## FORAGE CACTUS COMBINED WITH DIFFERENT SILAGES AS DIETS FOR LACTATING RED SINDHI COWS<sup>1</sup>

# ACIR JOSÉ DA SILVA SOBRAL<sup>2</sup>\*, EVANDRO NEVES MUNIZ<sup>3</sup>, RAFAEL DANTAS DOS SANTOS<sup>4</sup>, JOSÉ HENRIQUE ALBUQUERQUE RANGEL<sup>3</sup>

**ABSTRACT** - Forage cactus (*Opuntia ficus-indica* Mill) is an important forage resource in the Northeast region of Brazil due to its adaptation to the edaphoclimatic conditions of the region, mainly to the drought periods. The objective of this work was to assess the effects of diets with forage cactus combined with different sources of fibers on dry matter and water intakes, digestibility, microbial protein production, balance of nitrogen compounds, and performance of Red Sindhi cows in the Semiarid region of Brazil. The silages used were prepared from plants of *Atriplex nummularia* Lindi., *Sorghum bicolor* (L.) Moench, *Gliricidia sepium* (Jacq.) Walp., and *Manihot pseudoglaziovii* Pax & K. Hoffm. They were included in forage cactus-based diets at a roughage to concentrate ratio of 58:42. The cows were distributed in a 4×4 double Latin square design with 12 days for each period. Water intake presented differences (p<0.05) when using the diet with *A. nummularia* silage (19.42 L d<sup>-1</sup>). The diet with *S. bicolor* silage resulted in lower (p<0.05) coefficient of digestibility of dry matter (48.71%) than the diet with *A. nummularia* silage (68.46%). The diet with *A. nummularia* silage resulted in higher (p<0.05) microbial synthesis than that with *G. sepium* silage, 115.01 and 80.07 g CPmic kg<sup>-1</sup> TDN, respectively. The silages evaluated, combined with forage cactus, can be used as diets for lactating Red Sindhi cows without affecting the daily dry matter intake, milk production, fat content, microbial protein synthesis, and balance of nitrogen compounds.

Keywords: Milk cow. Cactaceae. Intake. Forage. Semiarid.

### PALMA FORRAGEIRA ASSOCIADA À DIFERENTES SILAGENS EM DIETAS PARA VACAS DA RAÇA SINDI EM LACTAÇÃO

RESUMO - A palma forrageira (Opuntia ficus-indica Mill) apresenta-se como forrageira importante para o Nordeste Brasileiro, devido a sua adaptação às condições edafoclimáticas da região, especialmente nos períodos de estiagens. O presente estudo teve objetivo de investigar os efeitos de dietas baseadas em palma forrageira associada a diferentes fontes de fibras no consumo (matéria seca e água), digestibilidade, produção de proteína microbiana, balanço de compostos nitrogenados e desempenho de vacas leiteiras sindi criadas em regiões semiáridas. As silagens utilizadas foram: atriplex (Atriplex nummularia Lindi.), sorgo (Sorghum bicolor (L.) Moench), gliricídia (Gliricidia sepium (Jacq.) Walp.) e manicoba (Manihot pseudoglaziovii Pax & K. Hoffm.), que foram incluídas nas dietas à base de palma forrageira (relação 58:42 volumoso: concentrado). As vacas foram distribuídas em delineamento Quadrado Latino duplo 4x4 com duração de 12 dias cada período. Para consumo de água houve diferenças (P<0.05) para dieta contendo silagem de atriplex (19.42 L/d). A dieta com silagem de sorgo apresentou menor coeficiente (P<0.05) digestibilidade da matéria seca (48,71 %) em relação ao tratamento de silagem de atriplex (68,46 %). A síntese microbiana foi maior (P<0,05) para a dieta com silagem de atriplex em relação ao tratamento que continha silagem de gliricídia (115,01 e 80,07 g de PBmic/kg de NDT), respectivamente. As silagens avaliadas associadas a palma forrageira podem ser utilizadas em dietas para vacas da raça Sindi em lactação sem influenciar o consumo diário de matéria seca, sem alterar a produção e gordura do leite, a síntese de proteína microbiana e o balanço de compostos nitrogenados.

Palavras-chave: Bovino de leite. Cactáceas. Consumo. Forragem. Semiárido.

<sup>\*</sup>Corresponding author

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<sup>&</sup>lt;sup>2</sup>Post graduate program of Animal Science, Universidade Federal de Sergipe, São Cristóvão, SE, Brazil; acirsobral@gmail.com - ORCID: 0000-0003-3013-7377.

<sup>&</sup>lt;sup>3</sup>Embrapa Tabuleiros Costeiros, Aracaju, SE, Brazil; evandro.muniz@embrapa.br - ORCID 0000-0003-2806-229X, jose.rangel@embrapa.br - ORCID 0000-0002-7373-1838.

<sup>&</sup>lt;sup>4</sup>Embrapa Semiárido, Petrolina, PE, Brazil, rafael.dantas@embrapa.br - ORCID: 0000-0002-5844-6780.

#### **INTRODUCTION**

In the Northeast region in Brazil, forage cactus (*Opuntia ficus-indica* Mill) is an important forage resource, mainly in drought periods, due to its adaptation to the regional edaphoclimatic conditions (RAMOS et al., 2017), in addition to its high non-fibrous carbohydrate contents and, mainly, water, which is a limiting factor for milk production systems in semiarid areas (ARAÚJO et al., 2008).

However, some limitations of this Cactaceae species include deficiencies in crude protein and neutral detergent fiber contents; this low fiber content may cause metabolic disturbances, such as diarrheas and depression in milk fat content (GALVÃO JÚNIOR et al., 2014). However, in the Semiarid region of Brazil, different roughage sources can be combined with forage cactus, generating more options of diets with positive bioeconomic value.

Some viable species are among these alternative forages that present potential for roughage production, including *Atriplex nummularia* Lindi., *Sorghum bicolor* (L.) Moench, *Gliricidia sepium* (Jacq.) Walp., and *Manihot pseudoglaziovii* Pax & K. Hoffm. (CAVALCANTI et al., 2008). These different fiber sources combined with forage cactus can compose diets to meet the nutritional needs of dairy cows and possibly achieve good zootechnical performances.

However, evaluating the effect of diets on the balance of nitrogen and production of microbial protein in the rumen is important to acquire information on protein demands, which can be important to avoid production, reproduction, and environmental losses caused by excessive supplying of protein or inappropriate energy-protein synchronicity in the rumen (PESSOA et al. 2009).

Supplying amino acids through microbial protein is essential for protein metabolism in ruminants. According to the National Research Council, the protein synthetized by rumen microorganisms has an excellent amino acid profile and a little variable composition (NRC, 2001). Therefore, studying mechanisms connected to microbial efficiency and protein synthesis is important because they are directly related to the performance of animals.

The objective of this work was to assess the effects of diets with forage cactus combined with different sources of fibers on the dry matter and water intakes, digestibility, milk production and composition, microbial protein synthesized in the rumen, and balance of nitrogen compounds of lactating Red Sindhi cows in the Semiarid region of Brazil.

#### MATERIAL AND METHODS

The experiment was carried out in the Brazilian Agricultural Research Corporation (Embrapa Semiarid), in Petrolina, Pernambuco, Brazil (09°09"S, 40°22"W, and altitude of 36.5 m). The mean annual rainfall in the region is 570 mm, and the mean annual maximum and minimum temperatures are 33.46 and 20.87 °C, respectively.

Four Red Sindhi cows with mean body weight of  $260\pm15$  kg, initial milk production of 7 kg, and a 56-day lactation period were distributed in a  $4\times4$  double Latin Square design, with 12 days for each period: seven for adaptation to the diets and five for data and sample collections; the total experimental time was 96 days.

The treatments consisted of full diets with forage cactus (*Opuntia ficus-indica* Mill cv. Gigante) combined with silages from different species (*Atriplex nummularia*, *Sorghum bicolor*, *Gliricidia sepium*, and *Manihot pseudoglaziovii*) and concentrate consisted of a mixture of soybean meal, ground maize grains, urea, and mineral salt (Tables 1 and 2).

The diets were formulated following recommendations of the NRC (2001) to meet the demand of lactating cows with a milk production of 10 kg day<sup>-1</sup> with 4% fat, according to the weight of the animals.

Table 1.	Bromatological	composition	of ingre	dients of the	e experimental	diets.

				Ingree	lients		
Component	Forage cactus	Atriplex nummularia	Sorghum bicolor	Gliricidia sepium	Manihot pseudoglaziovii	Soybean meal	Ground maize
DM (%)	7.70	27.63	24.97	28.15	22.63	89.70	88.20
OM	85.38	76.10	88.55	92.08	92.15	94.84	98.91
СР	5.40	11.50	5.07	15.47	14.34	50.00	9.00
EE	1.49	1.60	1.63	2.83	2.91	2.35	4.34
NDF	32.51	42.49	66.34	43.88	46.56	18.19	15.04
ADF	17.47	17.09	27.05	13.04	13.68	12.55	8.50
TCH	78.48	62.92	81.73	73.68	74.80	42.49	85.57
NFCH	45.97	20.43	15.39	29.80	28.24	24.30	70.53

DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; TCH = total carbohydrates; NFCH = non-fibrous carbohydrates.

A pre-experimental period of 10 days was used for adaptation of the animals to the new feed management and facilities. The animals were weighed at the beginning and end of each experimental period; the cows were maintained in  $6-m^2$  individual stalls with concrete floor and troughs and water drinkers to control individual feed and water intakes. The feed was provided ad libitum as a complete mixture twice a day, at 7:00 a.m. and 4:00 p.m., with water ad libitum. Leftovers were collected daily, in the mornings, and weighed to maintain them at approximately 10% of the total feed, and used to calculate the mean daily intake. The daily intake was determined by the difference between the weights of the offered feed and the leftovers for each animal.

 Table 2. Percentages and nutritional composition of the experimental diets.

T 1' /	Treatments						
Ingredients	Atriplex nummularia	Sorghum bicolor	Gliricidia sepium	Manihot pseudoglaziovii			
Forage cactus (%)	30.00	32.18	33.00	33.00			
Atriplex nummularia (%)	24.00	-	-	-			
Sorghum bicolor (%)	-	25.00	-	-			
Gliricidia sepium (%)	-	-	25.00	-			
Manihot pseudoglaziovii (%)	-	-	-	25.00			
Soybean meal (%)	17.39	18.88	8.44	15.9			
Ground maize (%)	27.11	22.44	32.26	24.85			
Urea (%)	0.5	0.5	0.3	0.25			
Mineral salt (%)	1.0	1.0	1.0	1.0			
Total	100	100	100	100			
	Nutritional Composition						
Dry matter	18.94	17.67	17.71	17.00			
Organic matter <sup>1</sup>	86.36	88.95	90.4	90.13			
Crude protein <sup>1</sup>	16.94	15.90	13.63	16.28			
Ether extract <sup>1</sup>	2.42	2.30	2.80	2.67			
Neutral detergent fiber <sup>1</sup>	23.55	25.66	23.85	24.52			
Acid detergent fiber <sup>1</sup>	12.99	14.47	12.09	12.65			
Total carbohydrates <sup>1</sup>	67.00	70.75	73.97	71.18			
Non-fibrous carbohydrates <sup>1</sup>	43.45	45.09	50.12	46.66			
Total digestible nutrients <sup>1, 2</sup>	70.81	72.65	76.24	77.98			

<sup>1</sup>(% in the dry matter); <sup>2</sup>Estimated according to Sniffen, O'connor and Van Soest (1992).

In the five days of collection of each experimental period, samples of feces were directly collected using a rectal vial, soon after the morning and afternoon milking. Samples of the feed provided and leftovers were daily collected in these periods, pre-dried in a forced air-circulation oven at 55 °C, and homogenized, originating composite samples for each period and animal, and then taken to the laboratory for analyses.

The composite samples were ground in a Wiley mill with a 1 mm mesh sieve, and representative aliquots were withdrawn and analyzed for dry matter (DM), crude protein (CP), and ether extract (EE), according to the methodology described by Silva and Queiróz (2002). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methodology described by Van Soest (1967) and adapted by Detmann et al. (2012), using nonwoven fabric bags (100 g m<sup>-2</sup>).

Total carbohydrate (TCH) and non-fibrous carbohydrate (NFCH) contents were estimated according to Sniffen, O'connor and Van Soest (1992) and Mertens (1997), using the equations: TCH = 100 - (CP + EE + Ashes), and NFCH = 100 - (NDF + CP + EE + Ashes), respectively.

The animals were evaluated for daily intake of dry matter, crude protein, ether extract, neutral detergent fiber, acid detergent fiber, total carbohydrates, non-fibrous carbohydrates, organic matter, total digestible nutrients (TDN), and water; as well as digestibility of dry matter, crude protein, ether extract, neutral detergent fiber, total carbohydrates, non-fibrous carbohydrates, and organic matter.

TDN intake (kg) and TDN contents were estimated according to Sniffen, O'connor and Van Soest (1992), by the equations: TDN intake = (ingested CP - fecal GP) + 2.25 (ingested EE –fecal EE) + (ingested TCH - fecal TCH); and TDN contents (%) = (TDN intake / DM intake) × 100.

The estimated fecal DM was obtained using the indigestible ADF as indicator. Aliquots of 1.0 g of the silages, forage cactus, soybean meal, ground maize, leftovers, and feces were placed in nonwoven fabric bags (100 g m<sup>-2</sup>) and incubated in the rumen of an adult male bovine for 144 hours, according to the methodology described by Berchielli et al. (2005). The incubated material was taken to the Laboratory of Animal Nutrition, washed in running water, and subjected to extraction with acid detergent; the residue was considered indigestible ADF.

Water was provided ad libitum, using buckets with capacity for 15 kg refilled three times a day, at 8:00 a.m., 12:00 p.m., and 3:30 p.m. The water intake (kg) was calculated by the difference between the amounts of water offered and leftovers minus the water lost by evaporation. The loss of water by evaporation was determined using buckets containing the same amount of water for each treatment, randomly distributed in the experimental facility: their differences in weight within 24 hours were used to quantify the average loss by evaporation.

The cows were manually milked twice a day and the productions were measured individually, evaluating the milk fat and protein contents and feed efficiency. Milk samples from the two daily milking on the eleventh and twelfth days of each experimental period were placed in containers with preservative (bronopol) and sent to the Laboratory of Milk of the Department of Zootechnics of the Federal Rural University of Pernambuco to determine protein, fat, and total solid contents. Fat and protein contents were determined using an infrared absorption device model Bentley 2000, Fat and protein contents were determined using an infrared absorption device model Bentley 2000, and total solid content was determined by the sum of values of the previous components (IDF, 1996). The milk production corrected to 4% fat (CMP) was determined through the equation: CMP = (0.432 + $0.1635 \times F$ ) × MP, where F is the percentage of fat in the milk and MP is the milk production in kg day<sup>-1</sup> (SKLAN et al., 1992).

Spot urine collections were carried out on the third day of collection of each experimental period, at approximately 4 hours after the morning feed, during spontaneous urination of each animal. A 10-mL aliquot was diluted in 40 mL of sulfuric acid (0.036 N) and measured for pH, which was adjusted to values lower than 3 with concentrate sulfuric acid to avoid bacterial destruction of purine derivatives and precipitation of uric acid. The samples were stored in a freezer at -20 °C for determinations of creatinine, urea, allantoin, and uric acid.

The samples were thawed to quantify the purine and creatinine derivatives at the end of the experiment; commercial kits (Labtest<sup>®</sup>) were used for tests by the method of final point, using picrate and acidifying to determine creatinine content, and uricase and hydrogen peroxide to determine uric acid content. Allantoin concentrations were determined as described by Chen and Gomes (1992). All analyses were carried out by the colorimetric method.

Milk samples from the two daily milking of each animal were collected on the fourth and fifth day of collection; 10 mL of milk was mixed with 5 mL of trichloroacetic acid at 25%, filtered in paper-filter, and stored in a freezer at -20  $^{\circ}$ C for analysis of urea and allantoin.

The analyses of urea in urine and in deproteinate milk, and creatinine and uric acid in urine were carried out using commercial kits (Human do Brazil<sup>®</sup>), as described by the manufacturer. The analyses were carried out in the Laboratory of Chromatography of the Brazilian Agricultural Research Corporation (Embrapa Dairy Cattle) in Juiz de Fora, Minas Gerais, Brazil. Nitrogen (N) contents in feces were determined according to the methodology described by Silva and Queiroz (2002). The balance of N was calculated through the difference between the ingested nitrogen and excreted nitrogen in feces, urine, and milk.

The mean daily urine volume was estimated for each animal by multiplying the respective body weight by the daily excretion of creatinine (mg kg<sup>-1</sup> of body weight) and dividing it by the concentration of creatinine in the spot urine (mg L<sup>-1</sup>), using the value 24.4 mg kg<sup>-1</sup> of body weight of creatinine found by Pereira (2003) for cows within the initial and mid thirds of lactation.

The analyses of allantoin in urine and in deproteinate milk were carried out by the colorimetric method described by Chen and Gomes (1992). The excretion of total purine derivatives (mmol day<sup>-1</sup>) was calculated by the sum of amounts of allantoin and uric acid excreted in urine.

Absorbed purine (X, mmol day<sup>-1</sup>) was calculated from the excretion of purine derivatives (Y, mmol day<sup>-1</sup>) through the equation  $X = [Y - (0.385 \times PV^{0.75})] / 0.85$ , where 0.85 is the recovery of absorbed purine as purine derivatives, and 0.385 ×  $PV^{0.75}$  is the endogenous contribution to purine excretion (VERBIC et al., 1990).

The ruminal synthesis of nitrogen (Y, g N day<sup>-1</sup>) was calculated in relation to absorbed purine (X, mmol day<sup>-1</sup>), using a modification of the equation described by Chen and Gomes (1992), replacing the purine N to total N ratio in the bacteria of 0.116 with 0.134, as described by Valadares et al. (1999):  $Y = 70X / (0.83 \times 0.134 \times 1000)$ , where 70 is the purine nitrogen (mg N mol<sup>-1</sup>); 0.134 is the purine N to total N ratio in bacteria; and 0.83 is the digestibility of microbial purine. The efficiency of microbial crude protein synthesis (MCPS) was calculated as follows: CPmic = g of microbial crude protein per kilo of TDN intake.

The results were analyzed through analysis of variance and the means were compared by the Tukey's test at 5% probability level, using the program SAS (2003).

### **RESULTS AND DISCUSSION**

Dry matter intake was not affected (p>0.05) by the different roughages used, presenting a mean

of 8.355 g day<sup>-1</sup> and 21.72 g kg<sup>-1</sup> body weight (Table 3). Dry matter intake presented no significant difference (p>0.05), probably due to similarity in nutrients in the diets, mainly neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Table 2). When forage cactus is included in the diet and processed, it favors the adhesion of ingredients of the diet to its mucilage (SILVA et al., 2007), facilitating

the ingestion of little palatable ingredients (ALMEIDA, 2012) and favoring the intake of the nutrients in the diet when provided as a complete feed (WANDERLEY et al., 2012); in addition, it presents good acceptability by animals and has low fiber contents and a high passage rate (COSTA et al., 2012).

**Table 3**. Mean daily intake of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), total carbohydrates (TCH), non-fibrous carbohydrates (NFCH), total digestible nutrients (TDN), and water (WA).

Item		Tre	eatment		
Item	Atriplex nummularia	Sorghum bicolor	Gliricidia sepium	Manihot pseudoglaziovii	CV (%)
DM (g day <sup>-1</sup> )	8.690	8.470	7.970	8.290	6.54
$OM (g day^{-1})$	5.505	6.397	6.772	6.867	9.84
$CP (g day^{-1})$	1.350 a	1.270 ab	1.160 b	1.300 ab	5.67
$EE (g day^{-1})$	155 b	165 b	208 a	205 a	5.11
NDF (g day <sup>-1</sup> )	2.420	2.220	2.120	2.320	8.39
ADF $(g day^{-1})$	1.270	1.230	1.250	1.310	10.31
TCH (g day <sup>-1</sup> )	4.345 b	5.170 ab	5.600 a	5.465 ab	5.60
NFCH $(g day^{-1})$	1.925 b	2.950 ab	3.480 a	3.145 a	11.92
TDN (g day <sup>-1</sup> )	4.690 b	5.235 ab	5.722 a	5.947 a	5.73
WA (L day <sup>-1</sup> )	19.42 a	10.86 b	8.0 b	7.57 b	32.5
		Consumption (g kg	g <sup>-1</sup> body weight)		
$DM (g kg^{-1})$	28.6	28.4	27.1	28.3	8.22
NDF $(g kg^{-1})$	7.8	7.6	7.3	7.8	11.38

Means followed by different letters in the rows are different from each other by the Tukey's test (p < 0.05); CV = coefficient of variation.

The intakes of organic matter (OM), NDF, and ADF (Table 3) were not affected (p>0.05) by the silage treatments, which is explained by the absence of effects on the dry matter (DM) intake. According to the NRC (2001), NDF has low digestion rate and is the first dietetic constituent associated with decreases in DM intake. In the present work, there was no limitation to DM intake.

NDF intake (g kg<sup>-1</sup> body weight) was not significantly affected (p>0.05) by the treatments (Table 3). According to Mertens (2001), the ruminal filling factor is connected to fiber contents in the diet; the effect of filling is expressed in diets with NDF intake above 1.2% body weight, which was not found in the results. The mean NDF intake found was 7.6 g kg<sup>-1</sup> body weight, or 0.76% body weight, for the treatments with *Atriplex nummularia* and *Manihot pseudoglaziovii* silages.

The diet with *A. nummularia* silage resulted in higher (p < 0.05) crude protein (CP) intake (1.350 g day<sup>-1</sup>) than the diet with *Gliricidia sepium* silage (1.160 g day<sup>-1</sup>) (Table 3). This lower CP intake is connected to the lower protein content in this diet (Table 2), resulting in a lower intake than the other treatments.

Ether extract intake was different (p < 0.05) between treatments; the diet with *G. sepium* silage

resulted in higher intake (208 g day<sup>-1</sup>) than those with *M. pseudoglaziovii*, *Sorghum bicolor*, and *A. nummularia* silages, which resulted in 205, 165, and 155 g day<sup>-1</sup>, respectively (Table 3).

Total carbohydrates (TCH) intake presented significant differences (p<0.05); the diet with *G. sepium* silage resulted in higher TCH intake (5.600 g day<sup>-1</sup>) than that with *A. nummularia* silage (4.345 g day<sup>-1</sup>) (Table 3). This result is attributed to the higher participation of maize on the diet (Table 2), as it presents greater TCH contents.

Non-fibrous carbohydrates (NFCH) intake presented significant differences (p<0.05); the diet with *G. sepium* silage resulted in higher mean intake (3.480 g day<sup>-1</sup>) than that with *A. nummularia* silage (1.925 g day<sup>-1</sup>) (Table 3). This difference is attributed to the high NFCH content in the diet with *G. sepium* (Table 2) due to the higher proportion of ground maize in the composition when compared to the others.

The TDN intake presented significant difference (p<0.05), being lower in the diet with *A. nummularia* silage, presenting values of 4.690 (g day<sup>-1</sup>) and higher in the diet with *M. pseudoglaziovii*, presenting 5.947 (g day<sup>-1</sup>). This fact is attributed to the lower energy content in the diet with *A. nummularia* when compared to the other

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treatments (Table 3).

Water intake was statistically different (p<0.05) between treatments, with higher intake in the diet with *A. nummularia* silage (Table 3). This result may be due to the salt contents in the plants, as their leaves have high salt concentrations, mainly sodium chloride (BEN SALEM et al., 2005), which can indirectly increase water intake in dairy cows (DAHLBORN; AKERLIND; GUSTAFSON, 1998). The lower water intake in the other treatments can be explained by the water supply through the feed.

The highest coefficient of DM digestibility was found for diets with *M. pseudoglaziovii* and *A. nummularia* silages: 69.13% and 68.46%

respectively (Table 4). This result can be attributed to increases in cell wall constituents in the diet, mainly ADF. Many factors can affect DM digestibility, such as food intake, proportion, cell wall digestibility, diet composition, and food preparation, in addition to factors dependent on the animals and nutritional level (McDONALD; EDWARDS; GREENHALGH, 1993). According to Muniz et al. (2011), forage cactus is different from other forages; it has low NDF contents and presents a fast passage rate due to a high ruminal fermentation rate and, consequently, a similar intake to the concentrate.

**Table 4**. Coefficients of digestibility (%) of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), total carbohydrates (TCH), and non-fibrous carbohydrates (NFCH).

			Treatments			
Nutrients	Atriplex nummularia	Sorghum bicolor	Gliricidia sepium	Manihot pseudoglaziovii	Mean	CV (%)
DM	68.46 a	48.71 c	56.47 b	69.13 a	60.70	6.92
OM	81.67	81.30	78.93	79.91	80.45	1.96
СР	79.33	79.23	77.43	78.20	78.55	2.22
EE	75.15 a	75.58 a	67.62 ab	64.33 b	70.67	5.35
NDF	53.34	48.63	48.96	44.85	48.94	20.03
ТСН	82.60	82.11	79.90	81.02	81.41	2.15
NFCH	97.17	97.45	96.85	96.92	97.10	2.08

Means followed by different letters in the rows are different from each other by the Tukey's test (p < 0.05); CV = coefficient of variation.

The diet with *S. bicolor* silage resulted in higher (p<0.05) coefficient of EE digestibility (75.58%) when compared to that with *M. pseudoglaziovii* silage (64.33%) (Table 4). Silva et al. (2007) evaluated lactating Holstein cows fed with forage cactus combined with different roughage sources (sugarcane bagasse, sorghum silage, *Pennisetum purpureum* hay, and Tifton hay) and found that EE digestibility was not affected by the addition of forage cactus.

The coefficients of digestibility of MO, CP, NDF, TCH, and NFCH were not statistically different (p>0.05) between treatments, presenting means of 80.45%, 78.55%, 48.94%, 81.41%, and 97.10%, respectively (Table 4). These results are consistent with those of Oliveira et al. (2007a), who found that the coefficients of digestibility of nutrients were not affected by the addition of forage cactus in the diet, except for NDF. Andrade et al. (2002) evaluated Holstein cows fed with forage cactus in replacement to *S. bicolor* and found that NDF digestibility and NFCH digestibility were affected by the addition of forage cactus in the diet.

The milk production corrected to 3.5% fat presented no significant difference (p>0.05) between

treatments, with a mean of 7.28 kg day<sup>-1</sup> (Table 5), which can be explained by the absence of differences in DM intake (Table 3). The uptake of nutrients for the breast gland was probably similar, resulting in concentrations close to the milk nutrient contents (CARDOSO et al., 2017) in the levels evaluated.

Milk fat content was not affected by the addition of the different roughages (p>0.05), presenting a mean of 57.9 g kg<sup>-1</sup> (Table 5). According to the NRC (2001), a minimum NDF of 25% (with 19% from roughage) and a maximum NFCH content of 44% are needed in the feed composition to avoid changes in the ruminal environment and decreases in milk fat. All nutrient contents found were within the limits established by the NRC (2001), except for NFCH in the diet with *G. sepium* silage, which was above the recommended limit.

Oliveira et al. (2007b) evaluated diets with differences of up to 14.96% in NFCH and found no differences in the mean milk production corrected to 3.5% fat, denoting that this result was due to the energy-protein balance in the diets, which presented similar TCH and CP contents.

Idam		Tı	reatments		
Item	Atriplex nummularia	Sorghum bicolor	Gliricidia sepium	Manihot pseudoglaziovii	CV (%)
CMP (kg day <sup>-1</sup> )	7.47	7.30	7.56	6.78	7.12
$FC (g kg^{-1})$	59.1	58.1	58.0	56.50	2.35
$PC (g kg^{-1})$	40.3 b	41.4 ab	38.9 c	41.9 a	1.35
FE (CMP/DM)	0.85	0.86	0.94	0.81	8.45

Table 5. Milk production corrected to 3.5% fat (CMP), fat content (FC), protein content (PC), and feed efficiency (FE).

Means followed by different letters in the rows are different from each other by the Tukey's test (p < 0.05); CV = coefficient of variation; DM = dry matter intake.

Milk protein content presented significant differences (p<0.05) between treatments, with mean of 40.6 g kg<sup>-1</sup> (Table 5). The diet with *G. sepium* resulted in lower protein content (38.9 g kg<sup>-1</sup>), probably due to the lower crude protein content in this diet. Therefore, the diets that resulted in higher milk protein content were due to the higher CP intake, which increased the microbial protein synthesis and the protein flow to the small intestine (PAIVA et al., 2013). Thus, it resulted in a higher availability of amino acids in the blood chain, which are the main precursors for milk protein synthesis in the breast gland.

The balancing of diets with a higher proportion of NFCH results in a greater supply of fermentable carbohydrates and in a higher milk protein content (ABRAHAMSE et al., 2008). This is due to the high proportion of propionate formed in the rumen, which increases the availability in the liver, since the propionic acid in animal cells is a precursor of glucose. Thus, when this acid increases, it is more absorbed and reaches the animal cell for the synthesis of glucose, making it available for formation of milk protein in the breast gland (NEIVA et al., 2006).

Feed efficiency in relation to milk production corrected to 3.5% fat and to dry matter intake was not affected (p>0.05) by the addition of the roughages (Table 5). It can be attributed to the dry matter intake, which was not changed as a function of the different silages.

The urine volume (p < 0.05) found in cows under the diet with *A. nummularia* silage (8.43 L) was higher than that found in those under diet with *G. sepium* (5.31 L) (Table 6). This result is connected to the higher water intake in this treatment (p < 0.05) and, consequently, to a higher urine excretion.

 Table 6. Urine volume and excretion of purine derivatives, absorbed purine, and efficiency of microbial crude protein synthesis.

Trans.	Experimental diet					
Item	Atriplex nummularia	Sorghum bicolor	Gliricidia sepium	Manihot pseudoglaziovii	CV (%)	
Estimated urine volume (L) Water intake (L day <sup>-1</sup> )	8.43 a 19.42 a	6.28 ab 10.86 b	5.31 b 8.00 b	6.00 ab 7.57 b	27.05 32.50	
	_		Urine excretion			
Allantoin (mmol day <sup>-1</sup> )	103.49	91.47	97.12	99.23	15.04	
Uric acid (mmol day <sup>-1</sup> )	22.78 a	19.10 b	13.92 c	17.35 b	11.69	
Total purine derivatives (mmol day <sup>-1</sup> )	126.27	110.57	111.04	116.58	11.49	
Allantoin to purine ratio	81.95 b	82.72 b	87.46 a	85.11 ab	3.63	
Absorbed purines (mmol day <sup>-1</sup> )	118.72	100.25	100.8	107.31	14.72	
Microbial nitrogen (g day <sup>-1</sup> )	86.31	72.88	73.28	78.02	14.69	
Microbial crude protein synthetized in the rumen $(g \ day^{-1})^{-1}$	539.44	455.5	458.04	487.62	14.71	
Efficiency of microbial crude protein synthesis (g CPmic $kg^{-1}$ TDN) $^2$	115.01 a	87.09 ab	80.07 b	82.39 b	25.91	

Means followed by different letters in the rows are different from each other by the Tukey's test (p<0.05); CV = coefficient of variation; <sup>1</sup>Microbial crude protein synthetized in the rumen; <sup>2</sup>Efficiency of microbial protein synthesis.

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These results are consistent with those found by Ben Salem et al. (2005), who reported that *A*. *nummularia* presents high ash contents in the dry matter, which can contribute to a higher water intake as a physiological resource to excrete the ingested salts.

Uric acid was affected (p>0.05) by the roughages used (Table 6), presenting higher values (p>0.05) for cows fed with forage cactus combined with A. nummularia silage, followed by S. bicolor and M. pseudoglaziovii silages, and lower values (p>0.05) for animals subjected to diet with G. sepium silage. This result can be explained by the higher crude protein content in the diet (Table 2), which caused a greater nitrogen intake by the animals (AGUIAR et al., 2015).

Regarding the allantoin to purine derivative ratio, the animals fed with forage cactus combined with G. sepium silage presented differences (p < 0.05) from the animals fed with forage cactus combined with A. nummularia or S. bicolor silages (Table 6). The means of the diets were 97.82 mmol day<sup>-1</sup> for urine total allantoin excretion, 18.28 mmol day-1 for uric acid, and 116.11 mmol day<sup>-1</sup> for total purine derivatives, representing 84.24% of allantoin and 15.74% of uric acid in relation to the total purine derivatives. These data are within the range reported by Chen and Gomes (1992), who found total excretions of 80% to 85% of allantoin and 15% to 20% of uric acid in bovine urine, denoting the efficiency of microbial nitrogen synthesis. However, this amplitude of excretion of uric acid and allantoin in the urine is dependent on the physiological stage of the animal and the dietetic treatments used.

The experimental diets had no effect (p>0.05) on absorbed purine, microbial nitrogen, and microbial crude protein synthetized in the rumen (Table 6), showing means of 116.77 mmol day<sup>-1</sup>, 77.62 g day<sup>-1</sup>, and 485.15 g day<sup>-1</sup>, respectively. The results highlighted the balance between the fibrous and non-fibrous carbohydrate supplies in the experimental diets. Aguiar et al. (2015) evaluated increasing contents of forage cactus in the diet of dairy heifers (<sup>3</sup>/<sub>4</sub> Holstein-Zebu) in feedlots and found no differences for absorbed purine, microbial nitrogen, and microbial crude protein synthetized in the rumen, with means of 103.06 mmol day<sup>-1</sup>, 74.93 g day<sup>-1</sup>, and 468.34 g day<sup>-1</sup>, respectively.

The diet with *A. nummularia* resulted in higher (p < 0.05) efficiency of microbial crude protein synthesis (115.01 g CPmic kg<sup>-1</sup> TDN) than the diet with *G. sepium* (80.07 g CPmic kg<sup>-1</sup> TDN) (Table 6). The results found for CPmic kg<sup>-1</sup> were lower than those found by Valadares Filho et al. (2006), who recommended the use of 120 g CPmic kg<sup>-1</sup> TDN as reference for efficiency of microbial synthesis under

tropical conditions, based on data of researches carried out in Brazil; it was lower than that recommended by the NRC (2001), 130 g CPmic kg<sup>-1</sup> TDN.

Important aspects of forage cactus-based diets include the balance between fibrous and non-fibrous carbohydrate supplies. Regarding the efficiency of microbial synthesis, the diets with *A. nummularia* and *S. bicolor* had better results when compared to the other diets; it may be connected to the profile and quality of the fibrous fraction in these foods when compared to the other treatments, which probably resulted in a greater chewing stimulus, contributing to increases in saliva production and dilution of ruminal content, thus increasing the passage rate and escape of microorganisms. According to Valadares Filho et al. (2006), the passage rate is one of the factors that affect the ruminal fermentation and microbial synthesis.

The highest (p < 0.05) intake of nitrogen compounds and balance of nitrogen was found for cows fed with diets containing *S. bicolor* silage, and the lowest (p < 0.05) for animals subjected to diet with *G. sepium* silage (Table 7).

The increases in nitrogen intake are connected to the high intake of dry matter or crude protein content provided by the diet, as also found in other studies (VALADARES et al., 1997; ÍTAVO et al., 2002; CAVALCANTE et al., 2006; CRUZ et al., 2006; RENNÓ et al., 2008; AGUIAR et al., 2015).

The mean percentage of N intake was 39.15% (Table 7), which is consistent with the results of Pessoa et al. (2009), who found higher nitrogen intake by the higher crude protein concentration in the diet of heifers fed with forage cactus, sugarcane bagasse (roughage), and concentrate with soybean or cotton meal (0.5% body weight) when compared to concentrate containing wheat bran and cotton seed. It reflects the high nitrogen contents in these supplements.

According to the NRC (2001), the crude protein demand for lactating cows (with weight of approximately 260 kg, milk production of 10 kg a day, and mean daily gains of approximately 300 g day<sup>-1</sup>) is 1300 g day<sup>-1</sup> i.e., approximately 208 g day<sup>-1</sup> of N. The nitrogen intakes observed in the present work were within these requirements, except for that found for the diet with *S. bicolor* (212.84 g day<sup>-1</sup>).

The excretion of nitrogen through urine, feces, and milk presented no differences (p>0.05) between the diets used; the N excretion through feces was the one that contributed the most to N excretion, corresponding to, on average, 89.70% of the total N excreted (Table 7).

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			Diet		
Item	Atriplex nummularia	Sorghum bicolor	Gliricidia sepium	Manihot pseudoglaziovii	CV (%)
Ingestion of nitrogen (g day <sup>-1</sup> )	206.52 ab	212.84 a	182.95 b	207.90 ab	10.32
N in the feces $(g day^{-1})$	93.95	98.29	84.18	82.39	17.82
N in the urine $(g day^{-1})$	10.74	9.97	9.13	11.13	31.05
N in the milk $(g day^{-1})$	23.03	22.36	24.19	22.69	15.49
Balance of nitrogen (g day <sup>-1</sup> )	78.78	82.21	65.43	91.68	31.65
% Ingested nitrogen	38.14	38.62	35.76	44.09	25.35

Table 7. Ingestion of nitrogen compounds and balance of nitrogen.

Means followed by different letters in the rows are different from each other by the Tukey's test (p < 0.05); CV = coefficient of variation.

According to Van Soest (1994), increases in N intake are associated with a greater production of urea in the liver and a higher excretion through urine, whereas a low N intake leads to a decrease in urine excretion for the maintenance of the plasmatic pool, which is under homeostatic physiological control.

The balance of nitrogen compounds was not affected (p>0.05) by the different silages used for feeding the lactating cows, presenting a mean of 78.55 g day<sup>-1</sup> (Table 7). This result may be due to the use of diets with silages that had low protein contents, whereas those diets with greater protein contents increase the excretion of nitrogen, mainly through the urine (PESSOA et al., 2009).

## CONCLUSIONS

The addition of 25% silage (*Atriplex nummularia*, *Sorghum bicolor*, *Gliricidia sepium*, or *M. pseudoglaziovii*) in diets based on 30% forage cactus and 45% concentrate, as a total feed, can maintain the daily dry matter intake with no changes in the digestibility and in the milk production and fat.

The crude protein intake was lower than that established by the NRC (2001) in the treatments with *S. bicolor* and *G. sepium*, probably due to the low crude protein content and presence of antinutritional compounds, respectively, in the treatments with these roughages, requiring attention on the correction of crude protein contents in diets when using these foods.

*A. nummularia* silage is a potential alternative feed for ruminants; however, it should be studied, mainly because it can lead to increases in water intake by the animals due to high salt concentrations in its composition.

Diets with forage cactus combined with silages of *A. nummularia*, *S. bicolor*, *G. sepium*, or *M. pseudoglaziovii* and concentrates can potentially be used for feeding lactating cows without compromising the microbial crude protein synthesis and the balance of nitrogen compounds.

Increasing roughage alternatives for feeding dairy cows in the Semiarid region of Brazil is

important for improving production systems in this region.

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