

# Levels of leucaena hay (*Leucaena leucocephala* *leucocephala*) associated with cactus pear (*Nopalea cochenilifera* Salm Dyck) in diets for crossbred steers

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## Research Article

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# Abstract

Our objective was to evaluate the effects of leucaena hay combined with cactus pear on the nutritional parameters and nitrogen metabolism of crossbred steers. Eight crossbred steers, with an average age of 12 months, initial weight of  $267.5 \pm 15$  kg, were distributed into two simultaneous 4x4 Latin squares according to a 2 x 2 factorial arrangement, in which the first factor evaluated two different proportions of inclusion of hay from leucaena (50 or 70% dry matter basis), and the second factor was the total replacement of ground corn grain with cactus pear. Data were analyzed by the MIXED procedure of SAS. Significance was set at  $p \leq 0.05$ . There was lower dry matter (DM) intake ( $P < 0.05$ ) in animals fed 70% hay. There was an interaction ( $P < 0.05$ ) between the factors for DM digestibility, in which the animals fed cactus pear did not differ regardless of the inclusion of leucaena, but for animals without cactus pear, the greater inclusion of leucaena hay decreases DM digestibility. The inclusion of cactus decreased ( $P < 0.05$ ) free water intake. On the other hand, there was interaction ( $P < 0.05$ ) for total water intake, in which the animals fed without cactus pear presented similarity for this, while in the inclusion of cactus pear, the animals fed 70% of leucaena presented higher water intake. Also, lower values ( $P < 0.05$ ) of chewing (number/bolus and number/d) were observed for animals fed cactus pear.

## Introduction

Traditionally, soybean meal and corn grain are used as a source of protein and energy, respectively, in the ruminant's diets. However, in semiarid regions, the availability and cost of these feeds constitute obstacles to the production of ruminants. Thus, there is a need to replace them with energy and protein feed sources with lower cost and greater tolerant to the dry season.

Spineless cactus use can reduce the use of concentrate feed and effective cost of the diet, and it is still able withstand prolonged droughts and having high production potential in these regions. Additionally, it has energy potential for feeding ruminants, due to the high proportion of non-fibrous carbohydrates, but it has a low crude protein (CP) content (Dubeux Jr et al., 2021), and it is used as a water source (Albuquerque et al., 2020). Moreover, it is low in fiber (Borges et al., 2019), requiring correction of the neutral detergent fiber (NDF) in the diet to maintain the dry matter (DM) intake and activities of chewing and rumination (Li et al., 2014).

Studies carried out in the tropics have shown that a diet balanced in protein and energy can promote interactive effects on the metabolism of nitrogenous compounds and increase nitrogen body accretion (Palma et al., 2023). There are several sources of fiber and protein with potential for use in cactus pear diets, especially leucaena hay (Wanderley et al., 2012). Leucaena is a tropical legume, also tolerant to the dry season, with perennial growth, being one of the most used legumes in tropical regions, mainly due to good protein content and fiber (Moreira et al., 2020), which encourages its use in feeding ruminants in association with cactus pear. However, the feasibility of using alternative feeds for ruminants, depends among others, on the nutritional quality and responses in animal production when compared to the traditional feeds (Halmemies-Beauchet-Filleau et al., 2018).

The literature (Moreira et al., 2020) showed the potential of this association for sheep. Thus, suppose it would also be applied to cattle. Our hypothesis is that the total replacement of ground corn grain with cactus pear in association with the inclusion of 70% leucaena hay (partially replacing the soybean meal) increase the dry matter intake, apparent digestibility, nitrogen retention and maintains feeding behavior. Therefore, we aimed to evaluate the effects of the association between leucaena hay and cactus pear in the diet of crossbred steers on feed intake and apparent digestibility, water intake, nitrogen balance, microbial protein synthesis and feeding behavior.

## **Materials and methods**

### **Animals, experimental design, and management**

The experiment was carried out at the Bela Vista farm, located in the Encruzilhada, Bahia, Brazil. All practices involving the use of animals were approved by the Institutional Animal Care Committee (Protocol no. 03/2016). At the beginning of the experimental period, the animals were identified and treated against endo- and ectoparasites. The experiment lasted 84 days, divided into 4 periods of 21 d, 16 d for the adaptation of the animals to the diets (Machado et al., 2016), and 5 d for data collection and samples.

Eight crossbred steers (Holstein/Zebu), with an average age of 12 months, initial body weight (BW) of  $267.5 \pm 10$  kg were used. The experimental design was simultaneous in two 4×4 Latin squares, being four diets, four experimental periods and four animals in each square. The animals were allocated to the different squares according to their initial BW. The steers were housed in individual stalls (2 x 6 m) equipped with concrete floors, individual feeders, and water drinkers, which assured unrestricted access to feed and water.

### **Treatments and feed processing**

The four experimental diets (treatments) were used: Diet 1, cactus pear associated with Leucaena hay in a proportion of 50% in the diet, as dry matter (DM) basis; Diet 2, cactus pear associated with Leucaena hay in a proportion of 70% in the diet (DM basis); Diet 3, without cactus pear, but with corn ground grain associated with Leucaena hay in a proportion of 50% in the diet (DM basis); Diet 4, without cactus pear, but with corn ground grain associated with Leucaena hay in a proportion of 70% in the diet (DM basis). The inclusion of leucaena hay partially replaced the soybean meal, a conventional protein source of high cost. On the other hand, the inclusion of cactus pear completely replaced ground corn grain in the diet (Table 1). The proportion of ingredients and chemical composition of experimental diets can be found in Table 2.

Table 1  
Chemical composition (g/kg DM) of the ingredients

| Item                                                                                                      | Ingredients  |             |             |              |
|-----------------------------------------------------------------------------------------------------------|--------------|-------------|-------------|--------------|
|                                                                                                           | Leucaena hay | Cactus pear | Ground corn | Soybean meal |
| Dry matter, g/kg as fed                                                                                   | 846          | 95          | 852         | 877          |
| Organic matter                                                                                            | 930          | 918         | 986         | 932          |
| Crude protein                                                                                             | 135          | 75          | 98          | 508          |
| <sup>1</sup> Neutral fiber detergent                                                                      | 568          | 231         | 171         | 177          |
| iNDF                                                                                                      | 378          | 81          | 31          | 20           |
| Lignin                                                                                                    | 187          | 73          | 6           | 3            |
| TDN                                                                                                       | 402          | 706         | 803         | 821          |
| <sup>1</sup> corrected for ash e protein; TDN = total digestible nutrients; NFC = non-fiber carbohydrates |              |             |             |              |

Table 2  
Proportion of ingredients and chemical composition of experimental diets

|                                                                             | Cactus  |         | No Cactus |         |
|-----------------------------------------------------------------------------|---------|---------|-----------|---------|
|                                                                             | 50% hay | 70% hay | 50% hay   | 70% hay |
| <i>Proportion of the ingredients in diet (g/kg DM)</i>                      |         |         |           |         |
| Leucaena hay                                                                | 500     | 700     | 500       | 700     |
| Cactus pear                                                                 | 408     | 232     | 0         | 0       |
| Ground corn                                                                 | 0       | 0       | 408       | 232     |
| Soybean meal                                                                | 72      | 48      | 72        | 48      |
| Mineral mixture                                                             | 20      | 20      | 20        | 20      |
| <i>Chemical composition (g/kg DM)</i>                                       |         |         |           |         |
| Dry matter                                                                  | 527     | 658     | 836       | 834     |
| Organic matter                                                              | 907     | 909     | 934       | 924     |
| Crude protein                                                               | 135     | 136     | 144       | 142     |
| <sup>1</sup> Neutral fiber detergent                                        | 391     | 460     | 367       | 446     |
| iNDF                                                                        | 223     | 284     | 203       | 273     |
| Lignin                                                                      | 124     | 148     | 96        | 132     |
| TDN                                                                         | 544     | 486     | 604       | 520     |
| <sup>1</sup> corrected for ash e protein; TDN = total digestible nutrients; |         |         |           |         |

The feeding was performed twice a day at 7:00 am and 1:00 pm to allow for *ad libitum* intake, allowing approximately 100 g/kg in orts (as fed). The diets were formulated to be isonitrogenous and to feed steers with an average of 300 kg BW and an expected gain of 1.5 kg/d (Valadares Filho et al., 2016). Leucaena hay was produced at the Universidade Estadual do Sudoeste da Bahia and at the Instituto Federal Baiano, Bom Jesus da Lapa, Bahia, both places the hay was produced from plants with stems up to 20 mm in diameter. The hay was subjected to processing by the stationar pick, set at 15mm, and after was inserted on canvas in the sun, being manually revolved, once a day, for drying. After dehydrated (moisture less that 15%), they were stored in bags and kept in an appropriate environment. The cactus pear (*Nopalea cochenillifera* Salm Dyck) was harvested daily after 2 years of planting.

## Sample colletions

## Voluntary intake and apparent digestibility

In each experimental period, feed intake was quantified from d 17 to d 21, through of the amount of diet offered from d 17 to d 21, and the orts obtained from d 18 to d 22. Daily, samples of ingredients and orts were collected and weighed individually for daily adjustment and calculation of feed intake. The weight of the animals was recorded at the beginning and at the end of each experimental period in order to calculate the average BW and relative feed intake.

The estimation of fecal output per animal per day, to determine digestibility, was made using indigestible neutral detergent fiber (iNDF) as marker. The calculation was performed by dividing the iNDF intake by the concentration of iNDF in the feces. The fecal samples were collected on the d 18 to d 22 of each period, immediately after each spontaneous defecation. Representative samples of feces and orts were collected, stored in plastic bags, and blended manually at the end of each period to obtain pooled samples per animal. Samples of diet ingredients were grouped by period. All samples were dried in a forced air oven (55 °C) for 72 h and ground in a knife mill (Tecnal, 132 Piracicaba, SP, Brazil).

The water intake was quantified by weighing the water offered in plastic buckets and subtracting the orts after a period of 24 hours, from the d 17 to the d 21, and the water lost through evaporation. To calculate the amount of evaporated water, plastic buckets with water were distributed in the same location of the bays where water was offered and weighed every 24 h, to calculate the loss of water by evaporation. Feeding water, in order to calculate the total water intake, was calculated by determining the dry matter content of feed and orts.

## Nitrogen compounds balance and microbial protein synthesis

Urine samples (*spot*) were collected on the last day of the collection period (21d) in spontaneous urination of the animals, four hours after the morning feeding (Valadares et al., 1999), in order to calculate urinary concentrations of total nitrogen compounds, creatinine, allantoin and uric acid. Immediately after sampling, aliquots of 10 mL were diluted into 40 mL of sulfuric acid at 0.036 N to avoid bacterial action, precipitation, and degradation of the purine derivatives and uric acid. Urine samples were stored frozen at -20 °C.

The daily urinary volume of each animal was estimated through the ratio between the amount of creatinine excreted daily by the concentration of creatinine (mg/L) in the spot sample. The mean value of 24.04 (mg/kg of BW) (Chizzotti et al., 2008) was used to total daily excretion of creatinine. The balance of nitrogen compounds was calculated as:  $N \text{ retained (g)} = \{N \text{ ingested (g)} - N \text{ fecal (g)} - N \text{ urine (g)}\}$ , where: Nitrogen balance = nitrogen retained in the animals; N intake = nitrogen ingested by the animal; N fecal = nitrogen excreted in feces and N urine = nitrogen excreted in urine. The daily urinary volume of each animal was estimated through the ratio between the amount of creatinine excreted daily by the concentration of creatinine (mg/L) in the *spot* sample.

Blood samples were collected on the last day (on the 21 day) of each experimental period, via puncture of the jugular vein, using 5mL test tubes (Vacutainer™) with EDTA (anticoagulant). After, the blood samples

were centrifuged out at 3.500 rpm for 10 minutes and, subsequently, plasma samples were taken, which were packed in eppendorf and stored at - 15°C for further analysis of urea.

Urinary purine derivatives methodology was used to estimate microbial protein synthesis (MPS). The excretion of total purines was estimated by the sum of the amounts of allantoin and uric acid excreted in the urine and the amount of absorbed purines (mmol/day), by the excretion of total purines (mmol/day), according to the equation of Verbic et al. (1990):  $AP = \{(total\ purines - 0.385 \times BW^{0.75}) / 0.85\}$ , where: AP = absorbed purines (mmol/day); 0.85 = urinary recovery rate for purines derivatives in the urine; and  $0.385 \times BW^{0.75}$  = endogenous fraction of urinary for purine derivatives. To estimate microbial protein production, purine bases (mmol/day) were used as a microbial indicator, whose quantification was performed according to the technique of Chen and Gomes (1992):  $MPS\ (g/day) = \{(70 \times AP) / (0.85 \times 0.116 \times 1000)\}$ , assuming the value of 70 for the nitrogen content in the purines (mg/mmol); 0.83 for intestinal digestibility of microbial purines and 0.116 for the NPURINE:NTOTAL ratio in bacteria.

## Feed behavior

For the evaluation of feed behavior, the steers were visually observed for 24 hours on the d 20 of each period, and observations were recorded every 5, including eating, ruminating, and total chewing times (min/d), being calculated by the number of observations multiplied by 5 min (Borges et al., 2019). The behavior variables were expressed as min/kg DM intake. The rumination efficiency was calculated according to the methodology of Burger et al. (2000).

## Laboratory analyses

Samples of ingredients, orts, and feces were processed to pass through a 1-mm sieve, were pooled per animal and experimental period, and analyzed with regards DM (method G-003/1), crude protein (CP; method INCT-CA N-001/1), ash (M-001/1), NDFap (neutral detergent fiber corrected for ash and protein was analyzed using a heat-stable  $\alpha$ -amylase; method F-002/1), lignin (method F-005/1) and ether extract (method G-004/1) according to the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann et al., 2012). Moreover, these samples were analyzed for iNDF (neutral detergent fiber indigestible), by of *in situ* incubation for 288 hours in the rumen (Valente et al., 2011). The non-fiber carbohydrates (NFC) content according to Detmann and Valadares Filho (2010):  $NFC = OM - CP - apNDF - EE$ . Total digestible nutrients (TDN) were estimated according to Weiss et al. (1999).

The concentrations of urea, creatinine and uric acid in the urine and urea in the plasma were determined using commercial kits (Bioclin, Belo Horizonte, MG, Brazil). The urinary contents of allantoin and uric acid were estimated by colorimetric methods (Chen and Gomes, 1992), and the total nitrogen in the urine estimated by the Kjeldhal method (Detmann et al., 2012). Similarly, the urea in blood serum was evaluated using the enzymatic-colorimetric (Bioclin, Belo Horizonte, MG, Brazil).

## Statistical analyses

The response variables were analyzed according to the model:

$$Y_{ijkl} = \mu + Q_i + T_j + A(i)k + P(i)l + (Q \times T)_{ij} + \varepsilon_{ijkl},$$

where  $Y_{ijkl}$  is the response measured on the animal  $k$  and period  $l$  within the Latin square  $i$  and, submitted to the treatment  $j$ ,  $\mu$  is the overall constant,  $Q_i$  is the effect of the Latin square  $i$  (random),  $T_j$  is the effect of the treatment,  $A(i)k$  is the effect of the animal  $k$  nested to the Latin square  $i$ ,  $P(i)l$  is the effect of the period  $l$  nested to the Latin square  $i$ ,  $Q \times T_{ij}$  is the interaction effect between Latin square  $i$  and treatment  $j$  (random), and  $\varepsilon_{ijkl}$  is the random error (NIID,  $\mu$ ,  $\sigma^2$ ). We performed an analysis of variance to assess the interaction between Latin square and treatment. Because no significant interaction was found ( $P > 0.05$ ), we removed it from the model (Vidal et al., 2022).

The SLICE option was used when interactions were significant to partition and test the simple main effects. Statistical analyses were performed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) and significances were declared at  $P < 0.05$ .

## Results

### Intake and apparent digestibility

There was no significant interaction ( $P > 0.05$ ) between the proportion of hay and the inclusion of cactus pear in the diet for intakes of DM (DMI), TDN and NFDap (Table 3). However, there was a significant effect ( $P < 0.05$ ) of the proportion of leucaena hay on intakes of DM and TDN (kg/d), with lower values for animals consuming 70% hay. There was a significant interaction ( $P < 0.05$ ) between the proportion of hay and the inclusion of cactus on DM digestibility. Interaction slicing showed that the addition of cactus pear to diets with 70% leucaena hay resulted in improved DM digestibility compared to the diet with 70% hay without cactus. On the other hand, diets with 50% leucaena hay do not show differences in DM digestibility when cactus pear is added or not (Table 3).

Table 3

– Voluntary intake and apparent digestibility by crossbred steers fed diets containing leucaena hay and cactus pear

| Item                                              | Cactus |       | No cactus |       | SEM   | P-value <sup>c</sup> |        |       |
|---------------------------------------------------|--------|-------|-----------|-------|-------|----------------------|--------|-------|
|                                                   | 50     | 70    | 50        | 70    |       | Cactus               | Hay    | C x H |
| <i>Intake, kg/d</i>                               |        |       |           |       |       |                      |        |       |
| Dry matter                                        | 7.23   | 7.10  | 8.35      | 6.70  | 0.402 | 0.35                 | 0.029  | 0.06  |
| Total digestible nutrients                        | 4.37   | 3.46  | 4.55      | 3.49  | 0.21  | 0.63                 | 0.0002 | 0.71  |
| Neutral detergent fiber <sup>a</sup>              | 2.90   | 2.77  | 2.72      | 3.05  | 0.22  | 0.77                 | 0.60   | 0.23  |
| <i>Intake, % BW</i>                               | 2.31   | 2.21  | 2.60      | 2.15  | 0.145 | 0.29                 | 0.017  | 0.11  |
| Dry matter                                        |        |       |           |       |       |                      |        |       |
| Neutral detergent fiber <sup>a</sup>              | 0.92   | 0.86  | 0.84      | 0.97  | 0.057 | 0.81                 | 0.54   | 0.11  |
| <i>Digestibility, g/kg</i>                        | 51.5a  | 53.7a | 51.7a     | 43.1b | 1.99  | 0.012                | 0.097  | 0.009 |
| Dry matter                                        |        |       |           |       |       |                      |        |       |
| Neutral detergent fiber <sup>a</sup>              | 43.5   | 45.9  | 40.4      | 39.8  | 2.22  | 0.036                | 0.68   | 0.47  |
| Crude protein                                     | 44.4   | 39.4  | 46.8      | 39.9  | 4.26  | 0.73                 | 0.18   | 0.82  |
| <sup>a</sup> corrected for ash e<br>protein ash e |        |       |           |       |       |                      |        |       |

## Water intake

Cactus pear inclusion decreased ( $P < 0.05$ ) free water intake and free water intake/DM intake ratio (Table 4). An interaction ( $P < 0.05$ ) was detected for total water intake, in which animals fed without cactus pear in the diet showed similar intake, regardless of the proportion of leucaena hay. However, in the cactus pear diet, the highest proportion of leucaena hay (70%) resulted in higher total water intake compared to the lowest inclusion.

Table 4  
– Water intake by crossbred steers fed diets containing leucaena hay and cactus pear

| Item                      | Cactus |       | No cactus |       | SEM  | P-value  |      |       |
|---------------------------|--------|-------|-----------|-------|------|----------|------|-------|
|                           | 50     | 70    | 50        | 70    |      | C        | H    | C x H |
| <i>Water Intake, kg/d</i> |        |       |           |       |      |          |      |       |
| Free water                | 3.56   | 2.66  | 22.0      | 19.9  | 1.32 | < 0.0001 | 0.24 | 0.63  |
| Total water               | 39.5b  | 48.4a | 24.0c     | 21.4c | 2.38 | < 0.0001 | 0.17 | 0.020 |
| Free water/DMI            | 0.48   | 0.34  | 2.60      | 2.59  | 0.21 | < 0.0001 | 0.72 | 0.76  |

## N balance

No interaction ( $P < 0.05$ ) was detected between the inclusion of cactus pear and the proportion of leucaena hay for variables related to N metabolism (Table 5). However, animals fed 70% of leucaena hay had a higher intake of N, fecal and urinary nitrogen excretion ( $P < 0.05$ ). Furthermore, the inclusion of cactus pear in the diet resulted in lower ( $P < 0.05$ ) urinary N excretion and higher ( $P < 0.05$ ) efficiency of microbial protein synthesis (g NMIC/g N intake). No differences were observed between treatments ( $P < 0.05$ ) for body N accretion and rumen microbial production.

Table 5  
Nitrogen metabolism of crossbred steers fed diets containing leucaena hay and cactus pear

| Item                          | Cactus |      | No Cactus |      | SEM   | P-value <sup>c</sup> |       |       |
|-------------------------------|--------|------|-----------|------|-------|----------------------|-------|-------|
|                               | 50     | 70   | 50        | 70   |       | Cactus               | Hay   | C x H |
| N intake, g/d                 | 137    | 162  | 167       | 200  | 15.1  | 0.006                | 0.013 | 0.69  |
| Fecal N, g/d                  | 42.9   | 54.0 | 42.2      | 62.6 | 10.1  | 0.56                 | 0.035 | 0.50  |
| Urine N, g/d                  | 70.2   | 88.4 | 94.5      | 103  | 5.88  | 0.008                | 0.048 | 0.69  |
| N retention, g/d              | 25.3   | 19.9 | 29.8      | 34.1 | 7.50  | 0.19                 | 0.99  | 0.47  |
| <i>Microbial production</i>   |        |      |           |      |       |                      |       |       |
| g/d                           | 48     | 44.5 | 48.7      | 43.4 | 4.11  | 0.94                 | 0.11  | 0.73  |
| g NMIC/g N intake             | 0.31   | 0.33 | 0.22      | 0.27 | 0.026 | 0.015                | 0.18  | 0.50  |
| g CP/ kg TDN                  | 72.1   | 77.3 | 70.8      | 75.9 | 3.62  | 0.061                | 0.59  | 0.89  |
| g NMIC = g microbial nitrogen |        |      |           |      |       |                      |       |       |

## Feeding behavior

There was no interaction for any of the variables ( $P > 0.05$ ) of feeding behavior between the proportion of leucaena hay and the inclusion of cactus pear in the diet, except for the rumination time per kg of DM

(Table 6). The slicing of this interaction showed that in diets without cactus pear the addition of 50% leucaena hay decreased the rumination time per kg of DM. On the other hand, when there is cactus in the diet, the different proportions of hay and leucaena hay did not influence this variable. Also, lower values ( $P < 0.05$ ) of chewing (number/bolus and number/d) were observed for animals fed cactus pear.

Table 6  
– Feeding behavior of crossbred steers fed diets containing leucaena hay and cactus pear

| Item         | Cactus |        | No cactus |       | SEM  | P-value |       |       |
|--------------|--------|--------|-----------|-------|------|---------|-------|-------|
|              | 50     | 70     | 50        | 70    |      | Cactus  | Hay   | C x H |
| Feeding      |        |        |           |       |      |         |       |       |
| min/d        | 210    | 231    | 198       | 210   | 23.8 | 0.43    | 0.43  | 0.81  |
| min/kg DM    | 30.3   | 34.7   | 23.3      | 31.9  | 3.9  | 0.15    | 0.063 | 0.52  |
| min/kgNDFap  | 77.3   | 89.4   | 75.9      | 70.8  | 11.3 | 0.33    | 0.73  | 0.39  |
| Rumination   |        |        |           |       |      |         |       |       |
| min/d        | 477    | 432    | 445       | 456   | 33.9 | 0.85    | 0.48  | 0.25  |
| min/kg DM    | 61.8ab | 66.5ab | 54.1b     | 69.3a | 4.57 | 0.56    | 0.24  | 0.033 |
| min/kgNDFap  | 168    | 161    | 181       | 152   | 19   | 0.92    | 0.35  | 0.56  |
| Chewing      |        |        |           |       |      |         |       |       |
| number/bolus | 58.0   | 56.9   | 65.3      | 67.9  | 7.11 | 0.026   | 0.84  | 0.62  |
| number/d     | 53.5   | 51.2   | 58.6      | 63.2  | 5.72 | 0.035   | 0.72  | 0.35  |
| min/d        | 687    | 663    | 643       | 666   | 38.2 | 0.54    | 0.99  | 0.49  |
| min/kg DM    | 96.7   | 96.5   | 77.4      | 101   | 7.17 | 0.27    | 0.089 | 0.082 |
| min/kgNDFap  | 245    | 251    | 257       | 223   | 27.4 | 0.75    | 0.59  | 0.46  |
| Idleness     |        |        |           |       |      |         |       |       |
| min/d        | 752    | 776    | 796       | 773   | 38.2 | 0.54    | 0.99  | 0.49  |

## Discussion

Total dry matter intake is one of the most important variables in ruminant nutrition and depends on many factors, such as chemical composition, characteristics, and palatability of feeds. The feasibility of using alternative feeds for ruminants, depends among others, on the nutritional quality and responses in animal production when compared to the traditional feeds (Halmemies-Beauchet-Filleau et al., 2018).

Due to the higher iNDF content in diets with higher proportions of leucaena hay, we expected lower DM intake for animals with 70% leucaena hay compared to the 50% hay level. This hypothesis was confirmed by the lower DMI and TDN intake of animals receiving 70% hay. Indeed, dietary undigested fiber may explain variations in voluntary intake of ruminants (Detmann et al., 2014a). However, despite the lower DMI of animals with 70% hay, the nitrogen accretion in animal's body, which indicates weight gain, it was not altered by treatments. Similarly, Moreira et al. (2020), evaluating different proportions of cactus pear and leucaena hay in the diet of rams, did not find differences in nitrogen retention.

In situations in which the availability of nitrogen (N) becomes much higher than the availability of energy in the animal organism, there is a depression in voluntary consumption due to the deficiency of hepatic ATP caused by the excessive use of the urea cycle (Vissek, 1984), and by the increased body heat production (Poppi & McLennan, 1995). Therefore, it suggests, based on the higher urinary excretion of nitrogen, that the lower DM intake obtained in animals with greater replacement of soybean meal with leucaena hay also was due to excess N circulating in the animal organism.

The inclusion of 70% leucaena hay in diets without cactus pear resulted in a decrease in dry matter digestibility. However, when cactus was included to the diet with leucaena hay, levels of leucaena hay did not affect DM digestibility, demonstrating the potential of association between these feeds. It is likely that there was greater synchronism between the protein from leucaena hay and the energy of cactus pear for ruminal microorganisms, which can be confirmed by the greater efficiency of microbial production of the animals fed cactus pear (g NMIC/g N intake). Indeed, an adequate synchronization between dietary energy and protein in the rumen is essential to maximize microbial efficiency, promoting an improvement in dry matter digestibility (Barros e Silva et al. 2022).

Notably, the inclusion of cactus in the diet with 70% leucaena hay improved the ruminal synchronism between protein and energy, confirmed by the higher DM digestibility in these diets. By contrast, the addition of cactus pear did not prevent the decline in feed intake in diets with 70% leucaena hay. This result agrees with some study (Detmann et al., 2014b; Weston, 1996), which claim that the metabolic effects of protein in regulation intake cannot be evaluated exclusively by the decrease in ruminal fill (evidenced by the greater DM digestibility) and/or rumen events, without considering post-digestive effects.

The inclusion of cactus pear in the diet promoted greater fiber digestibility, which agrees with the study of Costa et al. (2012), who found greater apNDF digestibility in ram diets with higher levels of cactus pear replacing ground corn grain. It was proposed that the increase in the soluble NDF fraction in diets with the inclusion of the pear cactus might be associated with the improvement in the digestibility of dietary NDF (Lins et al. 2016). Furthermore, the higher efficiency of microbial synthesis (g NMIC/g N intake) may explain this result.

The ingestive behavior of animals can be influenced by several factors, including those related to diet, such as the quantity and quality of fiber, feed particle size, and feed moisture (Riaz et al., 2014). The lower DM content of the diets containing cactus pear, in addition to the higher fiber digestibility of these

diets in relation to the corn grain diets, implies less time needed to moisten the bolus. In fact, in these diets there was a lower number of chewed bolus and the number of chews per bolus, which according to Borges et al. (2019). On the other hand, the superior values of rumination time per kg of DM for animals fed cactus pear and 50% leucaena hay compared to animals with 50% hay without cactus pear, suggests that the total replacement of ground corn grain is promising in improving the ruminal health. This fiber stimulated rumination, ensuring a suitable ruminal environment for the development of microbial populations.

Water restriction due to long periods of drought and rainfall irregularities have been reported to reduce feed intake and performance in ruminants (Salem, 2010). The increasing scarcity of this precious natural resource has drawn concern from different segments of society, who seek solutions for rational and sustainable use of this resource (Schlecht et al., 2011). Several studies with small ruminants and dairy cows (Borges et al., 2019; Albuquerque et al., 2020) have reported the great potential of cactus pear in supplying feed water to animals, contributing to the sustainability of the system.

Indeed, this study showed that the inclusion of cactus pear in the diet can generate savings in the supply of free water (water troughs), since the animals fed cactus pear had lower free water intake. Similarly, Nobre et al. (2023) verified that animals that were given diets containing cactus silage had less need to seek water from the drinking fountain, as they ingested more water via feed. Additionally, we verified that the total water intake in diets with cactus is increased when 70% of hay is included in the diet, demonstrating another relevant associative effect between these feeds.

Normally, when protein degradation is faster than microbial synthesis, ammonia accumulates in the ruminal fluid and is absorbed into the blood transported to the liver and converted to urea and excreted in the urine. Therefore, optimized use of dietary energy and protein in the rumen favors microbial protein synthesis and generates less nitrogen (N) loss in the urine (Reynolds et al., 2008). Indeed, cactus-fed animals showed a higher microbial efficiency (g NMIC/g N intake), which caused less urinary nitrogen loss, without affecting body N retention. These results allow inferring a better use of N by animals fed cactus pear compared to animals with ground corn grain, a response pattern obtained in another study (Mora-Luna et al., 2022). The reduction of N from urine has a positive effect regarding to environmental sustainability. The nitrous oxide (NO) is a prominent greenhouse gas and urinary N is an indirect emitter of NO (Cardoso et al., 2019).

The inclusion of cactus improves the digestibility of diets with a proportion of 70% of leucaena hay. In addition, the replacement of ground corn grain with cactus pear improves the efficiency of nitrogen utilization in the rumen. On the other hand, the inclusion of 70% leucaena hay in diets for growing cattle depresses voluntary intake.

## **Declarations**

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## Authors' contributions

W.A. Rodrigues led the research. G.S.S. David. contributed to draft and writing the manuscript and formal analysis. F.A. Teixeira, A. R. dos Santos, J.A.G. Azevêdo participated the critical review and to draft and writing the manuscript. A.J.V. Pires participated of conceptualization, methodology, the project administration, and the critical review. M.S.Nogueira participated of project conduction.

## Conflict of interest statement

The authors confirm that there are no conflicts of interest.

## Ethics approval

The research carried out in accordance with the Ethics Committee on the Use of Animals of UESB (Permit Number: 176/2018). The manuscript does not contain clinical studies or patient data.

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