE, Brazil
[3] Universidade Federal do Tocantins, Programa de Pós-graduação em Ciência Animal Tropical, Araguaiana – TO, Brazil
[4] Universidade Federal do Maranhão, Programa de Pós-graduação em Ciência Animal, Chapadinha – MA, Brazil

ABSTRACT

The aim was to evaluate the effect of the cactus pear levels (0, 15, 30, and 45%) in cassava shoot silages on the fermentation profile, chemical composition, and aerobic stability. Four treatments were tested with five repetitions, totaling 20 silos. The inclusion of cactus pear in cassava shoots silages promoted an increase in gas losses ($P < 0.001$), buffering capacity ($P < 0.001$), pH ($P = 0.033$), mineral matter ($P < 0.001$), total carbohydrates ($P < 0.001$), non-fiber carbohydrates ($P < 0.001$) and a decreasing in dry matter ($P < 0.001$), organic matter ($P < 0.001$), ether extract ($P = 0.002$), and crude protein ($P < 0.001$) content. A quadratic effect was observed for effluent losses ($P < 0.001$), with greater effluent losses (94.78 kg/t natural matter) obtained with the inclusion of 30% cactus pear in cassava shoots silages. Cactus pear inclusion in cassava shoots silage promoted a reduction in the pH during oxygen exposure ($P = 0.008$). Including cactus pear in up to 45% of cassava silage, shoots cause changes in the fermentation and nutritional characteristics. However, values found in the silages are by good quality standards.

Keywords: Dry matter. Fermentative profile. *Manihot esculenta* Crantz. *Opuntia stricta* Haw.

RESUMO

Objetivou-se avaliar o efeito de níveis de palma forrageira (0, 15, 30 e 45%) em silagens de brotos de mandioca sobre o perfil fermentativo, composição química e estabilidade aeróbica. Quatro tratamentos foram testados com 5 repetições, totalizando 20 silos. A inclusão de palma forrageira nas silagens de broto de mandioca promoveu aumento nas perdas por gases ($P < 0,001$), capacidade tampão ($P < 0,001$), pH ($P = 0,033$), matéria mineral ($P < 0,001$), carboidratos totais ($P < 0,001$), carboidratos não fibrosos ($P < 0,001$) e diminuição nos teores de matéria seca ($P < 0,001$), matéria orgânica ($P < 0,001$), extrato etéreo ($P = 0,002$) e proteína bruta ($P < 0,001$). Efeito quadrático foi observado para perdas por efluentes ($P < 0,001$), sendo as maiores perdas por efluentes (94.78 kg/t natural matter) obtidas com a inclusão de 30% de palma forrageira nas silagens de brotos de mandioca. A inclusão de palma forrageira na silagem de brotos de mandioca promoveu redução do pH durante a exposição ao oxigênio ($P = 0,008$). A inclusão de palma forrageira em até 45% na silagem de brotos de mandioca provoca alterações nas características fermentativas e nutricionais, porém, os valores encontrados nas silagens estão de acordo com os padrões de silagens de boa qualidade.

Palavras-chave: Matéria seca. Perfil fermentativo. *Manihot esculenta* Crantz. *Opuntia stricta* Haw.

Correspondence to:
Glayciane Costa Gois
Universidade Federal do Maranhão, Programa de Pós-graduação em Ciência Animal,
CEP: 65500-000, Chapadinha – MA, Brazil
e-mail: glayciane_gois@yahoo.com.br

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Introduction

Cactus pear (*Opuntia stricta* Haw) is a forage resource widely used in animal feed in arid and semi-arid regions due to its adaptation to soil and climate conditions (Silva et al., 2015), the ability to reduce water intake by animals and energy availability (Nascimento Junior et al., 2022). However, the cactus pear has mucilage composed of glycoprotein and organic acids, which confers the ability to retain water due to the hydrocolloid substance (Du Toit et al., 2019). When the cactus pear is ensiled with absorbent additives or prepared as mixed/complete silages, it aims to mitigate the effect of moisture on undesirable fermentation patterns (Brito et al., 2020). However, ensiling losses should not be restricted to the fermentation process alone. It should extend to the supply to animals with high aerobic stability (Köhler et al., 2019).

Cassava (*Manihot esculenta* Crantz) is a plant of the Euphorbiaceae family widely cultivated in the Brazilian semi-arid region due to its ability to adapt to low fertility soils and climatic adversities. It has good dry matter (DM) productivity (4,422 – 8,554 kg/ha) (Fernandes et al., 2016) and is considered the fourth most crucial commodity after rice, wheat, and corn (Tinini et al., 2021). During the cassava root harvest period, only 10% of the upper third of the plant is reused for a new planting. The remaining volume of the aerial part (branch and leaves) is discarded (Ferreira et al., 2009) due to the little knowledge of nutritional value and forms of use (Yabuta et al., 2021). Thus, due to the high content of crude protein (214.4 g/kg DM), neutral detergent fiber (440.9 g/kg DM) (Barrio et al., 2023), and total digestible nutrients (571.0 g/kg DM) (Oliveira et al., 2023) that the upper third of cassava has, it is considered a low-cost forage alternative that can be included in ruminant nutrition in natural form, hay, or silage (Barrio et al., 2023; Oliveira et al., 2023).

Characterizing the preservation of moist by-products using the ensiling technique on the productive, fermentation, nutritional, and food security aspects requires studies and characterizations (Daniel et al., 2019). Therefore, cassava shoots can be added in the ensiling process associated with cactus pear, increasing the dry matter, crude protein, and neutral detergent fiber content of the ensiled material, being able to improve the fermentation profile, the nutritional value and reduce effluent losses.

To the best of our knowledge, studies on the association of cassava shoots and cactus pear in the composition of mixed silages are scarce. However, they are necessary to generate knowledge about the forage resources in semi-arid regions, mainly considering the growing need for evaluations that enable inexpensive alternative feedstuffs for ruminants that can meet nutritional requirements. Thus, we hypothesize that the association of cactus pear and cassava shoots in the composition of mixed silages reduces effluent losses and improves the nutritional value of silages.

Thus, the aim was to evaluate the effect of the cactus pear levels (0, 15, 30, and 45%) in cassava shoot silages on the fermentation profile, chemical composition, and aerobic stability.

Material and Methods

The study was carried out at the Federal University of Agreste de Pernambuco (UFAPE), Garanhuns, Pernambuco - Brazil (8°53′25″ S, 36°29′34″ W, 96 m altitude). The climate is classified as tropical type Aw, with hot, dry summers and mild, wet winters. The average annual temperature and precipitation are 21.2 °C and 897 mm, respectively (Araújo et al., 2023).

A completely randomized design was adopted with four cactus pear levels (0, 15, 30, and 45%) in the cassava shoot and five replicates, totaling 20 silos.

Cassava shoots were cut 12 months after planting. The manual cut was 45 cm above the ground, and the plants had an average height of 1.5 cm. The upper third of the plants, with the tenderest leaves and stems, were harvested. The cactus pear (Orelha de Elefante Mexicana) was harvested from an established area for 12 months and maintained without irrigation. The cactus pear was cut, preserving the mother cladode. After the cactus pear harvest, the cladodes were processed in a razor cutter, which allows the cladodes to be cut into 2 x 2 cm cubes for ensiling.

The material was processed in a stationary forage (PP-35, Pine Machinery, Itapira, São Paulo, Brazil) chopper.

After, the sample of the fresh material was separated for laboratory analysis (Table 1). The chopped material was homogenized and ensiled in experimental silos made of polyvinyl chloride (PVC) tubes, 10 cm in diameter and 50 cm long, equipped with a Bunsen valve to allow gas outflow. At the bottom of the experimental silos, we placed a sand layer protected by a cotton cloth, preventing the forage from coming into contact with the sand and allowing the effluent to drain.

Experimental silos were opened at 90 days, discarding the top and bottom layers (10 cm). Samples were taken to determine the composition. Upon silo opening, density (D; kg/m^3), effluent losses (EL; kg/t natural matter), gas losses (GL; % dry matter), and dry matter recovery (DMR; %) (Araújo et al., 2022). The equations used to estimate are described below:

$$D = m / V \tag{1}$$

Where: D = density; m = weight of ensiled material; V = volume of ensiled material.

$$EL = \left[\left(WEBo - WEb \right) - \left(WEBc - Tb \right) \right] / FWs \times 1000 \tag{2}$$

Where: EL = effluent losses; WEBc= Weight of empty bucket + weight of sand at the closing; WEBo= Weight of empty bucket + weight of sand at the opening; WEb= Weight of empty bucket (kg); FWs = Forage weight at the sealing.

$$GL = \left(WSSc - WOs \right) / \left(FM \times FDM \right) \times 10000 \tag{3}$$

Where: GL = gas losses; WSSc = Weight of the sealed silo at the closing, WOs = Weight of the opened silo, FM = Forage mass (kg); FDM = Forage dry matter concentration.

$$DMR = \left(DMop \times 100 \right) / DMcl \tag{4}$$

Table 1 – Chemical composition of cactus pear and cassava shoot before ensiling

Variables	Cactus pear	Cassava shoot
Dry matter, g/kg NM	74.80	268.51
Mineral matter, g/kg DM	152.74	58.08
Organic matter, g/kg DM	847.26	942.30
Ether extract, g/kg DM	19.50	39.01
Crude protein, g/kg DM	74.79	219.21
Neutral detergent fiber, g/kg DM	280.71	427.94
Acid detergent fiber, g/kg DM	177.73	286.13
Total carbohydrates, g/kg DM	795.24	683.82
Non fiber carbohydrates, g/kg DM	545.49	255.90

DM = dry matter; NM = natural matter.

Where DMop = dry matter content at the opening; DMcl = forage dry matter content at closing

Upon opening, the silage mass was measured for temperature, pH, and ammonia nitrogen (N-NH3; %) for the fermentation profile. The silage temperature was measured using a digital thermometer (Thermometer, Tp101, Franca, São Paulo, Brazil), with an electrode inserted in the ensiled mass. To determine the pH of the silages, 25 g of sample were homogenized with 100 mL of distilled water. The solution remained at rest for 1 hour, and subsequently, the pH was measured with a portable digital meter (Marconi® MA-552, Piracicaba, São Paulo, Brazil) previously calibrated. For the ammonia nitrogen content, 50 g of silage sample was transferred to the blender and homogenized with 200 mL of potassium chloride solution (2 mol/L) for 10 min. The solution was filtered in a Whatman 54-type filter, and then 10 mL of the solution was transferred to digester tubes containing 250 mg of calcined magnesium oxide. Then, distillation was performed to capture ammonia (Bolsen et al., 1992). NH_3–The equation calculated n content about the total N (TN):

$$NH_3 - N = \left(ammonia\ N \times 100 \right) / TN \tag{5}$$

Where TN = obtained by dividing crude protein values by the factor 6.25.

Buffering capacity (BC) was determined according to the methodology of Playne & McDonald (1966), using 15 g fresh sample, expressed in milligram equivalent (e.g.,/100 g dry matter) of alkali, required to change the pH from 4.0 to 6.0 per 100 g dry matter, after correction for the titration value of 250 mL of distilled water, 15 to 20 g of silage were weighed and macerated in a blender with 250 mL of distilled water. The macerate was titrated to pH 3.0 with 0.1N HCl to release bicarbonates and CO_2 and then titrated to pH 6.0 with 0.1N NaOH. Subsequently, the BC value was calculated by the equation:

$$BC = \left[\left(0.1 \times \left(Va - Vb \right) \right) / DSW \right] \times 100 \tag{6}$$

Where: BC = buffering capacity; 0,1 = NaOH normality; Va = NaOH spent to change the pH of the sample from 4.0 to 6.0; Vb = NaOH spent to change blank pH from 4.0 to 6.0; DSW = dry sample weight = [(sample weight × dry matter)/100].

In chemical analyses, samples of the material before and after opening the silages were pre-dried in a forced ventilation oven at 55 °C for 72 h and processed in a knife mill (Wiley mill, Marconi, MA-580, Piracicaba, Brazil), using porosity 1 mm. The following were determined: dry matter (DM), mineral matter (MM), crude protein (CP), ether extract (EE) (Association of Official Analytical Chemists, 2016), neutral (NDF), and acid detergent fiber (ADF) (Van Soest et al., 1991). Total carbohydrates (TC) (Sniffen et al., 1992), non-fiber carbohydrates (NFC) (Hall, 2003), and hemicellulose (HEM) (Association of Official Analytical Chemists, 1995) were calculated using the equations:

$$TC = 1000 - (CP + EE + MM) \tag{7}$$

$$NFC = TC - NDF \tag{8}$$

$$HEM = NDF - ADF \tag{9}$$

Aerobic stability (AS; h) was evaluated following the methodology of Kung Júnior et al. (2000), in which each experimental unit consisted of a plastic container with a capacity of 4 L, with approximately 2 kg silage, kept in a closed room, under controlled temperature (24 ± 1 °C). The temperature (surface and internal) of the ensiled mass was monitored with the aid of a digital infrared thermometer (Benetech, Rio de Janeiro, RJ, Brazil), and the internal temperature (T, in °C) of the silages was measured at 1-h intervals for 120 hours. When the internal temperature of the silages reached 2 °C above the ambient temperature, the beginning of deterioration was considered. The pH of the silages was determined every 6 h during 96 h of exposure to the air of the ensiled masses. The following were determined: maximum pH, maximum temperature (MT; °C), time to reach maximum silage temperature (TRMST; h), maximum difference in the temperature of the silage about the environment (MDT; °C), and aerobic stability (AS, h).

The results obtained were analyzed using PROC GLM in the Software Statistical Analysis System (SAS University) and subjected to analysis of variance and regression at 5% probability. As a criterion for choosing regression models, the significance of the parameters estimated by the models and the values of the coefficients of determination were adopted. The following statistical model was used:

$$Y = \mu + Tj + eij \tag{10}$$

Where: μ = overall mean; Tj = effect of inclusion of cactus pear; eij = residual error.

Results

Cactus pear increased GL, with a higher GL for 30% inclusion (*P < 0.001*). A quadratic effect was observed for EL, with a maximum production of 94.78 kg/t natural matter with the inclusion of 30% cactus pear (*P < 0.001*). Including cactus, pear did not affect DMR and silage density (*P > 0.05*). Cactus pear increased pH, with a higher GL for 45% inclusion *(P = 0.033)*. The BC increases by 0.006 for every 15% inclusion of cactus pear (*P < 0.001*). NH_3-N and D were not influenced by the cactus pear inclusion (*P > 0.05*) (Table 2).

Cactus pear levels in cassava shoots silages reduced the DM, inversely proportional to MM content, which increased by 0.50% for each 1% cactus pear inclusion (*P < 0.001*). The OM, EE, and CP content decreased with the cactus pear inclusion (*P < 0.05*). The TC and NFC content increased with the cactus pear inclusion (*P < 0.05*). The use of cactus pear did not affect NDFap, ADF, HEM, and TDN (*P > 0.05*) (Table 3).

The aerobic stability of the silages was not affected by the inclusion of cactus pear (*P > 0.05*) (Table 4).

Discussion

The results of GL demonstrate that there was a greater respiratory activity of the plant cells and enzymes in the aerobic phase of the silo, which allowed for greater GL with the inclusion of 30% cactus pear and indicated higher activity of undesirable microorganisms, which converted

Table 2 – Fermentation losses and fermentation profile of silage of cassava shoots with inclusion of cactus pear levels

Variables	Cactus pear levels				SEM	P-value	
	0%	15%	30%	45%		L	Q
Gas losses, %DM[1]	12.15	14.36	17.16	17.03	0.74	<0.001	0.142
Efluent losses, kg/t NM[2]	62.64	92.74	94.78	91.43	3.98	<0.001	<0.001
Dry matter recovery, %DM	89.89	87.58	88.08	89.81	1.78	0.976	0.281
Density, kg/m[3]	601.60	580.72	616.75	607.97	17.2	0.489	0.732
Buffering capacity, e.g/100 g DM[3]	57.90	62.04	69.13	82.38	2.05	<0.001	0.047
pH[4]	3.94	4.14	4.14	4.26	0.087	0.033	0.637
Ammonia nitrogen, % total nitrogen	12.64	10.86	12.42	11.10	0.699	0.349	0.751
Temperature, °C	25.00	25.00	25.25	25.00	0.125	0.663	0.337

DM = dry matter; NM = natural matter; SEM = standard error of the mean; L = linear; Q = quadratic; Significant at the 5% probability level. Equations: [1]$\hat{y} = 12.559250 + 0.116367x$, $R^2 = 0.96$; [2]$\hat{y} = 63.779000 + 2.261767x - 0.037167x^2$, $R^2 = 0.96$; [3]$\hat{y} = 55.785222 + 0.536982x$, $R^2 = 0.93$; [4]$\hat{y} = 3.984500 + 0.006300x$, $R^2 = 0.86$.

Table 3 – Chemical composition of silage of cassava shoots with inclusion of cactus pear levels

Variables	Cactus pear levels				SEM	P-value	
	0%	15%	30%	45%		L	Q
Dry matter, g/kg NM[1]	287.91	258.10	230.64	204.51	3.99	<0.001	0.654
Mineral matter, g/kg DM[2]	62.43	63.50	74.84	83.81	1.98	<0.001	0.070
Organic matter, g/kg DM[3]	937.56	936.49	925.15	916.18	1.98	<0.001	0.070
Ether extract, g/kg DM[4]	33.13	29.61	25.12	22.91	2.03	0.002	0.754
Crude protein, g/kg DM[5]	228.42	189.21	169.03	149.69	5.39	<0.001	0.091
Neutral detergent fiber, g/kg DM	435.80	427.42	418.45	405.17	13.28	0.115	0.857
NDFap, g/kg DM	337.14	331.77	337.78	334.20	12.04	0.959	0.942
Acid detergent fiber, g/kg DM	285.37	286.68	272.14	295.72	13.33	0.786	0.420
Total carbohydrates, g/kg DM[6]	676.00	717.66	731.00	743.57	6.51	<0.001	0.046
Non-fiber carbohydrates, g/kg DM[7]	240.19	290.23	312.54	338.41	12.97	<0.001	0.370
Hemicellulose, g/kg DM	150.43	142.60	130.85	119.11	14.34	0.092	0.362
Total digestible nutrients, g/kg DM	755.64	761.35	767.45	776.48	9.03	0.115	0.857

SEM = standard error of the mean; L = linear; Q = quadratic; Significant at the 5% probability level; DM = dry matter; NM = natural matter; NDFap = neutral detergent fiber corrected for ash and protein. Equations: [1]$\hat{y} = 286.941500 - 1.850983x$, $R^2 = 0.99$; [2]$\hat{y} = 59.830750 + 0.503133x$, $R^2 = 0.92$; [3]$\hat{y} = 940.169250 - 0.503133x$, $R^2 = 0.92$; [4]$\hat{y} = 32.969500 - 0.234367x$, $R^2 = 0.98$; [5]$\hat{y} = 222.548250 - 1.709117x$, $R^2 = 0.96$; [6]$\hat{y} = 684.650750 + 1.440383x$, $R^2 = 0.90$; [7]$\hat{y} = 247.801250 + 2.113083x$, $R^2 = 0.96$.

Table 4 – Aerobic stability of silage of cassava shoots with inclusion of cactus pear levels

Variables	Cactus pear levels				SEM	P-value	
	0%	15%	30%	45%		L	Q
Maximum pH	4.18	4.77	4.25	4.46	0.155	0.670	0.247
Maximum temperature, °C	26.50	28.25	25.50	25.50	0.67	0.080	0.218
TRMST, h	55.50	78.00	17.75	1.25	12.99	14.17	2.251
MDT, °C	2.30	3.25	1.50	1.42	0.51	0.079	0.309
Aerobic stability, h	90.00	78.75	96.00	96.00	4.18	0.084	0.203

SEM = standard error of the mean; L = linear; Q = quadratic; Significant at the 5% probability level; TRMST = time to reach maximum silage temperature; MDT = maximum difference in the temperature of the silage about the environment.

soluble carbohydrates into the water, acetic acid, carbon dioxide, and ethanol (Reis et al., 2022). Including cactus pear promoted a greater supply of soluble carbohydrates with fermentative potential, increasing gas production.

Plants with high moisture content, when ensiled, are responsible for the high production of effluents during fermentation (McDonald et al., 1991), promoting the leaching of water-soluble components and an increase in the fiber components of silage. Nevertheless, the cactus pear has mucilage that is composed of glycoprotein and organic acids, which confers the water retention capacity (Du Toit et al., 2019) due to the hydrocolloid substance, which prevents the excessive loss of water due to the formation of an emulsifying gel. In this sense, silages with a 30% inclusion of cactus in the silage of cassava shoots promoted the most significant losses by effluents (94.68 kg/t NM).

The increase in BC may be associated with the presence of orthophosphates, sulfates, nitrates, and salts of organic acids (anions) (McDonald et al., 1991) in cactus pear. These factors alter the buffer dynamics of the silage. Buffering capacity refers to the resistance of the ensiled mass to pH oscillation. Thus, the higher the BC, the longer the ensiled mass remains with high pH, requiring a more significant activity of the lactic acid bacteria to reduce the pH. A reduced acidification rate makes it possible to increase the activity of microorganisms harmful to the quality of the silage (enterobacteria, fungi, and yeasts). The increased inclusion of cactus pear promoted a reduction in the dry matter content of the silage and an increase in the water potential, interfering with the silage BC (Araújo et al., 2020).

The increase in pH may be related to variations in the acidity of cactus pear, which changes according to the harvest period (Corrales-García et al., 2004). The high pH is associated with secondary fermentations, reducing silage quality (Cantoia Júnior et al., 2020). Faustino et al. (2003) observed values from 3.99 to 4.04 for silages of cassava shoots alone, while cactus pear silages combined with legumes presented pH values from 4.1 to 4.2 (Gusha et al., 2015), similar to the values found in this study.

Including cactus pear in up to 45% NM reduced DM contents by up to 28.96%. Thus, cactus pear is an option for the ensiling process, as it has high NFC concentrations, providing high fermentation capacity and low BC (Brito et al., 2020).

The increase in the MM content of silages with cactus pear is due to the high concentrations of water, minerals, and organic acid as a physiological adaptation to persist to water deficit. The higher values of MM in cactus pear (152.74 g/kg DM) about the percentages in cassava shoots (58.08 g kg^{-1} DM) positively affect the increase of this nutrient. The values observed in the present study are similar to Gusha et al. (2015) for cactus pear silage combined with hay from *Acacia angustissima* (10.61%), *Leucaena leucocephala* (91.9%), *Calliandra callothrysus* (9.62%) and *Macroptilium atropurpureum* (9.32%). According to Rodrigues et al. (2016), cactus pear averages 82.52 ± 9.55 g/kg of MM.

There was a decrease in the CP content of the silages with the increase in the levels of cactus pear in the composition, which was expected since cactus pear has a lower CP concentration than cassava shoots (Table 1). However, CP contents in silages were above the minimum necessary to ensure adequate ruminal fermentation (70 g/kg dry matter) (Pereira et al., 2019) without compromising the efficient use of fibrous carbohydrates. According to National Research Council (2001), when the CP content is below 7% in the feed offered to ruminants, low N availability occurs, which can reduce fiber digestion and intake due to the slow passage of feed through the rumen. Thus, the replacement levels of cassava shoot by cactus pear in silages are recommended to maintain CP levels above the minimum limit established by the National Research Council (2001).

The increase in the contents of TC and NFC is associated with the exposure of the cellular content of plants that confer on the release and activation of enzymes, such as amylase, that favor the catabolism of more complex biomolecules and provide substrates for lactic acid bacteria, besides the fact that cactus pear is a good source of pectin and NFC (Bispo et al., 2007; Macêdo et al., 2017).

During the exposure of silage to the aerobic environment, there was no effect of including cactus pear on the parameters related to temperature. This effect indicates that the decrease in silage dry matter attenuated the activities of harmful bacteria to the quality of the silage since it would be necessary to increase the exogenous heat of the silage in order to approach the temperature of the silage whose dry matter content was higher.

Conclusions

Including cactus pear in up to 45% of cassava silage, shoots cause changes in the fermentation and nutritional characteristics. However, values found in the silages are by the standards of good quality silages to be used in diets for ruminants.

Conflict of Interest

The authors declare that they have no competing interests.

Ethics Statement

Not applicable

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