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Intake, nutrient digestibility, nitrogen balance, and microbial protein synthesis in sheep fed spineless-cactus silage and fresh spineless cactus

Gildênia Araújo Pereira[a], Edson Mauro Santos[a], Juliana Silva de Oliveira[a], Gherman Garcia Leal de Araújo[b], Raniere de Sá Paulino[a], Alexandre Fernandes Perazzo[a,*], João Paulo de Farias Ramos[c], José Maria César Neto[a], Gabriel Ferreira de Lima Cruz[a], Guilherme Medeiros Leite[a]

[a] *Department of Animal Science, Federal University of Paraíba, UFPB, Areia, Paraíba, Brazil*
[b] *Brazilian Agricultural Research Corporation, EMBRAPA, Petrolina, Pernambuco, Brazil*
[c] *State Company of Agricultural Research of Paraíba, EMEPA, Soledade, Paraíba, Brazil*

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ABSTRACT

An experiment was carried out to compare the use of fresh cactus with cactus ensiled with and without a microbial inoculant in sheep diets by examining their feed intake, nutrient digestibility, nitrogen balance, and microbial protein synthesis and counting fecal enterobacteria. Twenty uncastrated mixed-breed sheep at approximately six months of age, with an average initial weight of 23.48 ± 2.40 kg, were used in the study. The animals were assigned to four treatments [fresh spineless cactus processed twice daily at the time of supply (FC2); fresh spineless cactus processed only once, in the morning, and supplied twice daily (FC1); spineless-cactus silage without inoculant (CS); and spineless-cactus silage with microbial inoculant (CSI)] in a completely randomized design with five replications per treatment. The experimental period was 21 days. The use of spineless cactus in the form of silage (CS and CSI) resulted in higher ($P < 0.05$) intakes of dry matter, organic matter, neutral detergent fiber, ether extract, non-fibrous carbohydrates, and total digestible nutrients; and higher digestibility coefficients of dry matter, organic matter, and total digestible nutrients. Lower counts of fecal enterobacteria were also observed with the ensiled cactus. By contrast, the diets did not influence ($P> 0.05$) nitrogen balance, microbial efficiency, urinary nitrogen losses, or fecal nitrogen losses. Regardless of inoculation, the ensiling of spineless cactus improves the sanitary quality of the diet, reducing the amount of enterobacteria in the cactus and resulting mainly in improved nutrient intake by sheep.

1. Introduction

Ruminant production in semi-arid regions of the world is affected by climatic fluctuations throughout the year. For this reason, farmers make use of plants adapted to water deficit conditions, which is the case of spineless cactus. Because this cactaceous species contains high levels of water and non-fibrous carbohydrates (NFC), it can partially replace the dietary concentrate (Aguilar–Yáñez et al., 2011; Costa et al., 2012; Rodrigues et al., 2016; Herrera et al., 2017). As a consequence, production costs are reduced, especially in feedlot systems.

Spineless cactus contains low levels of dry matter (DM) (8.32–10.8 %) and crude protein (CP) (5.42–7.54 %) and high concentrations of NFC (46.00–47.37%) and minerals (Costa et al., 2012; Lopes et al., 2017). These minerals include high levels of calcium (13–40 g/kg DM) but inadequate phosphorus contents (0.99–2.79 g/kg DM), resulting in a high calcium-to-phosphorus ratio, which may lead to kidney problems and decreased DM and nutrient intakes (Vazquez-Mendoza et al., 2016).

The use of cactus-based silages in small ruminant diets is already being studied in different regions of the world such as Tunisia (Abidi et al., 2013), Mexico (Miranda-Romero et al., 2018), Zimbabwe (Gusha et al., 2015a, b), and Brazil (Macêdo et al., 2017, 2018; Nobre et al., 2018). These studies reported that cactus-based silages have a good fermentation pattern and can therefore properly preserve the ensiled material. Some authors also found that well-fermented silage can be obtained when the plant is harvested after the second year of regrowth, owing to the presence of cactus mucilage, which reduces effluent losses

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* Corresponding author at: Department of Animal Science, Federal University of Paraíba, UFPB, Km 12, Rodovia PB-079, 58397-000, Areia, Paraíba, Brazil.
E-mail address: alexandreperazzo@hotmail.com (A.F. Perazzo).

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and the amount of soluble carbohydrates (Mokoboki et al., 2016; Monrroy et al., 2017; Sá et al., 2018).

When used in the form of silage, spineless cactus exhibits agronomic and operational advantages, since its ensiling process allows for the harvest of the entire plantation, providing uniform and increased crop growth capacity and, consequently, yield. Additionally, it allows for a reduction of man labor involved in the harvest and periodic supply during the drought period.

However, a recurrent problem observed in feedlot systems in which cactus is used as a diet ingredient is the occurrence of diarrhea associated with the feeding management, especially in young goats and sheep. Some authors relate this disorder to the low amount of neutral detergent fiber in the diet (Gebremariam et al., 2006; Costa et al., 2012; Pinho et al., 2018). Other authors consider that diarrhea may occur due to the presence of oxalate in spineless cactus. However, some studies point to the fact that, in a total diet, the amount of oxalate would not be sufficient to cause diarrhea (Gouveia et al., 2015; Pinho et al., 2017).

It is thus hypothesized that the presence of pathogenic microorganisms such as some enterobacteria could be one of the causes of these disorders, which might have negative effects on animal performance. Callaway et al. (2010) demonstrated that high-grain diets enabled the appearance of pathogenic species of *Escherichia coli* in the rumen and feces of steers, resulting in diarrhea and worsened animal performance.

Similarly to high-energy grains, spineless cactus contains high levels of NFC as well as high moisture, providing favorable conditions for the growth of enterobacteria when processed and supplied in the trough. This may represent an even more compromising situation when producers process the plant once daily to compose diets and supply them on various occasions. This management strategy may result in stimulus to the growth of various bacteria, especially under inadequate hygienic and sanitary conditions. Many of these bacteria may be pathogenic and result in diarrhea, thereby compromising feed intake and nutrient utilization in feedlot sheep.

The activity of enterobacteria could be mitigated by adequately managing the feed supply, but also by ensiling the cactus, since it is known that no enterobacteria are observed in environments with pH around 4.0 (Muck, 2010; Zheng et al., 2017; Rosa et al., 2018). pH values around that level have been found in cactus silage. In this way, it would be possible to avoid the inoculation of these microorganisms in the rumen of sheep fed this silage. Moreover, the ensiling process promotes a small reduction in the concentration of NFC (Muck, 2010), which might also reduce the risks of rumen acidosis (Oliveira et al., 2016) as well as prevent the proliferation of opportunistic enterobacteria. Therefore, it is also assumed that the use of microbial inoculant might further potentiate the beneficial effects of ensiling, especially if a *Lactobacillus* strain isolated from spineless cactus itself is used.

The present study was conducted to test the hypothesis that the elevated levels of NFC and moisture present in spineless cactus ground at different times induce fermentation and proliferation of pathogenic bacteria, causing diarrhea. Evaluating the use of spineless-cactus in lamb diets may indicate possible beneficial effects on their intake and nutrient digestibility stemming from a lack of digestive disorders caused by pathogenic bacteria, as compared with fresh spineless cactus.

We examined the effect of fresh cactus and cactus silage with and without microbial inoculant in the diet of mixed-breed sheep by evaluating feed intake, nutrient digestibility, nitrogen balance, and microbial protein synthesis as well as quantifying fecal enterobacteria.

2. Materials and methods

2.1. Location, animals and treatments

This study was submitted to and approved by the Ethics Committee in Animal Research at the Federal University of Paraíba (UFPB) (approval no. 004/2018/IPeFarM). Accordingly, all experimental procedures complied with the ethical principles of animal experimentation. The experiment was conducted from November to December 2017 on the Experimental Farm of Pendência, belonging to the Agricultural Research Corporation of Paraíba S.A (EMEPA), in the municipality of Soledade-PB, Brazil (7°8'18" S and 36°27'2" W; 534 m altitude). According to the Köppen classification, the climate of the region is a *Bsh* type (hot semi-arid) with rainfall occurring from January to April, an average temperature of 24 °C, relative humidity of approximately 68 % and average annual precipitation of 400 mm.

Twenty uncastrated mixed-breed sheep at approximately six months of age, with an average initial weight of 23.48 ± 2.40 kg, were used in the study. The animals were housed in individual stalls equipped with feeders and drinkers, in a masonry shed with concrete floors. Water and mineral supplement were available *ad libitum*. The experiment lasted 31 days, consisting of 10 days used for the animals to acclimate to the environment, management, and diets and 21 days of data collection. During the acclimation period, all animals were vaccinated against clostridial diseases and treated against endo- and ectoparasites. To preserve the health of the animals, the facilities underwent a daily cleaning procedure to remove droppings, which were stored on a dunghill.

The animal weights were used to calculate the amount of feed to be supplied and to adjust orts. Lambs were assigned to four treatments in a completely randomized design with five replications. The treatments consisted of fresh spineless cactus processed at the times of supply (0800 h and 1600 h) (FC2); fresh cactus processed only in the morning (FC1), at 0800 h, to be supplied twice daily (0800 h and 1600 h); cactus silage without inoculant (CS); and cactus silage with microbial inoculant (CSI).

2.2. Management and ensiling process

The cactus variety used for ensiling and to be supplied fresh was 'gigante' (*Opuntia ficus-indica*), which was harvested from the Experimental Farm of Pendência, belonging to the Brazilian Agricultural Research Corporation S.A (EMEPA), located in the municipality of Soledade - PB, Brazil. The plant was at the regrowth age of two years. All cladodes were collected, except for the main cladode and one primary cladode per plant.

To make the silage, the plants were processed through a chopper with a razor system in which the cladodes were chopped to 4-cm^3 cubes. Subsequently, the plants were weighed according to each treatment and their wet mass was ensiled in experimental bags in the amount of 25 kg of mass per bag.

The inoculant used in ensiling consisted of a homofermentative (*Weissella Confusa*) and a heterofermentative (*Lactobacillus Plantarum*) strain, which were chosen based on the fermentation profile of previous research with spineless cactus. The inoculants were previously reactivated in 10 L of MRS broth (De Man Rogosa and Sharpe, Oxoid, Cambridge, UK) for 24 h, at 30 °C, in accordance with the methodology described by Ávila et al. (2009). A total of 2.49 mL of MRS broth were diluted in 600 mL of sterile distilled water and sprayed over 25 kg of the mass at the time of ensiling, aiming at the application of 1×10^6 cfu per gram of forage. The silage that did not contain the inoculant received the same amount of distilled water at ensiling. Silos were opened after the storage period of 30 days.

2.3. Experimental diets and feed analysis

Sheep were fed diets composed of fresh spineless cactus or silages of spineless cactus with and without inoculant plus Tifton 85 grass hay, corn meal, soybean meal, urea, mineral supplement, ammonium chloride, and ammonium sulfate. A concentrate-to-roughage ratio of 40:60 was adopted (dry-matter basis). The roughage fraction was composed of 4.4 % Tifton hay and 95.6 % fresh or ensiled cactus (fresh-matter basis).

The diets were formulated to be isonitrogenous (10 % crude protein) and to provide an average daily weight gain of 150 g, following the

National Research Council (National Research Council - NRC, 2007).

The chemical composition of ingredients, orts, and feces collected throughout the experimental period was determined at the Laboratory of Feed Analysis and Animal Nutrition (LAANA) at the Center for Agricultural Sciences, Federal University of Paraíba (CCA/UFPB). Prior to these analyses, the samples were oven-dried at 55 °C for 72 h and ground through a Wiley mill to 1-mm particles.

Dry matter (DM; method 967.03), ash (method 942.05), crude protein (CP; method 981.10) and ether extract (EE; method 920.29) were determined as described by AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured as proposed by Van Soest et al. (1991), with modifications by Senger et al. (2008) for the use of an autoclave. The autoclave temperature was maintained at 110 °C for a period of 40 min. The samples were treated with thermostable alpha-amylase without the use of sodium sulfite. Neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP) values were obtained by following the recommendations of Licitra et al. (1996). Hemicellulose contents were calculated by subtracting NDFap (sum of cellulose, hemicellulose and lignin) from ADFp (sum of cellulose and lignin). Lignin was determined by treating the ADF residue with 72 % sulfuric acid (Silva & Queiroz, 2002) (Tables 1 and 2).

Neutral detergent fiber corrected for ash and protein (NDFap) was determined by the following equation:

$$\text{g/kg NDFap}_{DM} = \text{g/kg NDF} - (\text{g/kg NDIP}_{DM} + \text{g/kg ADIA}_{DM}).$$

The non-fibrous carbohydrate (NFC) content of the ingredients was determined by the following equation proposed by Detmann et al. (2012):

$$\text{NFC} = 1000 - (\text{g/kg CP} + \text{g/kg NDFap} + \text{g/kg EE} + \text{g/kg MM}).$$

Additionally, the pH and ammoniacal nitrogen content of the silages were measured in accordance with Bolsen et al. (1992) and the lactic and acetic acid levels in the silage were determined as proposed by Ranjit and Kung (2001).

The animals were fed twice daily (at 0800 h and 1600 h), in equal portions, attempting to maintain approximately 10 % orts. Feeds, orts, and feces were harvested on the 16th, 17th, and 18th days of the experimental period.

2.4. Analysis of intake, digestibility and nitrogen balance

Feces were collected (three days) on the 16th, 17th and 18th days of the experimental period, directly from the rectal ampulla of the animals, to determine nutrient digestibility. Samples were stored in a freezer at –20 °C until laboratory analyses. These were later homogenized and a composite sample was prepared per animal. Next, the material was dried in a forced-air oven at 55 °C for 72 h, after which time the material was ground through a Wiley mill to 1-mm particles that were then stored in containers with lid (Ferreira et al., 2009).

Table 1
Chemical composition of the ingredients used in the experimental diets.

Variable (g/kg DM)	FC2	FC1	CS	CSI	Tifton 85 hay	Corn meal	Soybean meal
DM	144	144	144	153	892	894	891
OM	905	916	922	910	922	960	932
Ash	94.4	83.5	77.4	89.4	73.5	37.5	67.1
CP	42.8	36.2	25.9	32.1	58.2	115.1	434.4
EE	8.0	6.7	12.3	14.5	16.3	152.4	24.1
NDF	221	236	239	240	754	331	140
NDFap	236	236	241	233	730	328	115
ADF	134	149	146	142	352	102	74.7
Cellulose	116	124	116	120	280	86.1	72.8
Hemicellulose	77.3	79.9	100	96.3	393	232	83.7
Lignin	17.7	24.5	29.6	21.6	66.8	16.6	1.9

FC2 = fresh spineless cactus processed at the time of supply, FC1 = fresh spineless cactus processed in the morning, CS = spineless-cactus silage without inoculant, CSI = spineless-cactus silage with inoculant, DM = dry matter, OM = organic matter, CP = crude protein, EE = ether extract, NDF = neutral detergent fiber, NDFap = neutral detergent fiber corrected for ash and protein, ADF = acid detergent fiber.

Table 2
Proportions of ingredients and chemical composition of the experimental diets used in the feeding of sheep, containing fresh spineless cactus or cactus silage with and without inoculant.

Variable, g/kg (DM)	Diet			
	FC2	FC1	CS	CSI
FC2	474	0.0	0.0	0.0
FC1	0.0	472	0.0	0.0
CS	0.0	0.0	474	0.0
CSI	0.0	0.0	0.0	474
Tifton 85 hay	126	126	126	126
Corn meal	204	204	204	204
Soybean meal	166	166	166	166
Urea	6.5	6.5	6.5	6.5
Mineral supplement	14.2	14.2	14.2	14.2
Ammonium chloride	8.3	8.3	8.3	8.3
Ammonium sulfate	0.7	0.7	0.7	0.7
	Chemical composition, g/kg DM			
Dry matter*	177	177	185	184
Organic matter	832	848	848	849
Crude protein	107	101	91.3	97.6
Ether extract	37.4	35.7	44.3	47.8
Neutral detergent fiber	438	440	444	444
Non-fibrous carbohydrates	289	290	263	260
Acid detergent fiber	233	235	236	235

* g kg^{-1} (as-is basis), FC2 = fresh spineless cactus processed at the time of supply, FC1 = fresh spineless cactus processed in the morning, CS = spineless-cactus silage without inoculant, CSI = spineless-cactus silage with inoculant.

The amount of excreted fecal dry matter, which was used to determine the apparent digestibility of the feed and the total digestible nutrients (TDN), was estimated from the concentration of indigestible neutral detergent fiber (iNDF), used as an internal marker. The marker was retrieved following the *in situ* incubation of the feed supplies (Cochran et al., 1986), orts and feces in a fistulated bovine for a period of 240 h (Casali, 2008). For this procedure, mini-bags [made of non-woven fabric (‘TNT’); 12.0 × 8.0 cm] containing approximately 4 g of the material in 2.0-mm particles were used in triplicate. After the incubation period, the mini-bags were washed in cold water and then in running water. Afterwards, they were oven-dried at 60 °C for 72 h (Casali et al., 2008). Subsequently, the samples were weighed to determine the fecal DM production (FDMP), and then iNDF was analyzed in accordance with the methodology described by Detmann et al. (2012).

Fecal dry matter production (FDMP) was determined by the following formula:

$$\text{FDMP} = \text{marker intake (kg)}/\text{concentration of marker in feces (\%)}.$$

Apparent digestibility was calculated as proposed by Berchielli et al. (2011). The digestibility coefficient (DC) of DM, OM, CP, and NDF was calculated based on FDMP, using the following formula:

$$\text{DC} = [(\text{g of nutrient consumed} - \text{g of nutrient in the feces})/(\text{g of nutrient consumed})] \times 100.$$

Total digestible nutrients were estimated by the formula proposed by Weiss (1999):

$$\text{TDN (g/kg)} = \text{dCP} + \text{dNDFap} + \text{dNFC} + \text{dEE} * 2.25,$$

where dCP: digestible CP; dNDFap: digestible NDFap; dNFC: digestible NFC; and dEE: digestible EE.

Spot urine samples were harvested from the animals on the 21 st day of the experimental period, at four hours after the first feeding, during spontaneous urination, using colostomy bags. The urine was then filtered and 10-mL aliquots were collected, immediately diluted in 40 mL 0.03 N sulfuric acid (Valadares et al., 1999) to prevent bacterial destruction of the purine derivatives and precipitation of uric acid, and stored at –15 °C in identified plastic jars for later analyses of creatinine, purine derivatives, and total nitrogen.

Each animal was assumed to excrete 17.05 mg of creatinine per kilogram of body weight (Pereira et al., 2013). The daily urinary volume (UV, L) was calculated based on the creatinine concentration in the spot urine sample (CCspot), as follows:

$$UV = DCE/CCspot.$$

The daily creatinine excretion was determined as follows:

$$DCE = CC \times UV/BW,$$

where DCE = daily creatinine excretion (mg/L) (total collection); UV = urinary volume (L); and BW = animal body weight (kg).

Nitrogen balance was determined as the difference between total nitrogen intake and total nitrogen excreted in the feces (fecal N) and in the urine (urinary N). The total nitrogen present in the feces and urine was determined by following the methodology described in AOAC (1990).

Urinary concentrations of allantoin, xanthine, and hypoxanthine were determined as suggested by Chen and Gomes (1992). The concentrations of uric acid and urea in urine were determined using commercial kits (Bioclin®).

The total excretion of purine derivatives was calculated by summing the concentrations of allantoin, uric acid, xanthine, and hypoxanthine. The amount of absorbed microbial purines (X, mmol/day) was estimated from the excretion of total purine derivatives (Y, mmol/day), using the following equation proposed by Chen and Gomes (1992), for sheep:

$$Y = 0.84X + (0.150\ LW^{0.75}\ \mathrm{e}\text{-}^{0.25X}).$$

The intestinal flow of microbial N (MN, in g/day) was estimated from the amount of absorbed purines (X, mmol/day), according to the equation described by Chen and Gomes (1992):

$$MN = X\ (\mathrm{mmol/d}) \times 70 = 0.727 \times 0.83 \times 0.116 \times 1000,$$

assuming a digestibility factor of 0.83 for the microbial purines, a 0.116 purine N-to-total purine ratio, and a purine N content of 70 mg/mmol.

The efficiency of microbial protein synthesis was obtained by dividing microbial protein synthesis (g/day) by TDN intake (kg/day).

During the 21 days of data collection, feces were harvested directly from the rectal ampulla. These samples were identified and stored in a freezer at –20 °C for later analysis.

2.5. *Enterobacteria counts and quantifications*

Enterobacteria were quantified in the samples of fresh and ensiled spineless cactus and fecal samples. All collections took place on the 14th day of the experimental period. Feces were harvested directly from the rectal ampulla two hours after the morning feed. In the case of the fresh plant, samples of the cactus processed in the morning and afternoon were collected before being supplied. All samples were stored in identified sterile containers. These were transported inside refrigerated bags to the laboratory where the microbiological count was performed.

For microbial count, 10 g of samples of feeds and feces were added to 90 mL of a previously sterilized saline solution. After agitation for 1 min, the obtained solution was serially diluted (10^{-1} to 10^{-9}), with the material always being homogenized before the aliquot was harvested. Violet red bile agar (VRB) was used as the growth medium, and plates were incubated for 24 h at 37 °C. Colonies on the plates that showed between 30 and 300 cfu were counted.

In the case of fresh samples, enterobacteria counts of 5.1 log cfu/g and 5.8 log cfu/g were found for the samples of cactus collected in the morning and afternoon, respectively. No enterobacterial growth was observed in either silage.

The experiment was laid out in a completely randomized design with four treatments and five replications, as follows: fresh spineless cactus processed at the time of supply and supplied twice daily (FC2); fresh spineless cactus processed only in the morning (FC1); spineless-cactus silage without inoculant (CS); and spineless-cactus silage with inoculant (CSI).

The following model was applied:

$$Yij = \mu + Ti + eij$$

where Yij = observed value of the dependent variable i; μ = overall mean; Ti = effect of diet i (i = 1–4); and eij = random error common to all observations.

The data were interpreted statistically by analysis of variance (ANOVA) and means were compared by Tukey's test at the 5% probability level.

3. Results

The following values were observed in the non-inoculated silages: pH: 4.1; ammoniacal nitrogen: 0.9 % of the total N; lactic acid: 50 g/kg DM; and acetic acid: 28 g/kg DM. For the inoculated silages, the following results were obtained: pH: 4.0; ammoniacal nitrogen: 0.8 % of the total N; lactic acid: 58 g/kg DM; and acetic acid: 23 g/kg DM.

The highest intakes ($P < 0.05$) of DM, OM, NDF, EE, NFC, and TDN, expressed in g/day, were shown by the animals fed the silages, with no differences between the treatments with and without inoculant (Table 3). On the other hand, no diet effect ($P > 0.05$) was observed for CP intake.

There was a diet effect ($P < 0.05$) for the digestibility coefficients of DM, OM, NFC and TDN (Table 4). However, the digestibility of CP, NDF, and EE was not influenced.

The animals fed the spineless-cactus silage without inoculant exhibited higher digestibility of DM (862 g/kg) than those fed the cactus processed only once daily (804 g/kg) and ensiled with inoculant (826 g/kg), but their result did not differ from those obtained by the animals fed cactus processed at the time of supply (836 g/kg) (Table 4). The same was observed for the digestibility of OM and NFC. As regards the TDN of the diets, the highest values ($P < 0.05$) were observed in the group that received untreated cactus silage and inoculated cactus silage (769 and 748 g/kg, respectively). However, the average TDN observed for the animals fed inoculated spineless-cactus silage was similar ($P > 0.05$) to that seen in the animals fed the cactus processed at the time of supply (724 g/kg) (Table 4).

Nitrogen balance, microbial production, and microbial efficiency were not influenced ($P > 0.05$) by the diets (Table 5).

Urinary and fecal nitrogen losses were not affected ($P > 0.05$) by the diets, which was likely because the diet formulation was identical for all

Table 3
Nutrient intake of sheep fed diets with fresh spineless cactus or spineless-cactus silages.

Variable	Diet[a]				
	FC2	FC1	CS	CSI	SEM
	g/day				
Dry matter	631b	601b	727a	750a	30.3
Organic matter	522b	506b	607a	633a	25.8
Crude protein	67.7	60.7	66.2	72.0	3.12
Neutral detergent fiber	281bc	269c	329ab	338a	12.8
Ether extract	24.0b	21.6b	33.5a	36.6a	1.20
Non-fibrous carbohydrates	149b	155ab	178ab	186a	9.03
Total digestible nutrients	456b	426b	559a	561a	20.7

[a] FC2 = fresh spineless cactus processed at the time of supply, FC1 = fresh spineless cactus processed in the morning, CS = spineless-cactus silage without inoculant, CSI = spineless-cactus silage with inoculant. Different letters in the row indicate significantly different means ($P < 0.05$) according to Tukey's test.

Table 4

Apparent digestibility coefficient of nutrients (g/kg) from diets with fresh spineless cactus or spineless-cactus silage with and without inoculant for mixed-breed sheep.

Digestibility coefficient (g/kg)	Diet				
	FC2	FC1	CS	CSI	SEM
Dry matter	836ab	804b	862a	826b	9.00
Organic matter	826ab	798b	860a	823ab	9.75
Crude protein	843	808	824	796	13.6
Neutral detergent fiber	801	786	834	803	12.2
Ether extract	855	853	902	881	12.2
Non-fibrous carbohydrates	859ab	801b	913a	856ab	23.7
Total digestible nutrients	724bc	708c	769a	748ab	8.38

FC2 = fresh spineless cactus processed at the time of supply, FC1 = fresh spineless cactus processed in the morning, CS = spineless-cactus silage without inoculant, CSI = spineless-cactus silage with inoculant. Different letters in the row indicate significantly different means ($P < 0.05$) according to Tukey's test.

Table 5

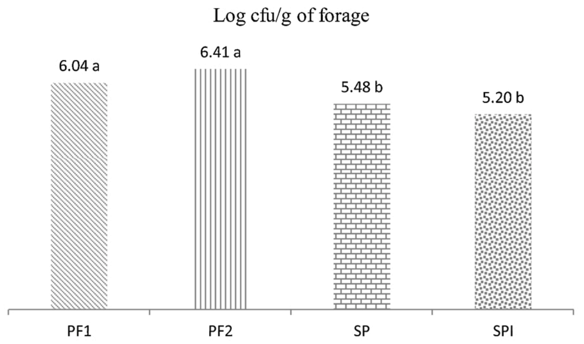
Nitrogen balance and microbial protein synthesis in sheep fed fresh spineless cactus or spineless-cactus silage.

Variable	Diet				
	FC2	FC1	CS	CSI	SEM
Nitrogen intake (g/day)	8.66	8.12	9.26	8.72	0.80
Fecal nitrogen (g/day)	1.33	1.54	1.62	1.70	0.23
Urinary nitrogen (g/day)	0.19	0.37	0.38	0.27	0.07
Retained nitrogen (g/day)	7.16	6.21	7.25	6.74	0.62
Microbial production (g/day)					
Microbial nitrogen	4.92	5.74	4.13	4.94	0.91
Microbial CP	30.7	35.8	25.8	30.9	5.71
Microbial efficiency					
g CP/kg TDN	82.6	100	55.0	72.0	16.4
SEM	2.34	4.90	1.29	2.93	

FC2 = fresh spineless cactus processed at the time of supply, FC1 = fresh spineless cactus processed in the morning, CS = spineless-cactus silage without inoculant, CSI = spineless-cactus silage with inoculant, CP = crude protein, NDF = neutral detergent fiber. Different letters in the row indicate significantly different means ($P < 0.05$) according to Tukey's test.

treatments (Table 2). Therefore, the protein intakes (Table 3) were balanced and so were the protein excretions in the feces and urine (Table 5). Microbial efficiency (g CP/kg TDN) also did not differ ($P > 0.05$) between the treatment groups.

As shown in Fig. 1, the material ensiled with and without inclusion of a bacterial inoculant led to lower ($P < 0.05$) counts of fecal enterobacteria (5.48 and 5.20) in comparison with the counts of enterobacteria found in the feces of sheep fed fresh spineless cactus. This finding corroborates the hypothesis of decreased contamination with enterobacteria resulting from the ensiling of spineless cactus.



FC2 = fresh spineless cactus processed at the time of supply, FC1 = fresh spineless cactus processed in the morning, CS = spineless-cactus silage without inoculant, CSI = spineless-cactus silage with inoculant. Different letters indicate an effect (P<0.05) according to Tukey's test.

Fig. 1. Count of enterobacteria in the feces of sheep fed fresh spineless cactus or spineless-cactus silage.

4. Discussion

Ensiling promotes a reduction in NFC due to the fermentation of soluble carbohydrates by microorganisms—lactic acid bacteria, mainly—, resulting in lactic acid production. However, the decreasing NFC content of the silage may induce a proportional increase in other non-fermentable nutrients when compared with the original material (Kung et al., 2018).

The ensiling process made it possible to improve the intake and digestibility of some nutrients, and some hypotheses can be raised to explain this phenomenon. Poorly fermented silages are known to possibly impair nutrient intake when compared with fresh silages. However, in the case of spineless cactus, an adequate fermentation profile is observed, with elevated lactic acid contents and reduced levels of acetic acid and ammonia, which are favorable to intake (Ellis et al., 2016). It is thus assumed that the tested silages did not have a negative effect on nutrient intake because of their fermentation characteristics.

On the other hand, the time during which the cactus was stored to be provided to the animals receiving FC1 promoted undesirable carbohydrate fermentations, which negatively influenced the digestibility of NFC, as compared with the CS diet. Because the estimation of TDN, DM and OM depends on the amount of NFC, when the NFC content of the diet is reduced, so are its TDN, DM and OM contents. These results demonstrate that the microorganisms naturally present in the cactus seem to provide more adequate fermentations for the carbohydrate from the ensiled cactus when compared with the inoculant studied here.

However, both CS and CSI favored the intake of most nutrients by the sheep when compared with FC1 and FC2. In many cases, decreases in nutrient intake may be due to nutritional factors such as elevated or low NDF contents or excess NFC (Santos et al., 2015; Sousa et al., 2018). In addition to having very similar chemical compositions, all diets were isonitrogenous. The NDF contents of all diets were above the minimum limit of 250 g/kg DM suggested by Kozloski et al. (2006), and their NFC levels were below the maximum limit of 440 g/kg DM proposed by Sousa et al. (2018).

Other authors assumed that the higher nutrient intakes observed in animals fed silage-containing diets are indeed due to the controlled growth of undesirable microorganisms in the plant, which prevent ruminal and metabolic disorders that might affect nutrient intake (Arthur et al., 2010; Adam and Brülisauer, 2010; Muck, 2010; Stanford et al., 2014; Dijkstra et al., 2016; Kafantaris et al., 2016). The reduced count of enterobacteria may be an indicator that the silage-fed animals might have had fewer problems associated with imbalance in their rumen microbiota, since lactic acid is an inhibitor of coliform development (Ostling and Lindgren, 1995).

Thus, the improved intake of spineless cactus in the form of silage when compared with its fresh version is likely not related to the digestibility of the dietary nutrients, but rather to the fermentation products of cactus, which can influence its acceptability by the animals (Nobre et al., 2018; Albuquerque et al., 2020).

Nobre et al. (2018) evaluated nutrient intake in mixed-breed lambs fed diets with different proportions of spineless-cactus silage and found that the inclusion of 42 % of the ingredient (DM basis) provided DM and NDF intakes of 839.46 and 344.51 g/day, respectively. These results indicate a higher DM intake in comparison with the present experiment, but similar NDF intakes in relation to the CS and CSI diets. On the other hand, Andrade et al. (2016) tested the supply of fresh and dried spineless cactus replacing Tifton 85 hay and observed DM intakes of 885 and 890 g/day for the partial (37.17 g/kg fresh cactus in the diet DM) and total (74.2 g/kg fresh cactus in the diet DM) replacements, respectively. In contrast, the group fed the diet without forage cactus showed a DM intake of 658 g/day, suggesting that nutrient intake was not affected even when the cactus diet was used as the exclusive roughage source, whereas TDN intake was higher in the cactus-based diet groups (619 and 673 g/day) than in control group (446 g/day).

In a study examining the replacement of corn with spineless cactus in the diet of Santa Inês sheep, Costa et al. (2012) found higher intakes of DM, CP, NDF, NFC and TDN than the current results, but similar EE intakes. These superior results are explained by the high body weight of the animals upon entering the feedlot (27.50 ± 0.48 kg).

In fact, the main problem related to intake was the lower TDN and DM intakes of the animals fed fresh cactus, suggesting that their performance would be compromised if they were reared in a feedlot system, since low nutrient intake typically results in decreased performance and feed efficiency in confinement (Brochier and Carvalho, 2008). This would be especially true if the amount of spineless cactus and/or concentrate were higher than those used in the present study. In addition to the agronomic and operational advantages stemming from the ensiling of cactus, the results obtained in the present study are worth mentioning. Further in-depth studies can thus be developed on the rumen microbiota and the expression of sub-clinical disorders that supposedly reduce the intake of animals fed fresh cactus.

Siqueira et al. (2017) evaluated the inclusion of spineless cactus at 588 g/kg replacing Tifton hay in the diet and obtained an apparent digestibility of 788 g/kg DM. In the present study, fresh spineless cactus and spineless-cactus silage were included in a lower proportion, at 474 g/kg of diet DM, and an average apparent digestibility of 832 g/kg DM was observed.

The high NDF digestibility observed in this study is due to the greater proportion of feedstuffs with a high degradation rate (844 g/kg) in the diet. Corn and soybean meal and spineless cactus have high NFC and low lignin contents (Rocha et al., 2003; Zambom et al., 2001; Batista et al., 2009), which favors the maximization of the rumen's fermentation capacity and promotes greater degradability of DM and fiber (Siqueira et al., 2017). In addition, the association of these factors with the presence of NDF in the diet provided by Tifton hay—a roughage feed with high NDF digestibility, above 600 g/kg, as reported by Lopes et al. (2019) and Rocha et al. (2003)—may have contributed to the non-alteration of the rumen microbial profile, favoring the fibrous carbohydrate-fermenting microorganisms and a lower passage rate (Barros et al., 2018; Dijkstra et al., 2012). It is important to highlight that Tifton hay, the ingredient of lowest digestibility in the diet, was present in a low proportion, at 126 g/kg of diet.

Agreeing with the present findings, Costa et al. (2012) reported increases in NDF digestibility after gradually replacing corn with spineless cactus in the diet of sheep. The authors obtained a maximum NDF digestibility of 77.5 % with the diet in which corn was fully replaced (28 % spineless cactus in the DM). They also reported 80.9 % digestibility of DM for that treatment, which is also close to the percentage obtained with the diets in the present study.

The digestibility results were not as significant as those observed for intake, which can be explained by the similar chemical composition of the diets. Digestibility is directly related to the rate of passage of the feed through the rumen (Fortius et al., 2014). In this way, because the animals fed fresh cactus consume less feed, they may show similar digestibility to those fed silage-based diets due to the longer retention time of the feed in the rumen. However, according to some authors, ensiling may lead to improved digestibility as a result of the acid hydrolysis of the hemicellulose that composes NDF (Lara et al., 2016).

In summary, considering the greater differences in intake without digestibility being compromised, the sheep fed cactus silage are benefited by the major advantage of having a higher TDN intake and, most likely, better production performance than those fed fresh cactus.

As stated by Moreno et al. (2010), adequately formulated diets result in similar nitrogen losses ($P > 0.05$). In this regard, Ma et al. (2015) mentioned that the synchronism between protein and energy reflects in lower nitrogen excretions. Also according to those authors, nitrogen intakes higher than nitrogen excretion through the feces are indicative of adequate diet balancing. In fact, a likely explanation for the similar nitrogen balance is the synchronism between the intakes of protein and energy, which shows that energy influences the apparent use of dietary protein, making it a critical factor for nitrogen balance (National Research Council - NRC, 1989). Since there were no alterations in nitrogen balance, the higher energy intake would have been the most relevant factor for the animals fed the spineless cactus-based diets.

In the experiments with feedlot lambs led by Silva et al. (2019), who evaluated spineless cactus-based diets and the replacement of hay with wheat bran, and Lins et al. (2017), who replaced wheat bran with spineless cactus, higher nitrogen retention values (above 10.0 and 12.2 g/day respectively) were found in comparison with the present study. However, the CP content of the experimental diets in these studies was above 12 %, whereas a much lower protein level was used in the present study. Abidi et al. (2013) evaluated silages composed of spineless cactus, olive cake and wheat bran and reported retained nitrogen values ranging from 8.0–8.9 g/day, which are slightly higher than the means found in the present study.

On the other hand, the evaluation of microbial efficiency is more effective for comparing nitrogen utilization because it consists of microbial synthesis based on TDN intake. Therefore, individual differences in diets from other studies are disregarded. The FC1 diet provided microbial efficiency results similar to those published by Silva et al. (2019), who reported a variation between 97.8 and 127.6 g CP/g TDN. In relation to the study by Lins et al. (2017), the FC1, FC2 and CS1 treatments were within the observed range for this variable, which varied from 70.8–130 g CP/g TDN across the studied diets. However, although the average observed in the present study for the CS diet was not within this range, it was statistically similar to those of the other diets, which may indicate that there was an adequate relationship between protein and energy in the diets, considering that energy and nitrogen are the main factors influencing rumen microbial growth (Clark et al., 1992). This is especially true if we also consider the lack of effects on urinary nitrogen excretion.

Another factor influencing nutrient intake is the presence of enterobacteria in the feed. These bacteria are active in the first hours after the plant is cut, causing protein degradation and producing amines, which are compounds that reduce the acceptability of feed by animals (Jobim et al., 1999). It is believed that the inclusion of high levels (> 50 % DM) of spineless cactus in the animal diet may result in laxative effects, making the feces moist or even causing non-pathogenic diarrhea due to the increased amount of water that has not been metabolized by the animal body (Gebremariam et al., 2006; Waal et al., 2006). Because of the high moisture and soluble carbohydrate contents of the plant, processing it and exposing it to oxygen for a long period may favor the proliferation of enterobacteria, which can be pathogenic, thereby compromising ruminant productivity (Elghandour et al., 2018).

As previously mentioned, no enterobacteria were detected in the silages, which likely reduced the possibility of disorders caused by the presence of this microbial group. Nonetheless, in the fresh samples of spineless cactus, the count indicated the presence of enterobacteria. In addition, there was proliferation and an increased count of enterobacteria in the cactus processed only in the morning (FC1 treatment), which corroborates the differences found for the count of enterobacteria in the animal feces (Fig. 1).

Enterobacteria are gram-negative microorganisms that can produce endotoxins, which cause fever, inflammation, diarrhea, and blood clotting (Muck, 2010). However, few studies have been undertaken to evaluate the presence of pathogens in the feed and their permanence in the rumen, influencing animal health (Dijkstra et al., 2016; Kafantaris et al., 2016).

However, the presence of enterobacteria in the feces is an indication of the existence of a foodborne pathogen, which leads to gastrointestinal problems, ultimately reducing DM intake. In this respect, according to Muck (2010), substantial concentrations of enterobacteria result in a reduction of dietary intake. To minimize the action of these deteriorating microorganisms, it is necessary to use additives that improve the fermentation profile of the silage (Durmaz et al., 2015). In the specific case of our study, the cactus silage without inoculant also exhibited an adequate fermentation pattern, which explains the lack of an effect on the animals. In this case, inoculation should be justified by the increased recovery of DM, and studies with experimental silos are warranted to investigate this hypothesis.

During ensiling, lactic bacteria produce baceriocins, which inhibit the action of microorganisms detrimental to the silage and also rumen microorganisms (Brashears et al., 2003; Weinberg et al., 2004a; Muck, 2010; Ni et al., 2016). Considering that the fermentation occurring in the silages was mostly lactic, there might have been a probiotic effect on the gastrointestinal tract of the animals, resulting in increased feed intake (Weinberg et al., 2004b). Coupled with this, lactic acid benefits microbial growth in the rumen unlike other end products such as acetic acid, which is generally not fermented and is directly absorbed by the rumen wall (Muck, 2010).

Considering the present results as a whole, the main indication of improved nutrient intake by the sheep fed spineless-cactus silage is the control of potential pathogens that might be affecting their ruminal balance and, subclinically, their health. This hypothesis has not been raised in the current literature, and the present findings open new possibilities for many studies and possible forms of preserving spineless cactus alone; mixtures of spineless cactus and other forages adapted to the semi-arid environment; or even the formulation of diets based on spineless cactus in the form of silage, as demonstrated by Macêdo et al. (2018). As long as adequate fermentation occurs in those silages, advantages can be attained from the agronomic and operational perspectives on farms, ultimately leading to improved results in feedlots and reduced risks to animal health.

5. Conclusion

Regardless of inoculation, the ensiling of spineless cactus improves the sanitary quality of the diet, reducing the amount of enterobacteria in the cactus and resulting mainly in improved nutrient intake by sheep.

Declaration of Competing Interest

The authors report no declarations of interest.

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