Spineless cactus as a replacement for wheat bran in sugar cane-based diets for sheep: intake, digestibility, and ruminal parameters

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ABSTRACT - The objective of this study was to evaluate the effect of replacing wheat bran by spineless cactus (0, 25, 50, 75, and 100%) in sugar cane-based diets on intake and nutrient digestibility and ruminal parameters of sheep. Five sheep (Santa Inês; average initial weight of 34.0±3.6 kg) were fitted with cannulas in the rumen and then assigned to a 5 × 5 Latin square design. The maximum dry matter (DM; 1414 g d^{-1}), digestible organic matter (658 g d^{-1}), and neutral detergent fiber corrected for ash and protein (NDFap; 425 g d^{-1}) intakes were estimated by replacing 80.2, 89.9, and 50.5% of wheat bran. Dry matter and crude protein digestibility increased by 0.60 and 0.85 g kg^{-1} of DM. With the replacement of 57.1 and 62.2% of wheat bran, a maximum rate of NDF ingestion (0.70 h^{-1}) and NDF ruminal pool (7.31 g) was obtained. Rumen pH increased with the inclusion of spineless cactus, while the maximum concentration of ammonia N (33.3 mg dL^{-1}) and total volatile fatty acids (57.7 μmol mL^{-1}) were estimated at the levels of 72.3 and 63.7% of replacement of wheat bran. It is recommended to replace 63% of wheat bran by spineless cactus in sugar cane-based diets, to promote better animal performance.

Key Words: animal nutrition, cactaceous, dry season, feed intake, rumen fermentation, semiarid

Introduction

In arid regions, small ruminants are the main producing species of meat and milk, not only for subsistence but also for the consumer market. Because of the high cost of production, farmers use little technology and feedstock, a fact that is aggravated by environmental conditions, such as severe droughts (Ben Salem and Smith, 2008). Another fact that suggests considerable concern among producers of milk and meat is the increased use of concentrate to maintain production in the dry season.

The ingredients that traditionally make up concentrate feed are difficult to produce in semiarid regions due to the rainfall irregularity. Thus, the replacement of wheat bran, a widely used ingredient in feed formulation (Melaku et al., 2005; Monteiro et al., 2014; Touno et al., 2014), could reduce production costs and make the animal production more sustainable.

The replacement of the concentrate or part thereof by spineless cactus could reduce feed costs. Currently, the spineless cactus has a cost to the producer of US$0.10, compared with the of US$0.17 of wheat bran, justifying the use of the cactus. However, the spineless cactus has a low neutral detergent fiber content (170-280 g kg^{-1} DM) (Ferreira et al., 2011), which suggests an association with forage containing effective fiber.

Among the available forages, sugar cane is traditionally used in Brazil because its production coincides with the period of forage scarcity (Mariz et al., 2013). The use of spineless cactus associated with sugar cane is scarce and justifies more studies. There is a hypothesis that the high concentration of non-fiber carbohydrates (640-710 g kg^{-1} DM) of spineless cactus with rapid degradation could allow the use of a higher amount of urea for protein correction (Ferreira et al., 2011). It also could allow a better use of the potentially digestible fiber from sugar cane.

In studies with beef cattle, Valadares Filho et al. (2014) found an urea content of up to 1.0% in the total dry matter of the diet, but higher levels of urea could be used, providing satisfactory performance. For sheep, a maximum level of urea inclusion that could adversely affect the performance of sheep has not been suggested.

In the literature, the urea content has been found to range from 1.5% (Alves et al., 2012) to 2.4% (Vidal et al., 2004) in the total dry matter of sheep diet, without compromising intake or performance.

Thus, we hypothesized that spineless cactus, high in non-fiber carbohydrates, could replace partially or totally wheat bran in sugar cane-based diets, evaluating the effects on intake and nutrient digestibility and ruminal parameters in sheep.

Material and Methods

The experiment was conducted in Recife - PE, Brazil.

The management and care of animals were performed according to the guidelines and recommendations of the Committee of Ethics on Animal Use (CEUA) of Universidade Federal Rural de Pernambuco. Five uncastrated male sheep of the Santa Inês breed with an average initial body weight of 34.0 ±3.6 kg and fitted with cannulas in the rumen were assigned to a 5 × 5 latin square design, with five treatments and five experimental periods. The animals were kept in indoor facilities with individual stalls provided with a feeder and drinker.

Experimental diets (Table 1) consisted of the replacement of wheat bran by spineless cactus (*Nopalea cochenillifera* Salm-Dyck) in the proportions of 0, 25, 50, 75, and 100% (Table 2). The diets were supplied *ad libitum*, as a complete ration, twice daily, at 08.00 and 16.00 h and adjusted daily, formulated to provide 13% as orts. The sugar cane (*Saccharum officinarum L.*) was corrected with urea and ammonium sulfate.

Each experimental period (five periods) lasted 15 days, with the first eight days dedicated to animal adaptation (Storry and Sutton, 1969; Menezes et al., 2011). Voluntary intake was evaluated from the 4th to 11th day of each experimental period with samples of ingredients, orts, and feces (total collection used a collection bag attached to the animals) collected for the three consecutive days of each period.

Rumen fluid was collected from the 11th to 13th day of each experimental period, before feeding and two, four, and six hours after feeding. The manual collection was performed at several locations in the ruminal environment, taking a representative sample of the content (100 mL), which was filtered through cotton fabric. After collection, pH was measured with a digital potentiometer. The ammonia nitrogen ($N-NH_3$) concentration was determined after samples were centrifuged at 3,000 rpm/15 min, using the supernatant for analysis by Kjeldahl distillation (Detmann and Valadares Filho, 2010). Aliquots for the assessment of the main volatile fatty acids (acetate, propionate, and butyrate) were defrosted and centrifuged at 15,000 × g, at 4 °C for 60 min. Analysis of volatile fatty acids (VFA) was performed using a gas chromatograph equipped with a flame ionization detector and auto-injector and coupled to a GP column (30 m × 0.250 mm, 0.25 μm; Chromosorb WAW).

On day 13, the rumen was completely emptied four hours after the morning diet was provided to determine the rates of indigestion and ruminal pool for each diet using the technique described by Allen and Linton (2007). On day 15, the rumen was emptied immediately before the morning feeding. After emptying the rumen, the total weight of the digesta was determined, followed by filtering through four layers of cheesecloth to separate the solid and liquid phases. A representative sample of both phases was collected and

Table 1 - Chemical composition of ingredients on a dry matter basis

	Sugar cane	Corn	Wheat bran	Spineless cactus
Dry matter[1]	275	888	908	99.1
Organic matter[2]	968	983	891	851
Crude protein[2]	24.0	88.7	159	51.0
Ether extract[2]	9.10	46.0	50.3	13.5
NDFap[2]	440	190	434	232
Non-fiber carbohydrates[2]	495	658	247	555

NDFap - neutral detergent fiber corrected for ash and protein.
[1] g kg−1 as fed.
[2] g kg−1 dry matter.

Table 2 - Proportion of ingredients and chemical composition of the diet

	Replacement level (%)				
	0	25	50	75	100
Ingredient[1]					
Sugar cane	369	369	373	375	377
Corn	79.3	79.9	80.6	80.8	80.8
Wheat bran	529	396	262	127	0
Spineless cactus	0	125	249	375	492
Urea/ammonium sulfate	13.4	19.3	26.3	32.4	39.4
Mineral	7.28	7.33	7.38	7.41	7.40
Sodium chloride	2.36	2.41	2.46	2.46	2.49
Chemical composition					
Dry matter[2]	490	314	232	184	154
Organic matter[1]	943	932	923	913	903
Crude protein[1]	136	137	140	141	146
Ether extract[1]	30.4	26.3	22.1	17.8	13.8
NDFap[1]	407	379	351	323	296
iNDF[1]	160	189	201	199	180
Non-fiber carbohydrates[1]	392	422	453	484	513

NDFap - neutral detergent fiber corrected for ash and protein; iNDF - indigestible neutral detergent fiber.
[1] g kg−1 dry matter.
[2] g kg−1 as fed.

frozen (–20 ºC) for later evaluation of dry matter (DM), neutral detergent fiber (NDF), and indigestible neutral detergent fiber (iNDF) contents. After sampling, the phases were again mixed, and the remaining digesta was returned to the rumen.

At the end of each period, a composite sample was prepared from the leftovers, feces, and ruminal digesta based on the air-dried weight of the samples for each animal, and the samples were then properly identified and stored in plastic containers for further analysis. At the end of the sampling period, the ingredients of diets, orts, fecal samples, and ruminal content samples were oven-dried (60 ºC) and ground.

Dry matter, organic matter (OM), and crude protein (CP) analyses were performed according to the AOAC (1990), method number 934.01 for DM, 930.05 for OM, and 981.10 for CP. Ether extract (EE) was analyzed by Soxhlet extraction with petroleum ether, according to the AOAC (1990), method number 920.39. The concentration of NDF was assayed with heat-stable amylase and corrected for ash and nitrogen compounds [aNDFom(n)] by using techniques described by Mertens (2002), with corrections for protein according to Licitra et al. (1996) and thermostable alpha-amylase added. Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) (Licitra et al., 1996) were measured using the Kjeldahl method. Non-fiber carbohydrates (NFC) were calculated according to Hall (2000), as follows:

$$NFC\ (g\ kg^{-1}) = 1000 - [(CP - urea\text{-}derived\ CP + urea) + NDFap + EE + ash],$$

in which CP = crude protein; NDFap = neutral detergent fiber corrected for ash and protein; and EE = ether extract.

The experiment was analyzed as a 5×5 Latin square design using the MIXED procedure of SAS (Statistical Analysis System, version 9.4), according to the following model:

$$Y_{ijk} = \mu + D_i + a_j + p_k + \varepsilon_{ijk},$$

in which μ is the overall constant; D_i is the fixed effect of diet I; a_j is the random effect of animal j; p_k is the random effect of experimental period k; and ε_{ijk} is the random unobservable error.

After analysis of variance, the significance of the linear and quadratic effects of the replacement of the total of wheat bran in the basal diet by spineless cactus was evaluated. A significance value of 0.05 was adopted as the critical value of the probability of type-I error. Rumen pH, ammonia nitrogen (NH_3-N), and volatile fatty acids (VFA) were considered the effect of sampling time as repeated measures in time.

Results

A quadratic effect (P<0.05) was found on the intakes of DM and nutrients (Table 3). The intakes of DM (1414 g d⁻¹), OM (1281 g d⁻¹), CP (204 g d⁻¹), NDFap (425 g d⁻¹), NFC (657 g d⁻¹), and digestible OM (658 g d⁻¹) were achieved at the levels of 80.2, 75.3, 88.6, 50.5, 89.9 and 89.9% of replacement of wheat bran respectively. However, the digestibility of DM and CP had a linear effect (P<0.05) (Table 3). The experimental diets had no effect (P>0.05) on the apparent digestibility of OM, NDF, and NFC.

Similarly to DM intake, a quadratic effect (P<0.05) was found for rate of ingestion of NDF, with maximum point (0.70 h⁻¹) estimated at 57.1% of replacement of the wheat bran (Table 3). Conversely, the minimum ruminal pool (P<0.05) of NDF (7.31 g) was achieved with 62.2% of replacement of wheat bran (Table 3). No effect (P>0.05) was found for the ruminal pool of iNDF.

The experimental diets had an effect on the ruminal parameters (Table 4). The rumen pH increased linearly (P<0.05) as the spineless cactus increased, but conversely reduced (P<0.05) with respect to collection time. Conversely, a quadratic effect (P<0.05) was found for ammonia N (N-NH_3), compared with the diets (33.3 mg dL⁻¹ achieved with 72.3% of wheat bran replacement) and ruminal collection time (42.8 mg dL⁻¹ achieved three hours after feeding). Similarly, a quadratic effect (P<0.05) was found for short-chain fatty acids (SCFA), concentrations of acetate, propionate and butyrate, and acetate/propionate (A/P) ratio. The maximum point was found with 57.7 µmol mL⁻¹ of SCFA, 34.5 µmol mL⁻¹ of acetate, 15.3 µmol mL⁻¹ of propionate, 7.66 µmol mL⁻¹ of butyrate, and an A/P ratio of 2.16, with replacement levels of 63.7, 70.6, 54.2, 57.5, and 46.5% (Table 4).

Discussion

The replacement of wheat bran by spineless cactus provided a higher nutrient intake, explained by the increase in the concentration of easily fermentable carbohydrates (NFC) and the lower iNDF content of the diets, promoting better dry matter digestibility (Table 3). The spineless cactus can be considered a good source of fermentable carbohydrates of rapid degradation (Batista et al., 2009), improving digesta flow through the gastrointestinal tract with a consequent increase in intake.

The non-fiber carbohydrate contents of the diets (Table 2) were above 30%, which, according, to Hoover (1986) is sufficient to reduce intake. However, in this study, the DM intake increased until 80% replacement of wheat bran. The inclusion of spineless cactus allowed for the balancing of

the ruminal environment, evidenced by the average rumen pH of 6.8 (Table 4), which remained at adequate levels (6.2 to 7.0), according to Hoover (1986), with the aid of the effective fiber presence (sugar cane). The pH values for all replacement levels were close to the acceptable range for microbial growth and ruminal fiber digestion. According to Abidi et al. (2009), the mucilage in spineless cactus cladodes may stimulate salivation, thus preventing pH decrease.

A possible hypothesis for the quadratic effect of nutrient intake is the excess of rapidly degradable nitrogen in the rumen (urea), which not only affects the palatability of the diet but also compromises ruminal fermentation. Another hypothesis is the high moisture content in the spineless cactus causing rumen filling (Gebremarian et al., 2006). In the present study, a DM content of spineless cactus of 99.1 g kg^{-1} was found (Table 1), and, according to Minson

Table 3 - Intake and digestibility of nutrients, rate of ingestion (ki) of NDF, and pool sizes of the diet components in sheep fed diets containing different levels of replacement of wheat bran with spineless cactus

Item	Replacement level (%)					SEM	P-value
	0	25	50	75	100		
Intake (g d^{-1})							
Dry matter[1]	949	1170	1348	1426	1376	88.5	0.014
Organic matter[2]	896	1091	1243	1303	1246	73.9	0.013
Crude protein[3]	124	158	190	204	202	15.3	0.009
NDFap[4]	380	406	429	415	377	10.1	0.046
Non-fiber carbohydrates[5]	363	497	595	658	649	55.3	0.009
Digestible organic matter[6]	634	765	945	998	954	68.9	0.009
Digestibility (g kg^{-1})							
Dry matter[7]	687	680	740	724	740	12.9	0.028
Organic matter	708	701	760	751	766	13.6	0.128
Crude protein[8]	794	803	848	854	875	15.5	0.001
Neutral detergent fiber	538	500	567	538	557	11.5	0.959
Non fibrous carbohydrates	927	931	954	946	941	4.91	0.170
ki_{NDF} (h^{-1})[9]	0.046	0.064	0.075	0.061	0.060	0.004	0.004
Ruminal pool (g)							
iNDF	4.54	3.60	3.29	4.12	3.74	0.22	0.096
NDF[10]	10.24	7.74	6.98	8.52	7.81	0.55	0.039

SEM - standard error of the mean; NDFap - neutral detergent fiber corrected for ash and protein; iNDF - indigestible neutral detergent fiber; NDF - neutral detergent fiber; P-value - significance level (α = 0.05).
[1] $\hat{Y} = 940.34 + 11,78X - 0.0734X^2$.
[2] $\hat{Y} = 887.89 + 10.449X - 0.0694X^2$.
[3] $\hat{Y} = 121.84 + 1.85X - 0.01044X^2$.
[4] $\hat{Y} = 376.98 + 1.90X - 0.0188X^2$.
[5] $\hat{Y} = 359.94 + 6.6006X - 0.0367X^2$.
[6] $\hat{Y} = 616.46 + 8.9434X - 0.0545X^2$.
[7] $\hat{Y} = 683.78 + 0.6039X$.
[8] $\hat{Y} = 792.47 + 0.8453X$.
[9] $\hat{Y} = 0.04739 + 0.0008136X - 0.00000713X^2$.
[10] $\hat{Y} = 9.9111 - 0.0834X + 0.0006709X^2$.

Table 4 - Ruminal parameters of sheep fed diets containing different levels of replacement of wheat bran with spineless cactus

Item	Replacement level (%)					SEM	Diet effect		Time effect	
	0	25	50	75	100		L	Q	L	Q
pH[1]	6.61	6.68	6.71	6.89	6.91	0.10	0.001	0.877	<0.001	<0.001
N-NH$_3$ (mg dL^{-1})[2]	24.5	27.7	32.9	33.9	31.2	1.76	0.009	0.018	0.135	<0.001
Volatile fatty acid (mmol L^{-1})										
Total[3]	38.9	56.9	56.4	52.7	54.9	1.88	0.007	0.005	<0.001	<0.001
Acetate[4]	24.1	33.6	33.6	32.2	34.7	1.79	0.006	0.033	0.005	<0.001
Propionate[5]	10.1	15.6	15.8	12.8	13.6	0.95	0.174	0.002	<0.001	<0.001
Butyrate[6]	4.69	7.75	6.96	7.71	6.68	0.57	0.031	0.005	0.088	<0.001
Acetate/propionate ratio[7]	2.41	2.18	2.14	2.5	2.60	0.10	0.169	0.044	<0.001	<0.001

SEM - standard error of the mean; N-NH$_3$ - ammonia nitrogen; L - linear effect; Q - quadratic effect.
[1] $\hat{Y} = 6.594 + 0.0034X$.
[2] $\hat{Y} = 23.861 + 0.2601X - 0.0018X^2$.
[3] $\hat{Y} = 41.464 + 0.5087X - 0.004X^2$.
[4] $\hat{Y} = 26.635 + 5.1004X - 0.7353X^2$.
[5] $\hat{Y} = 9.4814 + 4.1062X - 0.5873X^2$.
[6] $\hat{Y} = 5.8054 + 1.105X - 0.1688X^2$.
[7] $\hat{Y} = 2.3774 - 0.0093X + 0.0001X^2$.

(1990), water contents in forages exceeding 780 g kg^{-1} can compromise voluntary intake.

The presence of minor NDFap amounts in the diets explains the reduction of the intake of this nutrient after the level of 50% wheat bran replacement, as well as the presence of increasing amounts of NFC. However, approximately 31% of NDFap in the diet favored the maximum DM intake. For lactating cows, the NRC (2001) recommends a maximum of 44% of NFC in DM, and at least 25% of NDF, with an effective fiber source. From this result, it can be inferred that sheep are more tolerant to higher NFC levels than cattle, which may be related to the eating habits of the animals.

The increase in non-protein nitrogen (NPN) in the diets explains the higher digestibility of crude protein, since it is completely solubilized in the rumen and rapidly converted to ammonia by bacteria attached to the ruminal wall (Eustaquio Filho et al., 2008). Although soluble nitrogen in the ruminal environment can increase NDF digestibility (Detmann et al., 2008), this effect was not observed for this nutrient (Table 3).

The replacement of a fibrous feedstuff like wheat bran (43.4% NDF) by the spineless cactus (23.2% NDF) explains the increase in ingestion rate of NDF (ki) up to 50% of replacment of wheat bran. The effect of wheat bran on the intake rate can be observed by the higher presence of NDF resident mass in the rumen (NDF ruminal pool) of the control diet (100% wheat bran). The increased nutrient intake and the reduction in fiber ruminal retention time in the presence of the spineless cactus corroborate Huhtanen et al. (1995), who reported inverse relations between DM intake and retention time.

Melaku et al. (2005) found fewer particles flowing through the rumen and a higher fiber total retention time in sheep fed wheat bran. Therefore, it is important to note that the use of spineless cactus provided a higher particle flow in the rumen, with less NDF retention (7.31 g) with the replacement of 62.2% of wheat bran (Table 3).

The estimated concentration of ammonia-N (33.3 mg dL^{-1}) was achieved with 72.3% replacement of wheat bran. According to Leng (1990), the minimum amount of ammonia N necessary for maximum microbial growth ranges from 10 to 20 mg dL^{-1}. The increase in the intake of digestible organic matter (Table 3) and NPN derived from urea probably resulted in higher concentrations of ammonia N, which, according to Van Soest (1982), creates a suitable ruminal environment for cellulolytic microorganisms to ferment fiber effectively. In this study, there was no effect on the digestibility of fiber, probably because the replacement of the NPN amount at all levels

(with a minimum concentration of 24.5 mg dL^{-1} at 0% of replacement) was sufficient to allow for fiber digestion.

The peak production of rumen NH$_3$-N (42.8 mg dL^{-1}) occurred three hours after feeding, probably due to the presence of low rumen-degradability fiber originating from sugar cane. Also, because of the presence of wheat bran, microbial fermentation became slower, as evidenced by the increased retention of food (Table 3), thus delaying the ammonia-N peak.

When there is a predominance of fibrous carbohydrates in diets, ruminal microorganisms direct the production of short-chain fatty acids (SCFA) to acetate production. However, a higher concentration of acetate was observed at the higher wheat bran replacement levels (Table 4), indicating that soluble carbohydrates and pectin in the spineless cactus were preferably fermented. When soluble carbohydrates are abundant, bacteria produce both acetate and ethanol as formate, and, if the rapidly fermentable substrate is reduced, the final products are acetate and propionate (Antunes et al., 2011).

The high degradability of spineless cactus has become known for maximizing the rumen fermentation capacity and increasing microbial protein synthesis, SCFA production and, consequently, nutrient conduction for animals (Ferreira et al., 2009). It was found that maximum production of total SCFA (57.7 μmol mL^{-1}, Table 4) was obtained with 63.7% replacement of wheat bran by spineless cactus.

Conclusions

The spineless cactus has proven to be a food alternative in semi-arid regions, replacing up to 80% of wheat bran in sheep diet without affecting dry matter intake. To promote better animal performance, it is recommended to replace 63% of wheat bran by spineless cactus in sugar cane-based diets because of the optimal ruminal fermentation and higher volatile fatty acids synthesis for the animal.

Acknowledgments

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