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Forage cactus associated with different fiber sources for lactating Sindhi cows: intake, digestibility and microbial protein production

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ABSTRACT - This experiment was carried to evaluate the effect of forage cactus with different fiber sources (elephant grass hay [EGH], corn straw [CS], hay of cassava shoots [HCS], fresh sugarcane bagasse [FSB], and hydrolyzed sugarcane bagasse [HSB]) on intake, apparent digestibility and macrobiotic protein production of cows. Five cows with 265 kg average body weight, 4.95 kg average daily milk yield and 5.22 kg 4% fat-corrected milk yield were randomly allocated to a 5 × 5 Latin square design. The animals fed cactus associated with CS, EGH and HCS showed higher intake of DM and nutrients. However, regarding the digestibility, diets with sugarcane bagasse showed higher digestibility compared with those with CS and hay. For microbial protein, the excretion of purine derivatives, synthesis of microbial N and microbial protein production were higher in the treatment with CS than in the treatment with sugarcane bagasse. On the other hand, the treatment with EGH and HCS did not have statistical differences compared with the other cases. The mean efficiency of microbial protein synthesis of the experiment was 131 g microbial crude protein/kg of TDN. The treatment with CS showed better efficiency; however, it was statistically superior only compared with FSB. The other treatments did not show statistical difference compared with the other cases. Diets with cactus associated with corn straw, hay of elephant grass or hay of cassava shoots provide higher intake of dry matter and nutrients and better metabolic response compared with diets with cactus associated with sugarcane bagasse for lactating Sindhi cows.

Key Words: *Nopalea cochenillifera*, purine derivatives, roughage, zebu

Introduction

The productivity of ruminants depends on their ability to consume and extract useful energy from the available foods. According to Zeoula and Geron (2006), intake is the component that plays the role of greatest importance in animal nutrition, as it will determine the amount of ingested nutrient and hence performance. In addition to the nutrient intake, determining the digestibility of feeds or diets is important because it corresponds to the portion of feed consumed that was not excreted in the stools.

The microbial protein synthesis in the rumen is primarily responsible for meeting the protein requirements of ruminants and this depends largely on the availability of carbohydrates and nitrogen (N) in the rumen, and an asynchronism results in inefficient use of fermentable substrates and reduced synthesis of microbial protein (NRC, 2001). The lack of simple and accurate methods for measuring microbial protein is an obstacle to its deeper understanding. According to Chen and Gomes (1992), the method of excretion of purine derivatives overcomes other methods to determine microbial protein production for being simple, non-invasive and only requiring a total urine collection. In recent years, research has been developed with the aim of making this technique simpler, from a single sampling of urine for about four hours after morning feeding of animals, called spot collection, using serum creatinine as a marker to estimate the urine volume.

In the Brazilian Northeast, forage cactus (*Opuntia ficusindica* Mill *Nopalea cochenillifera* Dyck) is one of the main forages used in dairy cattle feeding, especially in the dry season. All cactus cultivars are rich in non-structural carbohydrates and have a high moisture content. The main limitations of this cactus are the nitrogen compounds and neutral detergent fiber content, requiring supplementation with other sources of nitrogen and fiber. Soluble urea, a result of the high carbohydrates content, is an important alternative for protein correction of forage cactus, besides

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its being associated with a source of forage with high effective fiber.

Zebu are rustic animals well-suited to adverse conditions of tropical regions, and Sindi is the recommended breed for the northeast of Brazil.

Therefore, the objective of this study was to evaluate the effect of combining forage cactus with different forages on intake, digestibility and estimated production and efficiency of microbial protein synthesis in Sindhi cows.

Material and Methods

The experiment was conducted at the Alagoinha Experimental Station, belonging to the State Company for Agricultural Research of Paraíba State (EMEPA, Empresa Estadual de Pesquisa Agropecuária da Paraíba S.A.), located in the municipality of Alagoinha/PB, Brazil.

Five Sindi cows (265 kg average live weight, first order of delivery, between 60 and 90 days of lactation) were housed in individual stalls provided with individual troughs and drinkers for feed-intake control. The experimental design was a Latin square with five cows, five periods and five treatments. Each period lasted 15 days during which 10 days were for the animals to adapt to diets and five for collecting data and samples, totaling 75 days of experimental period. The experimental diets were formulated according to the NRC (2001) to meet the requirements of animals of low-yield potential, average 6 kg milk/day, containing around 10.3% CP in their formulation. The feed was given to the animals twice daily (6.00 h and 15.00 h) as a complete diet, allowing for leftovers of 10 to 20% of total dry matter provided as a means to maintain the levels of ingredients in the diets. Animals were weighed at the beginning and end of the harvest period, thereby determining the change in weight according to the experimental periods.

The experimental treatments consisted of spineless cactus (*Nopalea cochenillifera* Salm-Dyck) Miúda (variety), associated with different fiber sources (Tables 1 and 2). The treatment of hydrolyzed sugarcane bagasse consisted of three kilos of hydrated lime dissolved in 50 L of water for every 100 kg of bagasse (Mattos et al., 2000).

At harvest time samples of feed ingredients and leftovers were taken and at the end of each period composite samples were made per animal and stored at −20 °C to be analyzed later. The dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE) and lignin contents were determined according to Silva and Queiroz (2002); concentrations of NDF and ADF were measured according to the non-sequential procedures of Van Soest et al. (1991) using amylase because of the high content of non-fiber carbohydrates in the forage cactus, but not sodium sulfite.

On the 11th and 13th days of each experimental period before food supply in the morning and afternoon, a fecal sample was collected directly from the rectum of animals and stored at −20 °C for further analysis and determination of diet digestibility. At the end of the experiment samples of ingredients, leftovers and feces were thawed and dried in a forced-air oven at 55 °C for 72 to 96 hours, with the

Table 1 - Proportions of ingredients in the diet

	Experimental diets				
Ingredients	Forage + EGH	Forage + CS	Forage + HCS	Forage + FSB	Forage + HSB
Forage cactus[1]	499.0	471.0	500.0	500.0	461.0
Elephant grass hay[1]	440.0	-	-	-	-
Corn straw[1]	-	448.0	-	-	-
Hay of cassava shoots[1]	-	-	450.0	-	-
Fresh sugarcane bagasse[1]	-	-	-	399.0	-
Hydrolyzed sugarcane bagasse[1]	-	-	-	-	438.0
Soybean meal[1]	30.0	50.0	20.0	70.0	70.0
Urea + ammonium sulfate[1]	16.0	16.0	15.0	16.0	16.0
Mineral mix[1]	15.0	15.0	15.0	15.0	15.0
Nutritional composition					
Dry matter (g/kg fresh weight)	194.6	204.2	190.0	212.9	195.6
Organic matter[1]	837.5	854.1	864.7	873.7	869.7
Crude protein[1]	113.1	123.4	105.7	119.4	117.3
NDFap[1]	502.5	473.7	430.9	504.0	520.7
Acid detergent fiber[1]	374.0	316.8	344.1	363.7	372.3
Lignin[1]	69.0	54.7	94.0	66.1	71.1
Ether extract[1]	17.8	18.4	16.7	17.7	14.6
NFCap[1]	239.7	272.4	337.0	269.4	251.1
Total digestible nutrients[1,2]	597.6	642.2	620.9	605.7	599.0

[1] g/kg DM.
[2] Patterson et al. (2000).
EGH - elephant grass hay; CS - corn straw; HCS - hay of cassava shoots; FSB - fresh sugarcane bagasse; HSB - hydrolyzed sugarcane bagasse.
NDFap - neutral detergent fiber corrected for ash and protein; NFCap - non-fibrous carbohydrates corrected for ash and protein.

Table 2 - Chemical composition of the nutrients in ingredients of experimental diets

	Forage	EGH	CS	FSB	HSB	HCS	Soybean meal
DM	110.8	848.0	826.3	709.6	526.3	835.1	865.0
OM[1]	843.9	882.1	914.1	966.9	946.7	942.0	943.0
MM[1]	156.1	111.9	86.0	58.0	33.1	53.3	56.9
CP[1]	58.3	51.6	53.1	18.5	17.3	52.2	539.2
EE[1]	18.9	17.3	18.4	16.2	09.3	14.9	25.3
NDF[1]	315.0	774.7	709.7	833.0	833.4	601.0	148.7
ADF[1]	249.7	559.4	432.4	579.3	569.6	482.4	109.9
NFC[1]	427.5	44.3	132.8	99.2	86.6	273.8	229.9
LIG[1]	34.0	117.2	84.5	120.4	124.0	170.4	15.2

[1] g/kg DM.
EGH - elephant grass hay; CS - corn straw; HCS - hay of cassava shoots; FSB - fresh sugarcane bagasse; HSB - hydrolyzed sugarcane bagasse.

exception of forage cactus, which required a longer period. These were then processed in a grinder with sieve fitted with a 1 mm sieve and stored for subsequent analysis.

The indigestible neutral detergent fiber was used as internal marker to determine the apparent digestibility of the feed, which was packed in bags made of non-woven fabric (TNT fabric) (100 g/m^2) according to the methodology described by Casali et al. (2008), except for the feces, which were processed through a 1 mm sieve.

The total carbohydrates (TC) were calculated according to Sniffen et al. (1992): TC = 100 – (%CP + %EE + %ash). The content of non-fiber carbohydrates (NFC) was obtained by the formula NFC = TC – NDFap, in which NDFap = neutral detergent fiber corrected for ash and protein. The total digestible nutrients (TDN) were calculated by the formula TDN = (CPI – fecalCP) + (TCI – fecalTC) + 2.25 (EEI – fecalEE), in which CPI = crude protein intake; TCI = total carbohydrate intake; and EEI = ether extract intake.

On the 12th day of each experimental period the urine spot collection was performed for analysis of allantoin in urine by the colorimetric method, according to the technique described by Chen and Gomes (1992); the same procedure was performed in milk without protein. Analyses of creatinine and uric acid in urine were performed using commercial kits (Labtest). The average daily urine volume was estimated for each animal by multiplying their body weight by the daily excretion of creatinine (mg/kg BW) and dividing that product by the creatinine concentration (mg/L) in the spot urine, using the value of 24.4 mg/kg of BW creatinine found in cows by Pereira (2003) in the early and middle thirds of the lactation period.

The purine derivatives were calculated by adding allantoin and uric acid in urine and milk allantoin, expressed in mmol/day. The microbial purines absorbed and the intestinal flow of nitrogenous compounds were calculated from the equations described by Chen and Gomes (1992). The estimate of microbial CP (MCP) was obtained by multiplying the synthesis of microbial nitrogen by 6.25, while the efficiency of microbial protein synthesis was determined by the formula: EMCPS (g/kg) = MCP (g)/TDNI (kg), in which TDNI = intake of total digestible nutrients.

The animals were divided in a in 5 × 5 Latin square design with five treatments and five experimental periods, according to the model: Yijk = m + ai + bj + dk + eijk, in which m = overall constant; ai = fixed effect related to treatment i; bj = random effect related to animal j; dk = random effect related to trial period k; and eijk = random error associated with each observation, assumption NID (0, s ^{2}).

The analysis of variance was performed using the SAS® statistical software, version 8.2 for Windows®, and the means for DM intake and nutrients, apparent digestibility and microbial protein production were compared by Tukey's test at 5% probability.

Results

Dry matter intake expressed as kg/day, %BW and g/BW$^{0.75}$ and OM intake were higher (P<0.05) for animals fed forage associated with EGH, CS or HCS compared with the treatments with sugarcane bagasse (Table 4).

Animals fed forage associated with CS had greater (P<0.05) CP intake compared with those on treatments HCS, FSB or HSB. The treatment with forage associated with EGH, although not different from treatments with CS and HCS, was higher compared with the treatment with FSB and HSB.

Lower NDF intake (kg/day and %BW) (P<0.05) was observed for treatments with forage associated with FSB and HSB compared with the treatments with CS and EGH; on the other hand, the treatment with HCS showed no statistical difference (P>0.05) compared with the others.

Increased NFC intake (P<0.05) was observed for animals fed forage associated with HCS, compared with the other treatments, which may be explained by the lower NDF and higher non-fiber carbohydrates in this treatment.

The intake of total digestible nutrients was higher (P<0.05) in animals fed forage associated with EGH, CS or HCS than those fed HSB; however, those animals fed FSB showed no statistical difference compared with the others.

Higher DM digestibility was observed (P<0.05) in the diets of animals fed FSB compared with the treatments with EGH, CS and HCS; however, the diets containing forage associated with HSB showed no difference (P>0.05) compared with those containing FSB and HCS (Table 4).

As regards NDF digestibility, the treatment with HCS was significantly lower (P<0.05) compared with all other treatments. On the other hand, diets with EGH and CS were statistically lower (P<0.05) compared with the FSB diet.

The allantoin excretion in the urine, purine derivatives, absorbed purines, synthesis of microbial nitrogen and microbial crude protein (Table 5) showed similar behaviors, that is, the respective variables were higher (P<0.05) for treatment with CS compared with the treatments with sugarcane bagasse. On the other hand, treatments with EGD and HCS showed no statistical difference (P>0.05) compared with the others.

The treatment with CS had higher milk allantoin excretion (P<0.05) than the treatment with HSB; the same behavior was observed for milk yield in the experiment (Table 3).

Table 3 - Milk yield and 4% fat-corrected milk yield of Sindhi breed cows fed diets with forage cactus associated with different fiber sources

	Experimental diets				
	Forage + EGH	Forage + CS	Forage + HCS	Forage + FSB	Forage + HSB
Milk yield (kg/day)	5.24	6.09	4.76	4.62	4.04
4% fat-corrected milk yield (kg/day)	5.94	6.23	5.00	4.72	4.24

EGH - elephant grass hay; CS - corn straw; HCS - hay of cassava shoots; FSB - fresh sugarcane bagasse; HSB - hydrolyzed sugarcane bagasse.

Table 4 - Average intakes and digestibilities of chemical components and coefficients of variation (CV) of Sindhi cows fed diets with forage cactus associated with different fiber sources

	Experimental diets					
	Forage + EGH	Forage + CS	Forage + HCS	Forage + FSB	Forage + HSB	CV (%)
			Intake			
DM (kg/day)	8.67a	9.08a	8.5a	6.22b	5.63b	13.07
DM (%BW)	3.33a	3.45a	3.23a	2.36b	2.14b	12.45
DM (g/$BW^{0.75}$)	133.77a	138.82a	129.99a	95.08b	86.12b	12.56
OM (kg/day)	7.45a	7.88a	7.48a	5.54b	4.93b	13.41
CP (kg/day)	0.98ab	1.15a	0.89bc	0.73c	0.66c	12.88
EE (kg/day)	0.16a	0.15ab	0.15ab	0.11bc	0.09c	15.62
NDF (kg/day)	4.21a	4.20a	3.44ab	3.01b	2.67b	14.24
NDF (%BW)	1.62a	1.59a	1.30ab	1.14b	1.01b	14.06
NFC (kg/day)	2.09bc	2.35b	3.04a	1.68c	1.51c	14.44
TDN (kg/day)	4.11a	4.10a	4.20a	3.71ab	3.01b	12.79
			Digestibility			
DM	52.00c	50.12c	53.07bc	63.99a	58.45ab	5.04
OM	52.48b	51.28b	53.94b	64.92a	59.71a	4.73
CP	70.06bc	65.34cd	61.99d	79.15a	74.78ab	5.45
EE	64.71ab	47.94c	58.42bc	74.40a	68.06ab	9.51
NDF	38.00b	36.20b	27.29c	48.96a	44.86ab	11.19
NFC	72.31b	70.84b	83.73a	86.52a	77.59ab	7.23

Means followed by different letters in the row differ by Tukey's test (P<0.05).
EGH - elephant grass hay; CS - corn straw; HCS - hay of cassava shoots; FSB - fresh sugarcane bagasse; HSB - hydrolyzed sugarcane bagasse.

Table 5 - Daily urinary excretion of uric acid (UA), milk allantoin (MAl), allantoin in urine (UAl), purine derivatives (PD), absorbed purines (AP), synthesis of microbial nitrogen (SMN), microbial crude protein synthesis (MCPS), efficiency of microbial crude protein synthesis (EMCPS) and coefficients of variation (CV) of Sindhi cows fed diets with forage cactus associated with different fiber sources

	Experimental diets					
	Forage + EGH	Forage + CS	Forage + HCS	Forage + FSB	Forage + HSB	CV (%)
UA (mmol/day)	10.45bc	15.64a	12.12ab	7.51c	7.85c	17.05
MAl (mmol/day)	2.35ab	2.67a	2.23ab	2.26ab	1.90b	15.57
UAl (mmol/day)	113.21ab	128.16a	117.29ab	82.85b	87.93b	18.56
PD (mmol/day)	126.02ab	146.48a	131.64ab	92.63b	97.67b	17.45
AP (mmol/day)	119.09ab	142.53a	125.31ab	79.23b	85.25b	22.00
SMN (g N/day)	86.58ab	103.62a	91.10ab	57.61b	61.98b	21.99
MCPS (g/day)	541.16ab	647.64a	569.42ab	360.05b	387.40b	21.99
EMCPS (g MCP/kg TDN)	132.23ab	158.17a	136.34ab	96.47b	131.88ab	20.39

Means followed by different letters in the row differ by Tukey's test (P<0.05).
EGH - elephant grass hay; CS - corn straw; HCS - hay of cassava shoots; FSB - fresh sugarcane bagasse; HSB - hydrolyzed sugarcane bagasse.

The urinary excretion of allantoin ranged from 82.85 to 128.16 mmol/day, averaging 105.88 mmol/day.

The average EMCPS of the experiment was 131 g MCP/kg TDN. The treatment with CS was the most efficient; however, it was statistically higher (P<0.05) only in relation to the FSB, while other treatments showed intermediate behavior.

The treatment with FSB had the lowest EMCPS (96.47 g of MCP/kg of TDN); this result is probably related to the fiber quality of FSB, which may have led to a lower passing rate and hence lower flow of protein to the abomasum.

Discussion

As regards dry matter intake, similar results to that reported herein were found by Mattos et al. (2000), wherein lower DMI was observed for Girolando cows fed forage associated with fresh and hydrolyzed sugarcane bagasse compared with treatments in which the forage was associated with sorghum silage and saccharin.

The higher DM intake (P<0.05) observed in this experiment in the treatments with EGH, CS and HCS is probably due to the greater palatability of these diets and fiber quality in diets with sugarcane bagasse, which had a high lignin content (Table 2), which may have caused a lower passage rate in the rumen-reticulum coupled with the filling effect, a factor that in these cases reduces intake by the animal.

A fact that should be stressed is the particle size of sugarcane bagasse; although it passed through the forage machine before being offered to the animals, it still remained with a larger particle size than the corn hay and stover, which also may have influenced the DMI. According to Allen (2000), the flow in the rumen-reticulum is limited by size and density of food particles, which leads to stretching of these organs. These authors also emphasize that when the stretch in the rumen-reticulum limits the DMI, a reduction in the particle size of the forage may result in increased DMI if the density of the consumed particles or the time available for rumination increases.

This difference in CP intake may be due to the higher DM intake in these treatments and consequently the nutrients, as well as higher content of nutrients mainly in the treatment with CS (Table 1). Although all diets were formulated to contain on average 10% CP, after laboratory tests at the end of the experiment some ingredients differed in their composition of nutrients, especially in CP and NDF.

The effect of diets on NDF intake may be caused by reduced DMI, and probably a result of greater rejection of these diets by the animals, besides the effect of bagasse particle size. According to Allen (2000), the physical and chemical characteristics of diet ingredients and their interactions can have a great effect on the DMI. In this case, the amount of ingested nutrients will be changed as well.

Unlike the results found herein, Silva et al. (2007) found no difference in NFC and TDN intake of Holstein cows fed diets with forage cactus associated with different forages, possibly because there was no difference in DMI and nutrient intake by the animals in the study, besides the similarity in diet composition.

Although the form of providing the feed in this experiment was in a complete diet, the different fiber sources associated with forage cactus affected the digestibility of nutrients and DM (Table 4). According to Van Soest (1994), digestibility coefficients can be relatively constant for certain environmental conditions, but the food can be more or less digestible if there is variation in intake or separate provision.

Although the diets with sugarcane bagasse showed higher digestibility, this was not sufficient to ensure the highest performance of the animals (Table 3), probably reflecting the lower intake. The characteristics of sugarcane bagasse fiber as a probable effect of rumen fill might have made this material remain longer in the rumen than the other diets, which led to a higher digestibility at the end, though possibly with lower degradability, which in this case also affected the intake.

Although the diet with HCS had lower NDF contents (42.40%) in its composition, the ADF percentage is higher than NDF, whose most influential component is lignin, which may have led to lower digestibility.

Leão (2007), working on increasing levels of cassava shoots hay in diets based on sugarcane + 1% urea (0, 25, 50, 75 and 100% DM), found decreased digestibility for all nutrients, besides increased DM intake at the level of 65% hay of cassava shoots. According to the authors, this phenomenon can be explained by the increased DM intake, which decreases the digestibility of nutrients, besides the direct ADF effect of cassava shoots hay on the digestibility of its components.

The acid detergent fiber fraction is composed of cellulose and lignin; as the latter component is increased the digestibility of DM, NDF and ADF decreases. The hay of cassava shoots has a high lignin content, which in this experiment was on average 17.04% (Table 2). Thus, although this was the treatment with the lowest NDF content, it had a high amount of lignin, a fact that may have contributed to reduced digestibility of the treatment in question. Van Soest (1994) points out that lignin is recognized as the main factor limiting digestibility, but this does not affect all components.

Pessoa et al. (2005) found mean coefficients of digestibility of 67.16, 68.96, 77.24, 52.97, 53.42, and 73.40% for DM, OM, CP, EE, NDF and NFC in an experiment using forage cactus. However, the NDF content in the aforementioned study was 30.30% below the value reported in the present study, whose NDF of treatments ranged from 42.40 to 52.70%. According to Van Soest (1994), decreased digestibility is proportional to the NDF and rate of passage and inversely related to the rate of digestion and therefore the characteristics of the cell wall are of great importance. This refers basically to the cell wall lignification, because as lignification increases, digestibility decreases.

As the microbial protein synthesis is dependent on the availability of substrate for rumen microorganisms, mainly protein and carbohydrates, the lower DM intake in diets with sugarcane bagasse possibly influenced the results, which reflected also in milk production (Table 3).

According to Gonda and Lindberg (1997), milk production influences the concentration and quantity of allantoin in milk, which probably accounts for the results found here.

Barbosa et al. (2006) observed an average of 92.33 mmol/day of allantoin in Nellore cows. According to Barbosa et al. (2006), data described in the literature indicate that the level of allantoin in cows is not constant in all physiological stages and dietary treatments, which also seems to happen in zebu cows.

The NRC (2001) established an average microbial protein synthesis efficiency of 130 g MCP/kg of TDN; however, Valadares Filho et al. (2006) recommends 120 g MCP/kg of TDN as a reference for tropical conditions, i.e., the findings of the present study are consistent with the literature.

Lower passage rates result in lower ruminal dilution rate, and according to Bach et al. (2005), higher microbial protein synthesis and EMCPS are associated with higher rates of dilution because microbial species with higher growth rates are selected and the maintenance requirements of rumen microorganisms are diluted. Moreover, the microbial protein synthesis increases with the availability of organic matter; hence the higher values of microbial protein and EMCPS in diets with CS, EGH and HCS. The treatment with HSB showed lower microbial protein synthesis; however, its EMCPS was within the recommended by NRC (2001), which was also a result of lower TDN intake.

Conclusions

The different fiber sources evaluated as alternative to diets with forage cactus influence nutrient intake, apparent digestibility and microbial protein synthesis. The use of forage associated with corn straw allows for better metabolic responses than its association with sugarcane bagasse as a fiber source; however, elephant grass and hay of cassava shoots are also a good alternative for use as fiber source in diets based on forage cactus for lactating Sindi cows.

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