Nutrient intake and digestibility, microbial protein synthesis and ingestive behavior of dairy steers fed with leucaena hay associated or not with spineless cactus

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Abstract

The aim of this study was to evaluate the use of leucaena hay and spineless cactus in the feed of confined crossbred steers on nutrient intake and digestibility, water intake, nitrogen balance, microbial protein synthesis and ingestive behavior. Eight crossbred steers, with a mean age of 12 months with an average initial weight of 267.5 ± 15 kg, were used, distributed in two simultaneous 4x4 Latin squares according to a 2x2 factorial scheme, in which the first factor evaluated two different proportions of leucaena hay inclusion (50 or 70% of dry matter base) and the second factor evaluated the effects of the replacement of corn by spineless cactus. The animals were kept in confinement for 84 days, divided into four periods of 21 days each. Dry matter intake (% of BW) was higher in diets with 50% hay, regardless of whether or not the cactus was included. The use of the cactus favored greater intake of total digestible nutrients (TDN) in diets with a proportion of 70% hay, whereas in the diets with 50% hay, the diet without the inclusion of cactus favored greater intake. The inclusion of the cactus promoted greater digestibility of dry matter (DM), NFC and TDN in diets with 70% hay. The intake of dietary water and total water was higher in diets with a proportion of 70% hay and with the inclusion of spineless cactus. The intake of nitrogen (N), urea levels in the urine and excretion of urea and N-urea with the urine were higher in diets with 50% hay. DM rumination and chewing time was longer in diets with 70% hay and the inclusion of spineless cactus, which also reduced the number of chewed cuds as well as the time spent per cud. The inclusion of the cactus improves the digestibility of diets with 70% leucaena hay content.

Keywords: Animal feed, Alternative Feeding, *Leucaena leucocephala*, *Nopalea*, *Opuntia*, Semi-arid

Introduction

In semi-arid regions, due to long periods of drought, animals are fed via troughs that increase production costs considerably, mainly due to the adoption of concentrated feed in this type of production system (Costa et al., 2016; Moraes et al., 2017). Thus, ranchers, especially those of low-income, seek local alternatives for concentrated feeds (Franzel et al., 2014).

Some studies have shown satisfactory results with the use of leucaena (*Leucaena leucocephala*) in animal feed, such as improved weight gain in cattle, goats and sheep (Seid & Animut, 2018; Ojo et al., 2014; Dahlanuddin et al., 2014; Soares et al., 2018; Gusha et al., 2015; Peniche-González et al., 2014; Khaing et al., 2016). Leucena is a tropical, drought-tolerant legume plant with arboreal-shrubby and perennial growth, considered to be one of the most used legume plants in tropical regions around the world, mainly due to its characteristics, such as a high supply of proteins, energy and minerals (Garcia et al. al. 1996).

Just like the leucaena, the spineless cactus (*Opuntia or Nopalea*) is a tropical climate plant. Despite its various uses, it has increasingly stood out as a forage source in the feeding of ruminants in semiarid regions, as it has good drought tolerance and is a source of non-fibrous carbohydrates (NFC) with satisfactory dry matter degradability (Barboza et al., 2019). However, it has protein and fiber limitations (Ferreira et al. 2012; Freitas et al. 2018; Santiago et al. 2019), which can be complemented by including leucaena in diets. When used in combination with different foods, the cactus shows satisfactory results in the performance of lactating cows (Borges et al., 2019; Inácio et al., 2019; Moraes et al., 2019), and also in the performance of small ruminants (Oliveira et al., 2017; Cardoso et al., 2019, Gusha et al., 2014).

Studies carried out in the tropics indicate that nutrition balanced between protein and energy can promote interactive effects on the metabolism of nitrogen compounds and increase nitrogen assimilation (Souza et al., 2010). In this sense, and considering good NFC content of the cactus and leucaena protein, we can assume that combining spineless cactus with leucaena increases the intake and digestibility of nutrients, as well as the synthesis of microbial protein, without affecting the ingestive behavior of the animals.

Thus, the objective of this study was to evaluate the effects of the inclusion of leucaena hay with or without spineless cactus in the feed of confined crossbred steers on nutrient intake and digestibility, water intake, microbial protein synthesis and ingestive behavior.

Materials and Methods

The experiment was carried out on the Bela Vista farm, located in the municipality of Encruzilhada, BA, latitude 15°32'48.0"S, longitude 40°45'27.8"W, at an altitude of 845 m.

Eight crossbred castrated dairy steers were used, with an average age of 12 months and initial average body weight of 267.5 ± 10 kg. The animals were kept in individual, partially covered pens with an area of 12 m^2 and a concrete floor. Each pen was equipped with individual feeding and drinking troughs. Water and experimental diets were provided *ad libitum* throughout the day. The experiment was divided in four periods, each period lasting for 21 days. During the first 16 days, all animals were fed with their respective diets and adapted to the management scheme, while the last 5 days were destined to data collection.

The steers were randomly distributed over a 4 × 4 Latin square design, in double and simultaneously, with a 2x2 factorial scheme, the first factor considering the proportions of leucaena hay (50 or 70% of dry matter base) and the second the inclusion or not of spineless cactus that replaced corn. Thus, in in the experimental diets, different sources and combinations, as well as proportions between bulky, energy and protein ingredients were evaluated as follows: (1) 50% leucaena hay combined with corn, (2) 50% leucaena hay combined with cactus, (3) 70% leucaena hay combined with corn, (4) 70% leucaena hay combined with cactus, all concentrates contained soybean meal and a mineral supplement.

The leucaena hay used in the diets was own production, originating from plants with stems of up to 20 mm in diameter. After harvesting, the plants were processed in a stationary chopper with average particle sizes up to 15 mm, and then dehydrated under the sun. The spineless cactus (*Nopalea cochenillifera Salm Dyck*) was cultivated on the territory of the property, being harvested daily and supplied *in natura* to the animals, after being minced in a stationary mincer (Table 1). Ground corn and soybean meal used in the diets were produced by Cargill® (Cargill Agrícola S/A, São Paulo, SP, Brazil), while the mineral supplement was by Matsuda® (Matsuda Seeds and Animal Nutrition, Alvares Machado, SP, Brazil).

The steers received two meals a day (7:00 a.m. and 01:00 p.m.) in order to allow for leftovers of approximately 10% of the initial amount. The experimental diets were calculated in order to be able to meet the nutritional requirements of crossbred steers, (Valadares Filho et al., 2016).

Samples of diets and total leftovers were collected in the morning and stored at -20°C for further analysis. In order to determine nutrient digestibility, animal feces were collected daily and, after weighing, a subsample of approximately 10% of the total amount was separated and stored at -20°C. Subsequently, feces and leftovers were dried in an oven with forced ventilation at a temperature of 55°C for 72 hours. A composite sample was prepared, which was proportional to the daily amount for both feces and leftovers.

The intake of nutritional components was determined by the amount of feed offered to the animals and leftovers during the experimental period. Thus, it was calculated by the difference between the amounts of nutrients present in the feed and the amount of nutrients in the leftovers. The apparent digestibility of the total tract of all nutrients was calculated using the following equation: [amount ingested - amount excreted in feces] / amount ingested. Total digestible nutrients (TDN) were estimated using the formula proposed by Weiss (1999).

Spot urine samples were obtained on the 21st day of each sampling period, approximately 4 hours after morning feeding, during spontaneous urination. Ten milliliters of aliquots from this sample were filtered and immediately diluted in 40 mL of H_2SO_4 0,036 N for creatinine analysis according to the method proposed by Oliveira et al. (2001). The aliquots were stored in plastic bottles, labeled and frozen for further analysis and quantification of creatinine, uric acid and allantoin.

Creatinine excretion (mg / kg of body weight) was used to estimate the urinary volume through spot samples obtained from each animal, according to the equation described by Chizzotti et al. (2008): DE = {32.27 - 0.01093 × BW}, where DE = daily creatinine excretion (mg / kg BW). Total daily urinary volume was estimated by dividing daily urinary creatinine excretions by the observed values of creatinine concentration in the urine (Valadares Filho et al. 2000).

In order to determine microbial protein synthesis, urinary allantoin and uric acid contents were estimated by colorimetric methods, as specified by Chen and Gomes (1992). Absorbed purines (PA) (X, mmol/day) were calculated from excretion of purine derivatives (Y, mmol/day) using the following equation X = [Y - (0.385 x PV0.75)]/0.85, where 0.85 is the recovery of absorbed purines as purine derivatives and 0.385 x PC0.75 is endogenous contribution to purine excretion (Verbic et al., 1990).

Ruminal microbial protein synthesis (Y, gN/day) was calculated in relation to absorbed purines (X, mmol/day) using a modified equation described by Chen & Gomes (1992), replacing the N- relation purine:N-total in bacteria of 0.116 by 0.134, as described by Valadares et al. (1999): Y = 70X/(0.83 x 0.134 x 1000), where 70 is purine nitrogen (mgN/mol); 0.134 is the total N-purine:N ratio of bacteria and 0.83 is the digestibility of microbial purines. The efficiency of microbial synthesis was calculated as follows: CPSEmic=[(0.629 x AP) x 6.25)]/TDNC, where TDNC is total digestible nutrients intake .

Water intake was assessed daily; the water was supplied in buckets with a capacity of 50 liters. After 24 hours, the drinking fountains were filled again, the difference being considered as water ingested by the animal. Two additional drinking troughs containing water were distributed near the animals' pens in the shed and monitored in order to determine daily evaporation. Total daily water intake was calculated as the sum of free water intake (from the drinking trough) plus dietary water minus evaporative loss and leftovers from the trough.

In order to assess ingestive behavior, eight steers were visually observed during 24 hours on the 21st day of each period, and the observations were recorded at 5-minute intervals, including feeding, rumination and idle time (Mezzalira et al., 2011). On the same day, three observations were made for each animal: in the morning, at noon and at night. Data were collected by trained observers using digital timers. During the night observation, the environment was kept under artificial lighting, with animals having had an adaptation period before. Dietary variables (feeding, rumination and idling) were obtained using equations adapted from Bürger et al. (2000)

Samples of ingredients offered to the steers during the experimental period were stored at –20°C. Then, at the end of the experiment, all samples were dried in a forced air oven at 55°C for 72 hours, ground in a Wiley mill (model 0.48 by Marconi, Piracicaba, Brazil), which allows for passing of the sampled material through 1mm sieves, and analyzed for dry matter contents (method G-001/1) Crude ashe (method M-001/1), crude protein (method N -001/1), EE (method G-004/1), neutral detergent fiber (method F-002/1) neutral detergent fiber corrected for ash and protein (method N-004/1 and M-002/1), acid detergent fiber (method F-004/1), Lignin (method F-005/1), and indigestible neutral detergent fiber (method F-009/1), all according to the methods described by Detmann et al. (2012).

Total carbohydrate (TC) contents were calculated using the equation proposed by Sniffen et al. (1992): TC = 100-(CP%+EE%+ash%), where CP = crude protein, EE = ethereal extract plus ash. The non-fibrous carbohydrates (NFC) of the samples were calculated according to the formula reported by

Detmann et al. (2010): NFC = 100 – (CP% + EE%+MM%+NDFap), where EE = ethereal extract and ash, and NDFap = neutral detergent fiber. Total digestible nutrients (TDN) were calculated according to NRC (2016): TDN = DCP + (DEE X 2.25) + DNDF + DNFC), Where: DCP = digestible crude protein; DEE = digestible ethereal extract; DNDF = digestible neutral detergent fiber; DNFC = digestible non-fibrous carbohydrates. The chemical composition of the ingredients and experimental diets can be seen in Table 1 and 2.

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

Item	Ingredients			
	Leucaena hay	Spineless cactus	Corn	Soybean meal
Dry matter[1]	846	95	852	877
Organic matter[1]	930	918	986	932
Crude protein[1]	135	75	98	508
Ethereal extract[1]	38	13	46	34
NDF_{ap}[1]	568	231	171	177
NDF_{i}[1]	378	81	31	20
Ash	70	82	14	68
Lignin	187	73	6	3
NFC[1]	222	626	682	213
TDN[1]	402	706	803	821

[1] g/kg of DM; estimated accordinf to NRC (2016)

Table 2. Proportion of ingredients and chemical composition of experimental diets.

	50% hay		70% hay	
	Without cactus	With cactus	Without cactus	With cactus
Proportion of ingredients (g/kg of DM)				
Leucaena hay	500	500	700	700
Spineless cactus	0	408	0	232
Corn	408	0	232	0
Soybean meal	72	72	48	48
Mineral mixture [1]	20	20	20	20
Chemical composition (g/kg of DM)				
Dry matter	836	527	834	658
Organic matter	934	907	924	909
Crude protein	144	135	142	136
Ethereal extract	40	27	39	31
NFC	405	382	324	311
NDF_{ap}	367	391	446	460

NDFi	203	223	273	284
Ash	46	73	56	71
Lignin	96	124	132	148
TDN[2]	604	544	520	486

[1] Assuarnce levels (per kg in active elements): calcium – 187 g; phosphorus – 85 g; magnesium – 15 g; sodium – 90 g; sulfur – 18 g; copper – 1350 mg; cobalt – 80 mg; iron – 1450 mg; iodine – 90 mg; manganese – 1700 mg; selenium - 22 mg; zinc – 5800 mg; fluorine max. – 850 mg; phosphorus (P) solubility in citric acid at 2% - 95% (minimum).[2] Estimated according to NRC (2016)

Subsamples of roughage and ingredients were collected, dried in a forced air oven at a temperature of 55°C for 72 h and then milled, forming particles of 2 mm (Wiley Mill, AH Thomas, Philadelphia, NY, USA). After that, they were placed in bags made of non-woven fabric (5×5 cm, pore size 50 µm, 20 g DM/m^2) and incubated in the rumen of 2 cannulated bulls, which were randomly selected to be used in this study. Thus, before incubation, the animals were adapted to a diet similar to that used in this experiment for 12 days. After 288 h, the bags were removed from the rumen, washed in running tap water and analyzed for neutral detergent fiber concentration (INCT CA-F 009/1 method described by Detmann et al. (2012) in order to determine the indigestible neutral detergent fiber (NDFi) concentration.

Data were analyzed using the SAS 9.3 MIXED procedure (SAS System Inc., Cary, NC, USA), using a Latin square design arranged in a 2 × 2 factorial scheme using the following statistical model: $Yijkl = \mu + \alpha_i + \beta_j + a_k + P_l + (\alpha\beta)ij + \varepsilon ijkl$, where Yijkl is the response variable; α_i = leucaena fixed effect; β_j = cactus fixed effect; a_k = random animal effect; P_l = random effect of the period; $(\alpha\beta)ij$ = effect of the interaction between leucaena and cactus; and eijk refers to the experimental error associated with all observations assuming an independent normal distribution (μ, σ^2)

Data were subjected to analysis of variance and compared using the F test. The error normality was verified using the Shapiro–Wilk test. Outliers were considered when the absolute values of the standardized residuals were outside the ± 3 range. In all analyses, 0.05 was adopted as tolerable for type I error

RESULTS

There was an interaction effect between the proportion of hay and the inclusion of cactus in the diet for dry matter intake (DMC) (P=0.037) and TDN (P=0.001) in kg/day^{-1} (Table 3), for digestibility of dry matter (P =0.011), NFC (P=0.001) and TDN (P=0.003) as observed in (Table 5), for dietary water intake (P=0.001) and total water (*P*=0.037) kg/day^{-1} (Table 7), for Rumination (min kg^{-1} of DM) (*P*=0.018), as well as for the efficiency of DM rumination in g h^{-1} (*P*=0.043), (Table 11).

In the decomposition of interactions, the DMC of steers fed 50% leceuna hay was higher when the diet did not contain spineless cactus (Table 4). On the other hand, when the diet had 70% cactus in it, the addition of cactus did not differ from the diet without same. In relation to TDN intake, diets containing 50% hay had higher intake when cactus was not used. In the diet containing 70% hay, TDN intake was higher with the inclusion of cactus. In relation to dry matter (DM), NFC and TDN digestibility, the diet with 50% hay presented higher values when cactus was not used. In turn, the animals fed 70% leucaena showed greater digestibility of DM, NFC and TDN when cactus was added to the diet. (Table 6).

There was an interaction between the factors for dietary (P = 0.001) and total (P = 0.037) water intakes (Table 7). In relation to total and dietary water intakes, higher values were observed when cactus was included in the Leucaena based diets. However, it is noteworthy that, despite the higher water intake in these diets, there was less water intake via the drinking trough, indicating an effective participation of the water contained in the feed (Table 8).

The different hay proportions and the inclusion of spineless cactus did not influence (P>0.05) the urinary volume, the microbial CP production nor the microbial efficiency, with mean values of 11.9 l.288.35 g/day^{-1} and 74.035 g NW/kg of TDN, respectively (Table 9).

For the DM intake expressed in % BW and g/kg$^{0.75}$, the diet containing 50% leucaena hay provided higher values, regardless of whether or not cactus was included. The intake of EE was higher in diets without the use of cactus, with an average value of 0.3 kg/day. The daily intakes of CP, NDFap and NFC were not influenced by the diets, with mean values of 0.95 kg, 2.85 kg and 2.9 kg, respectively, (P > 0.05).

The 50% proportion of leucaena hay in the diet increased (P < 0.05) N intake, as well as the level of N in the urine, in addition to a greater excretion of urea and ureic N in the urine. Regarding the inclusion of cactus in the diet, it influenced (P < 0.05) the results, with lower N intake and lower N level in the feces, besides a lower excretion of ureic N and urea via urine. The excretion of ureic N in plasma expressed in g/day^{-1} was also influenced (P < 0.05), with cactus promoting greater excretion in diets with 50% hay and less excretion in diets with 70% hay. The N balance was not influenced by the diets (P > 0.05).

The different hay proportions and the inclusion of spineless cactus did not influence (*P*>0.05) the feeding behavior of the animals, except for chewing in seconds/cud (*P* = 0.001), in which diets with the inclusion of cactus showed higher values (Table 11).

Discussion

As leucaena is an arboreal legume, the chemical composition of its hay, especially the fibrous fraction, can vary greatly depending on the height of cut, the age of the plant and the inclusion, as well as the diameter of the stem at the time of harvest. As the objective of this study was to make the most of the forage plant, they were harvested with stalks of up to 20 mm in diameter for making the hay, which resulted in lower CP and NFC content as well as higher NDF content when compared to hay with lesser share of stems originating from plants harvested with stems up to 10 mm (Abot et al., 2015). The cactus used in diets showed nutrient levels which were in line with those mentioned in other studies (Morais et al., 2019; Barros et al., 2018).

Table 3. Dietary dry matter and nutrient intake by crossbred steers fed diets containing leucaena hay and spineless cactus.

Item	Hay proportion		Cactus inclusion		SEM[1]	*P-value*[5]		
	50%	70%	Without	With		Hay	Cactus	Hay x cactus
Intake (kg/day)								
Dry matter	7.8	6.9	7.5	7.2	0.22	0.016	0.305	0.037
Crude protein	1.0	0.9	1.0	0.9	0.04	0.128	0.104	0.358
Ethereal extract	0.3	0.2	0.3	0.2	0.01	0.037	0.000	0.058
NDF_{ap}[2]	2.8	2.9	2.9	2.8	0.10	0.614	0.771	0.245
NFC[3]	2.9	2.9	2.9	2.9	0.17	0.952	0.984	0.972
TDN[4]	5.0	4.2	4.6	4.6	0.54	0.004	0.936	0.001
Intake (% body weight)								
DM	2.45	2.18	2.37	2.26	0.06	0.019	0.308	0.120
NDF_{ap}	0.88	0.91	0.90	0.89	0.04	0.571	0.823	0.125
Intake (% metabolic weight)								
DM	103.6	91.8	99.9	95.5	3.18	0.017	0.350	0.087

[1] Standard error of the mean, [2]Neutral detergent fiber corrected for ash and protein, [3]Non-fibrous carbohydrates, [4]Total digestible nutrients, [5] Probability

The observed intake of DM and TDN were lower than the values estimated by Valadares Filho et al. (2016) for crossbred steers with an average weight of 318.00 kg, which were 8.08 kg of DM/day^{-1}, corresponding to 2.5% BW, and 5.94 kg /day^{-1} of TDN/day^{-1}.

It was suggested by Mertens (1994) that the concentration of NDF in the diet limits intake as it is inversely related to the energy content of the diet, although the observed average NDF intake of 0.9% BW was below the value of 1.2% indicated by these authors, suggesting that the fiber content of the diet did not limit intake. Thus, the lower NDT content observed in the diets, especially those with a 70% proportion of hay, may have limited the energy supply via an overload of protein, creating an imbalance, causing low microbial growth, and consequently a lower intake of DM (Calsamiglia et al., 2010). Another factor that may have influenced DM intake are antinutritional factors and the presence of condensed tannins in leucaena hay, which can have a negative effect on DM digestibility and intake (Gusha et al., 2015; Giang et al., 2016; Piñeiro-Vázquez et al., 2017).

Radrizzani & Nasca, (2014) reported symptoms of intoxication in steers that grazed in an integration system that mixed Leucena with Marandu grass. The symptoms observed were lethargy, decreased appetite, excessive salivation, skin wounds and tail hair loss, and appeared during the last stages of the experiment. In this experiment, no symptoms of mimosin intoxication were observed. In addition, the process of dehydration reduces mimosin content (Fasae et al., 2011). Another factor that must be taken into account is that the mimosin level of the stem and bark of leucaena is only 20% of that found in the leaf material, so the dilution effect in diets with inclusion of the whole plant would actually reduce toxicity problems (Tesfay & Tesfay, 2013, Radrizzani & Nasca, 2014).

The intake of DM by ruminants can be influenced by different factors, which may be related to physical limitation (rumen filling), physiological factors related to the supply of diets and psychogenic factors that involve responses in the animal's behavior (Moraes et al., 2017). Likewise, Borges et al. (2019) observed an effect of including cactus in the diet of dairy cows, with a decrease in intake when cactus was used together with sorghum silage, which, according to the authors, is a result of the DM content of the diets.

Table 4. Decomposition of interactions for dry matter (DM) and total digestible nutrient (TDN) intake in crossbred steers fed diets containing leucaena hay and spineless cactus.

Proportion of hay	Without cactus	With cactus	SEM[1]
	DM intake kg/day		
50%	8.3 Aa	7.2 Ab	0,337

70%	6.7 Ba	7.1 Aa	
	TDN intake kg/day		
50%	5.5 Aa	4.5 Ab	0,262
70%	3.7 Bb	4.7 Aa	

[1] Standard error of the mean, Means followed by the same lowercase letter in the row and uppercase latter in the column do not differ by the f test (*P*<0.05).

The higher intake of TDN for the diet with 50% hay and without the inclusion of cactus can be explained by the composition of corn that has more TDN in relation to spineless cactus (Table 1). However, the interaction effect revealed that for diets with a high proportion of hay (70%) the intake of TDN ended up being higher in diets with inclusion of cactus. It is possible that the presence of mucilage in the cactus allowed a greater adherence of the components of the diets, reducing the selection and thus providing a greater intake of the cactus, and consequently of TDN.

The diet with 50% hay and without the use of cactus showed greater digestibility for DM, NCF and TDN (Table 6). These diets, in addition to having higher levels of these nutrients, also had lower levels of NDF and NDFi in their composition, which are components of the plant, which interfere a lot in food digestibility. Besides, according to Piñeiro-Vázquez et al. (2017), the effect of the use of leucaena on digestibility has been characterized by the interaction between condensed tannins and the imbalance in the energy-protein ratio, which has impaired the development of cellulolytic bacteria.

Table 5. Digestibility coefficient of dry matter and nutrients in the diet of crossbred steers fed with leucaena hay and spineless cactus.

Item	Proportion of hay		Cactus inclusion		SEM[1]	*P-value*[5]		
	50%	70%	Without	With		Hay	Cactus	Hay x cactus
DM	57.3	54,5	53.6	58.1	1.21	0.113	0.014	0.011
Crude protein	46.8	51,7	49.8	48.7	2.77	0.220	0.784	0.884
Ethereal extract	52.2	43,7	53.1	42.8	2.94	0.052	0.020	0.673
NDF_{ap}[2]	48.8	49,5	47.1	51.2	1.37	0.703	0.049	0.505
NFC[3]	76.5	71,9	71.2	77.2	1.52	0.042	0.011	0.001
TDN[4]	64.4	60,8	60.6	64.6	1.50	0.103	0.076	0.003

[1] Standard error of the mean[2]Neutral detergent fiber corrected for ashes and protein , [3]Non-fibrous carbohydrates, [4]Total digestible nutrients. [5] Probability

Regarding the use of spineless cactus, Almeida et al. (2017) observed a decrease in DM digestibility when corn was replaced by cactus in the supplementation of pasture-based heifers, which, according to the authors, was due to the increase in cell wall constituents in the diet as corn was replaced by cactus.

At the same time, in several studies, the cactus has improved the digestibility of diets when used to replace roughage (Barros et al., 2018, Cardoso et al., 2019). This behavior was possibly because the constituents of the cactus, especially DM, have a high degradation rate, thus favoring the maximization of the rumen's fermentative capacity due to the high content of NFC (Siqueira et al., 2017). Unlike in the diet with 50% hay, with the use of 70% leucaena hay the inclusion of cactus promoted greater digestibility of DM, NFC and TDN (Table 4), in addition to greater intake of the cactus due to the greater capacity that the cactus has of adhering to the dietary components.

Table 6. Decomposition of interactions for the coefficient of digestibility of dry matter (DM), non-fibrous carbohydrates (NFC) and total digestible nutrients (TDN) of the diet of crossbred steers fed leucaena hay and spineless cactus.

Proportion of hay	Without cactus	With cactus	Mean	SEM[1]
	DM digestibility (%)			
50%	57.4 Aa	57.2 Aa	57.3	1.71
70%	49.8 Bb	59.1 Aa	54.5	
Mean	53.6	58.1		
	NFC digestibility (%)			
50%	77.6 Aa	75.5 Aa	76.5	0.262
70%	64.8 Bb	78.9 Aa	71.9	
Mean	71.2	77.2		
	TDN (%)			
50%	65.9 Aa	62.9 Aa	64.4	2.12
70%	55.3 Bb	66.2 Aa	60.8	
Mean	60.6	64.6		

[1] Standard error of the mean, Means followed by the same lowercase letter in the row and uppercase latter in the column do not differ by the f test (P<0.05).

Diets with the use of cactus promoted a reduction in water intake by 85% as a result of the high amount of water present in the composition of this forage plant (Table 1). These are important results, especially for production systems located in semi-arid regions, where restrictions in the supply of water for animals can occur throughout the year, which may limit the performance of animals. In this case, saved water can be used for other activities.

Table 7. Water intake by crossbred steers fed diets containing leucaena hay and spineless cactus.

Water intake (kg/day)	Proportion of hay		Cactus inclusion		SEM[1]	*P-value*[2]		
	50%	70%	Without	With		Hay	Cactus	Hay x cactus
Free (water trough)	12.8	11.3	20.9	3.1	0.90	0.238	0.000	0.634

Diet	18.9	23.7	1.78	40.8	1.00	0.003	0.000	0.001
Total	28.7	29.9	22.0	36.5	1.48	0.460	0.000	0.037

[1] Standard error mean, [2] Probability

Table 8. Decomposition of interactions for dietary water and total water intake by crossbred steers fed diets containing leucaena hay and spineless cactus.

Proportion of hay	Without cactus	With cactus	Mean	SEM[2]
	Dietary water (kg/day)			
50%	2.0 Ab	35.9 Ba	18.9	1.42
70%	1.5 Ab	45.8 Aa	23.7	
Mean	1.78	40.8		
	Total water (kg/day)			
50%	23.2 Ab	34.2 Ba	28.7	1.60
70%	20.9 Ab	38.9 Aa	29.9	
Mean	22.0	36.5		

[1] Standard error of the mean, Means followed by the same lowercase letter in the row and uppercase latter in the column do not differ by the f test (*P*<0.05).

In other studies, the urinary volume was also not influenced by the use of cactus in the diet of heifers (Aguiar et al., 2015) or lactating cows (Moraes et al., 2019). In a study by Barros et al. (2018), the high humidity of the cactus was responsible for an increase in the urinary volume of heifers, which had the tifton hay replaced by cactus at the inclusion levels of 0.0, 167, 333 and 500 g kg^{-1}.

Microbial protein synthesis is important in ruminants, as it provides protein in necessary quantity and quality to the host animal, accounting for 50 to 80% of the total protein absorbed (Nguyen et al., 2017). The microbial efficiency observed in this study was below the value suggested by Valadares Filho et al. (2016) who, based on research data collected in Brazil, recommended the value of 120 g PB mic/kg of TDN as a reference for warranting microbial synthesis efficiency in tropical conditions. Microbial protein synthesis in ruminants is dependent on the availability of carbohydrates and nitrogen in the rumen (NRC, 2016), with energy supply being the first factor to limit microbial growth, whose main function is to release ATP in order to guarantee the use of ammonia for amino acid synthesis and microbial growth (Possenti et al., 2008).

Table 9. Urinary volume, microbial protein synthesis and microbial efficiency of crossbred steers fed diets containing leucaena hay and spineless cactus.

Item	Proportion of hay		Cactus inclusion		SEM[1]	*P-value*[2]		
	50%	70%	Without	With		Hay	Cactus	Hay x cactus
Urine (l day^{-1})	11.2	12.6	12.5	11.3	2.02	0.640	0.693	0.962

Microbial production (g/day)								
Microbial CP	302.2	274.5	287.6	289.0	12.3	0.125	0.943	0.739
Microbial efficiency								
g CP/kg TDN	68.9	79.1	72.7	75.4	3.62	0.059	0.598	0.895

[1] Standard error of the mean, [2] Probability

The intake of TDN below the recommended level observed in this study may have influenced the microbial efficiency, since all diets showed a crude protein content above 11%, which would be the minimum for optimal microbial growth to occur (Pathak 2008). On the other hand, the intake of large amounts of leucaena can have a negative impact on animal performance, mainly due to excess nitrogen in the diet, which causes an imbalance in the protein-energy ratio, resulting in inefficient microbial protein synthesis (Calsamiglia et al., 2010).

The higher N intake in diets with 50% hay and in diets without the use of spineless cactus is related to higher intake of DM and TDN, in addition to these diets having presented a higher CP content in their composition (Table 2). The lowest values of N in feces and urine in diets with cactus and with 70% of leucaena hay were due to lower nitrogen intake. Regarding the lower excretion of ureic N and urea in plasma and urine in diets with inclusion of cactus, it may be related to the reduction in protein intake, since cactus has a lower protein content compared to corn. Barros et al. (2018) also observed a reduction in the excretion of ureic N and urea in heifers when fed with cactus as a replacement for tifton hay at increasing levels, reaching a maximum value of 500 g/kg^{-1}.

Table 10. Balance of nitrogen compounds of crossbred steers fed diets containing leucaena hay and spineless cactus.

Item	Proportion of hay		Cactus inclusion		SEM[1]	*P-value*[2]		
	50%	70%	Without	With		Hay	Cactus	Hay x cactus
N balance, g/day^{-1}								
Ingested N	195.2	150.3	195.0	150.4	11.1	0.010	0.010	0.195
N in feces	95.3	85.5	97.5	83.4	4.4	0.133	0.036	0.967
N in urine	69.1	42.4	63.9	47.6	8.4	0.036	0.186	0.253
Retained N	30.7	22.3	33.7	19.4	7.5	0.441	0.195	0.495
N balance	15.0	14.2	15.6	13.6	3.3	0.875	0.661	0.683
Excretion g/day^{-1}								
Ureic N in plasma	0.08	0.09	0.10	0.08	0.005	0.394	0.001	0.025

Urea in urine	80.1	45.2	85.5	39.7	7.9	0.005	0.000	0.076
Ureic N in urine	3.56	2.51	3.99	2.0	0.35	0.044	0.001	0.439

[1] Standard error of the mean, [2] Probability

It was also reported by Nguyen et al. (2017) that N excretion increased when the leucaena supply was greater in the diets for steers and in buffaloes (Phesatcha & Wanapat, 2016).

The ingestive behavior of animals can be influenced by several factors, including those related to diet, such as the quantity and quality of fiber, food particle size, food moisture, exposure of soluble nutrients to fermentation and microbial colonization, in addition to factors inherent to animals and the environment (Riaz et al., 2014). In the present study, it is possible to observe the influence of the NDF and TDN levels in the diets, with the diet that had a lower proportion of hay and no inclusion of cactus requiring less time for rumination and chewing. The influence of the proportion of hay on feeding and chewing time is a result of the nature of the diet and seems to be proportional to the cell wall content of bulky foods, with fiber effectiveness being a key factor in stimulating chewing (Grant & Albrigth, 1995).

Table 11. Feeding behavior of crossbred steers fed diets containing leucaena hay and spineless cactus.

Item	Proportion of hay		Cactus inclusion		SEM[1]	*P-value*[2]		
	50%	70%	Without	With		Hay	Cactus	Hay x cactus
Time spent (minutes / day)								
Alimentation	204.4	220.6	204.4	220.6	11.6	0.338	0.338	0.765
Rumination	461.2	444.4	450.6	455.0	17.9	0.515	0.865	0.283
Idleness	774.37	775.00	785.00	764.37	18.1	0.989	0.438	0.380
Chewing								
number/cud	61.6	62.4	66.6	57.5	2.0	0.793	0.005	0.538
second/ cud	56.0	57.4	61.1	52.4	1.5	0.545	0.001	0.121
number/day	30329	28976	29274	30031	1181.7	0.428	0.655	0.534
Rumination								
Min kg^{-1} DM	60.2	65.5	64.1	61.6	2.7	0.187	0.522	0.018
Min kg^{-1} NDF_{ap}	32.0	30.9	31.3	31.6	11.9	0.306	0.912	0.522
Number of periods								
Alimentation	14.2	15.3	14.1	15.3	0.80	0.338	0.338	0.765
Rumination	16.1	16.5	15.8	16.8	0.83	0.793	0.379	0.379
Idleness	21.8	23.2	21.7	23.4	0.65	0.134	0.082	0.840
Feeding efficiency								

g DM h^{-1}	2695.8	2130.8	2545.7	2280.8	285.0	0.178	0.519	0.198
g NDF_{ap} h^{-1}	958.9	908.8	953.9	913.8	102.8	0.735	0.785	0.833
Ruminal efficiency								
g DM h^{-1}	1061.4	957.1	1060.6	957.7	60.4	0.237	0.245	0.043
g NDF_{ap} h^{-1}	383.8	404.8	409.6	379.1	26.9	0.587	0.435	0.899

[1] Standard error of the mean, [2] Probability

With the use of the palm, the number of cuds chewed per day, in addition to the time spent per cud, was lower. Possibly, the high humidity of the cactus, in addition to its effect of decreasing the DM content of the diet, ends up requiring less time to moisten the food cud, which eventually reduces both the chewing time spent to reduce the particle size and the number of cuds, allowing thus its rapid passage through the digestive system.

Regarding rumination efficiency, the shorter time spent for rumination of DM in the diet with 50% hay and without the inclusion of cactus ended up resulting in a greater overall efficiency of rumination in this diet, also reflected in the voluntary intake, which was higher for this same diet.

Conclusion

Given the obtained results, it can be concluded that diets with 50% leucaena hay without inclusion of spineless cactus provide greater consumption of nutrients. The use of cactus increases digestibility of diets with greater roughage content.

Credits

The authors of the present study thank CAPES (Coordination for Improvement of Higher Education Personnel) and the State University of Southwest of Bahia.

Universidade Estadual do Sudoeste da Bahia – UESB
Autorizada pelo Decreto Estadual nº 7344 de 27.05.98
Comitê de Ética No Uso de Animais – CEUA / UESB

CERTIFICADO

Certificamos que a proposta intitulada "**Feno de leucena e palma forrageira na alimentação de ruminantes**", registrada com o nº **176/2018**, sob a responsabilidade de Aureliano Jose Vieira Pires, UESB (Departamento de Tecnologia Rural e Animal – Campus de Itapetinga) - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8/10/2008, do Decreto nº 6.899, de 15/07/2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Estadual do Sudoeste da Bahia (UESB), em reunião de 15/04/2015.

Finalidade	( ) Ensino (**x**) Pesquisa Científica
Vigência da autorização	13/08/2018 a 29/03/2019
Espécie/linhagem/raça	Bovino Gir x Holandês
Nº de animais	08 animais
Peso/Idade	200 kg / 12 meses.
Sexo	Machos
Origem	Fazenda Bela Vista - Encruzilhada, Ba.

Lembramos ao pesquisador que:

- O responsável pela proposta encaminhará à CEUA, ao final do estudo, um relatório de uso de animais. O relatório deverá conter informações básicas acerca da proposta de acordo com o roteiro publicado em conjunto com a RN nº 4 do CONCEA publicado no DOU em 19/04/2012.

- No caso da necessidade da continuidade das propostas usando animais para fins científicos ou didáticos é obrigatório o envio do Relatório à CEUA acrescido da justificativa.

- Para os casos da continuidade de propostas, após a análise do relatório e de esclarecimentos adicionais, se necessário, a CEUA pode deferir, suspender, ou requerer modificação dos mesmos, dentro de suas atribuições.

Itapetinga, 13 de Agosto de 2018.

Sônia Martins Teodoro

Drª. Sônia Martins Teodoro
Coordenadora CEUA/UESB

DECLARATION OF CONFLICTS OF INTEREST

I declare that there is not conflict of interest between the authors of the article "Nutrient intake and digestibility, microbial protein synthesis and ingestive behavior of dairy steers fed with leucaena hay associated or not with spineless cactus" submitted for consideration in the journal Tropical Animal Health and production.

Itapetinga, Ba, Brazil, August 16, 2021.

Weudes Rodrigues Andrade
Author responsible for submission

CONTRIBUTIONS MADE BY EACH LISTED AUTHOR

WRA- Idealization of the experimente, Experiment planning, Conducting the Experiment, Data analysis and review and wrote the article

AJVP- Idealization of the experimente, Experiment planning, Data analysis and review

FAT- Contributed new reagents or analytical tools

JAGA- Data analysis and review

ARS- Idealization of the experimente, Data analysis and review

MSN- Conducting the Experiment

All authors read and approved the manuscript,

Itapetinga, Ba, Brazil, August 16, 2021.

Weudes Rodrigues Andrade

Author responsible for submission

AVAILABILITY DECLARATION OF DATA AND MATERIAL

I declare that datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Itapetinga, Ba, Brazil, August 16, 2021.

Weudes Rodrigues Andrade
Author responsible for submission

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