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Nutritional and mineral composition of *Opuntia stricta Haw*: Balance of macrominerals, renal function and blood metabolites in sheep

[*Composição nutricional e mineral da* Opuntia Sttricta *Haw: balanço de macrominerais, função renal e metabólitos sanguíneos em ovinos*]

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ABSTRACT

This study aimed to evaluate the effect of the inclusion of spineless cactus (*Opuntia stricta* Haw) in the diet of sheep on the balance of macrominerals, renal function, and blood metabolites. Five sheep cannulated in the rumen (61.5±9.5kg body weight) were used in a 5 × 5 Latin square composed of five diets and five experimental periods. The experimental period lasted 105 days, with five periods of 21 days each. Four diets containing levels of spineless cactus (121, 245, 371, and 500g/kg of dry matter (DM)), and a control diet were evaluated. Samples of the ingredients, orts, feces, urine, and blood were collected. Spineless cactus inclusion in sheep diets increased the DM intake, ash, oxalate, and all macrominerals intake (P < 0.05), but did not affect the urinary and fecal excretion of P, as well as the concentration of P in the blood (P > 0.05). It is possible to verify that the inclusion of spineless cactus, up to the level of 500g/kg of DM in the sheep diets, does not appear to cause damage to the animal’s health. Its inclusion does not compromise kidney function or blood metabolites evaluated herein.

Keywords: cactus, kidneys, microelements, oxalic acid

RESUMO

O objetivo deste estudo foi avaliar o efeito da inclusão de palma forrageira (Opuntia stricta *Haw) na dieta de ovinos sobre o balanço dos macrominerais, a função renal e os metabólitos sanguíneos. Cinco ovinos canulados no rúmen (61,5±9,5kg de peso corporal) foram distribuídos em um quadrado de 5 × 5 latinos, composto de cinco dietas e cinco períodos experimentais. O período experimental teve duração de 105 dias, sendo cinco períodos de 21 dias cada. Foram avaliadas quatro dietas contendo níveis de palma forrageira (121, 245, 371 e 500g/kg de matéria seca (MS)), e uma dieta controle. Foram coletadas amostras dos ingredientes, sobras, fezes, urina e sangue. A inclusão de palma forrageira na dieta dos ovinos aumentou o consumo de MS, cinzas, oxalato e todos os macrominerais (P<0,05), mas não afetou a excreção urinária e a fecal de P, bem como a concentração de P no sangue (P>0,05). É possível verificar que a inclusão de palma forrageira, até o nível de 500g/kg de MS na dieta de ovinos, não parece causar danos à saúde do animal. Sua inclusão não compromete a função renal ou os metabólitos sanguíneos avaliados.*

Palavras-chave: ácido oxálico, cactáceas, rins, microelementos

INTRODUCTION

The spineless cactus (*Opuntia* spp.) is an energetic food used in the diets of sheep in arid and semi-arid regions (Alhanafi *et al*., 2019), and presents in its composition a large amount of non-fibrous carbohydrates (NFC), water, and minerals (Oliveira *et al*., 2017; Siqueira *et al*., 2017). The minerals in the spineless cactus are mainly presented as oxalate (Rahman *et al*., 2013). The form of soluble oxalate is one of the anti-nutritional compounds of forage plants (Rahman *et al*., 2013) that exerts its effect, making calcium (Ca) and magnesium (Mg) unavailable for assimilation.

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In general, most forage plants have enough Ca for animal production, but this mineral may be unavailable through the gastrointestinal tract being excreted in the feces (Rahman *et al*., 2013). The presence of oxalic acid in the forage palm forms insoluble salts with Ca and can influence the intake and digestion in sheep (Ben Salem and Smith, 2008). The Ca (27.7 - 52.6g/kg), potassium (K; 19.0 - 47.8g/kg), and Mg (12.7 - 14.2 g/kg) contents of the spineless cactus are high, but the spineless cactus has low levels of phosphorus (P; 0.8 - 2.1g/kg) and sodium (Na; 0.5g/kg) (Cordova-Torres *et al*., 2015; Mayer and Cushman, 2019), resulting in high K:Na ratio and high Ca:P ratio. In ruminants, when the Ca:P ratio exceeds 7:1, or is less than 1:1, it may cause the appearance of renal calculus affecting the animal performance (Santos *et al*., 2009).

Although there are studies evaluating mineral supplementation for sheep, studies evaluating the effect of the imbalance of minerals in the spineless cactus on the balance of macrominerals and renal function of sheep are scarce. Thus, the present study aimed to evaluate the effect of the inclusion of spineless cactus (*Opuntia stricta* Haw) in the diet of sheep on the balance of macrominerals, renal function, and blood metabolites.

MATERIALS AND METHODS

Procedures used in this work agreed with the Ethical Principles of the Brazilian College of Animal Experimentation (code number 053/2015). Five adult sheep Santa Ines breed, castrated and cannulated in the rumen (61.5±9.5kg body weight (BW)), were used in a 5×5 Latin square composed of five diets and five experimental periods (21 days each). The experimental period lasted 105 days, with five periods of 21 days each. The first 14 days of each period were used for adaptation to the diets and the following seven days for data collection. Each animal was treated against endoparasites and ectoparasites and then kept in individual stalls (0.82 × 1.60m) equipped with feeders and drinking fountains, as previously described by [9].

Five diets were evaluated, being a control diet, consisting of only Buffel hay (*Cenchrus ciliares* L.) as forage (ground to pass through a 4 mm screen), and four other diets presenting the inclusion of spineless cactus (*Opuntia Stricta* Haw) at levels of 121, 245, 371, and 500 g/kg of DM. The spineless cactus was two years old at the time of cutting and came from an irrigated and fertilized area. Before feeding, the cactus was chopped to reach a particle size of approximately 1 cm as previously described by Araújo *et al.*, (2020) in a complementary study.

The diets (Tables 1 and 2) presented a forage:concentrate ratio of 65:35 and were formulated to be isoproteic and to meet the requirements of the castrated male sheep for 100g of average daily gain according to the Nutrient Requirements of Small Ruminants Council (NRC, 2007).

Feed was supplied twice a day, at 8:00 am (60%), and at 4:00 pm (40%), and the diet was offered as a complete mixed ration. The orts were weighed daily, and the amount of offered food was adjusted based on the previous day’s intake (allowing 20% orts).

During each experimental period, 20% of feces, orts, and the ingredients composing the diets were sampled. These samples were homogenized to obtain a composite sample for each animal per period. The material was pre-dried in a forced ventilation oven at 55°C for 72 h and milled with Willey-type knives using a sieve screen with 1 mm mesh for orts and ingredients and 2 mm mesh for feces.

The samples were analyzed following the protocols described in the Official Methods of Analysis (AOAC, 2005) for DM (method 934.01), crude protein (CP, method 990.13), ether extract (EE, method 920.85), and ash (method 942.05). The total oxalate of the ingredients and orts was estimated, following the procedure described by Moir (1953). The measurement of neutral detergent fiber (NDF) was performed using an ANKOM fiber analyzer (ANKOM200 Fiber Analyzer; ANKOM Technology Corporation, Fairport, NY, USA). The NDF was corrected for ash and nitrogen.

Table 1. Chemical composition of the ingredients of the experimental diets

Composition (g/kg DM)	Ingredients					
	Ground corn	Soybean meal	Spineless cactus[2]	Buffel hay	Mineral mix	Water[3]
Dry matter[1]	879	885	131	936.2	-	-
Organic matter	987	938	844	936.7	-	-
Ash	12.5	61.2	156	63.3	-	-
Crude protein	88.0	493	50.1	65.7	-	-
Ethereal extract	52.1	21.4	14.6	13.3	-	-
aNDFn[4]	199	153	230	651	-	-
Total carbohydrates	847	424	779	857	-	-
Non-fibrous carbohydrates	649	272	549	206	-	-
Oxalate	1.92	1.42	9.82	2.84	-	-
Calcium	0.19	1.27	23.9	1.81	136	22.1
Phosphor	3.35	5.53	2.41	0.86	94.7	0.24
Magnesium	0.53	2.41	8.16	0.99	11.8	9.02
Sodium	0.26	0.53	0.75	0.52	70.5	38.3
Potassium	1.87	22.6	27.8	10.2	0.12	7.00
Ox:Ca	9.79	1.12	0.41	1.56	-	-
Ca:P	0.05	0.23	9.93	2.09	-	-
K:Na	7.00	42.9	37.0	19.4	-	-
K:Ca	9.52	17.8	11.3	5.68	-	-
K:Mg	3.54	9.40	3.40	5.68	-	-

[1]g/kg natural matter. [2]*Opuntia stricta* Hall. [3]mg/L. [4]Neutral detergent fiber with thermolabile amylase corrected for ash and nitrogen.

Table 2. The proportion of ingredients and chemical composition of experimental diets

Ingredients (g/kg DM)	Inclusion of spineless cactus (g/kg DM)				
	Control	121	245	371	500
Ground corn	199.9	172.7	173.4	175.2	177.0
Soybean meal	132.0	166.6	168.3	170.0	170.7
Buffel hay	649.8	525.1	397.8	267.9	135.3
Spineless cactus	0.0	121.2	244.9	371.2	500.0
Urea	7.4	3.7	4.7	4.8	5.8
Ammonium sulfate	0.8	0.4	0.5	0.5	0.6
Mineral mix	10.1	10.2	10.3	10.4	10.5
	Chemical composition (g/kg)				
Dry matter	918.4	529.4	369.7	282.7	227.9
Organic matter	937.4	924.9	913.4	901.7	889.7
Ash	61.8	74.6	86.1	97.7	109.6
Crude protein	147.2	149.0	150.6	149.5	150.8
Ethereal extract	21.9	21.3	21.5	21.7	22.0
aNDFn[1]	483.3	429.8	375.8	320.9	264.7
Non-fibrous carbohydrates	285.9	325.2	366.0	410.1	453.0
Total carbohydrates	769.2	755.1	741.8	731.0	717.6
Oxalate	2.42	3.25	4.11	4.98	5.87
Calcium	2.76	5.48	8.22	11.02	13.87
Phosphor	2.91	3.21	3.42	3.64	3.85
Magnesium	1.19	2.12	3.01	3.92	4.84
Sodium	1.18	1.22	1.25	1.29	1.32
Potassium	10.05	12.86	15.03	17.25	19.48
Ca:P	0.94	1.67	2.30	2.84	3.33
K:Na	8.53	10.54	11.98	13.37	14.70
K:Ca	2.55	2.01	1.70	1.52	1.40
K:Mg	8.46	6.06	4.99	4.40	4.02
Ox:Ca	0.61	0.51	0.46	0.44	0.42

[1]Neutral detergent fiber with thermolabile amylase corrected for ash and nitrogen.

Metabolic water production was estimated according to the chemical analysis of the diets and calculated by multiplying carbohydrates, protein, and digestible ether extract intake by the factors 0.55, 0.42, and 1.07, respectively (Araújo *et al*., 2010).

To estimate the balance of macrominerals, the amount of minerals consumed, absorbed, and retained by the animals was counted. The mineral intake of the mineral mixture and drinking water was measured to obtain the intake values of the minerals. The mineral mixture presented the following levels of macrominerals: 136.1g Ca/kg DM, 94.91g P/kg DM, 11.79g Mg/kg DM, 70.50g Na/kg DM, and 0.127g K/kg of DM. The water presented the following levels of macrominerals: 22.10 mg of Ca/L, 0.241 mg of P/L, 9.02mg of Mg/L, 38.30mg of Na/L, and 7.0mg of K/L.

The intake of macrominerals was calculated by the difference between the amount of mineral offered and orts. Absorption was determined using the results of the amount of mineral ingested which was excreted in the feces, and to obtain the amount of mineral retained, the difference between the ingested mineral, and the mineral excreted in the feces and urine was used.

The urine samples were collected on the 5th day of the collection period by spontaneous urination, via "spot", 4 hours after morning feeding. To obtain the urinary volume (UrV) of each animal, creatinine was used as an indicator, BW was multiplied by the daily excretion of creatinine (mg/L), dividing the product by the concentration of creatinine (mg/L) in the urine. The average excretion of daily creatinine of 23.2mg/L was determined according to Kozloski *et al*. (2005) estimates in sheep.

Samples of the ingredients, orts, feces, and urine were subjected to digestion via the wet process, using nitric acid using a microwave accelerated reaction system, Model MARS®, (Technology inside) adapted from Horwitz (1997). Subsequently, a dilution was performed according to (Method 968.08, AOAC) (Official…, 2005).

The minerals Na and K in the samples were estimated using the DM-62 flame photometer. The minerals Ca and Mg were determined using atomic absorption spectrophotometry (AA240FS), and P was determined by colorimetric spectrophotometry.

Blood samples were collected on the 5th day of the experimental period, 4 hours after morning feeding, by jugular venipuncture, using a hypodermic needle (25 × 8 mm) in silicone tubes vacutainer®, without anticoagulants, and with anticoagulant (sodium fluoride with 10% EDTA), to obtain serum and plasma, respectively. Blood samples without anticoagulants were kept at room temperature to retract the blood clot for subsequent centrifugation, while samples with anticoagulants were homogenized, promptly refrigerated in an isothermal environment, and sent to the laboratory.

All tubes were centrifuged for 10 minutes at 3.500 rpm and then conditioned in polyethylene tubes with a capacity of 2mL and stored at -20°C. The urine was made by spontaneous urination of the animals, using a plastic bag used in colostomy (Mark Med®), and was applied to the preputial region by adhesion with adhesive glue. Immediately after urination, urine samples were centrifuged and aliquoted in conical polyethylene tubes with a capacity of 2 mL, followed by storage at –20°C.

The biochemical indicators evaluated in the blood serum were: fructosamine, total cholesterol, triglycerides, creatinine, urea, total protein, albumin, uric acid, Ca, P, Mg, Na, K, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), and alkaline phosphatase (AP). Those determined in blood plasma were: β-Hydroxybutyrate (βHB), unesterified fatty acids (UFA), and glucose. The determinations were determined using a commercial LABTEST® kit, and processing was carried out in an automated biochemical analyzer LABMAX 240®.

The estimative of urinary metabolite and mineral indexes was made adopting equations described by Garry *et al*. (1990); Lunn and McGuirk (1990). The endogenous creatinine clearance rate (ECr; Eq. 1), the urinary excretion rate of "Y" substance (UrEY; Eq. 2), and the fractional excretion rate of "Y" substances (FEY; Eq. 3), such as creatinine, urea, Ca, P, Mg, Na and K. For the determination of the respective indices, the analysis of metabolites and minerals ("Y" substance) were determined both in serum and urine, according to:

$$\mathrm{ECr}\ (\mathrm{mL/min/BW^{0.75}}) = [(\mathrm{CrUr} \times \mathrm{UrV/CrSr})] / \mathrm{BW^{0.75}} \quad \text{(Eq. 1)}$$
$$\mathrm{UrEY}\ (\mathrm{mMol/L}) = (\mathrm{UrY/CrUr}) \times \mathrm{BW^{0.75}} \quad \text{(Eq. 2)}$$
$$\mathrm{FEY}\ (\%) = [(\mathrm{Ury/Sry})/(\mathrm{CrUr/CrSr})] \times 100 \quad \text{(Eq. 3)}$$

Where: UrY = Y substance in urine; SrY = Y substance in the serum; CrUr = Urinary creatinine concentration; CrSr = Serum creatinine concentration; UrV = Urinary volume; $BW^{0.75}$ = Metabolic BW.

In all equations in which BW is expressed in kg, it was decided to change it to metabolic body weight ($BW^{0.75}$). The ECr corresponds clinically to creatinine clearance and is a useful measure to estimate the glomerular filtration rate of the kidneys. The UrEY corresponds to the amount eliminated from a substance in the urine corrected by urinary creatinine and weight (Fleming *et al*, 1991). The FEY refers to the fraction of material filtered by the glomerulus that is eliminated in the urine. This rate of any substance reflects both the effort of the kidneys to maintain homeostasis and defects in the ability to perform this function (Kaneko *et al.*, 2008).

The variables were analyzed using the PROC MIXED procedure in the SAS software program (Statistical Analysis System, version 9.2), adopting 0.05 as the critical level of probability or tendency (P < 0.10) for a type I error and using the mathematical model described below (Equation 04):

$$\gamma_{ijk} = \mu + \tau_i + \alpha_j + \beta_k + \varepsilon_{ijk}$$

Where γ_{ijk} is the dependent variable measured in animal j that was submitted to treatment i in period k; μ is the overall mean; τ_i is the fixed effect of treatment i; αj is the random effect of animal j; β_k is the random effect of period k; ε_{ijk} is the random error.
After analyzing the variance effects, the significance of the linear and quadratic effects of the inclusion of cactus was evaluated.

RESULTS

The inclusion of spineless cactus in sheep diets increased the intake of DM (from 1250.7 to 1748.2 g/d; *P* = 0.025), ash (from 76.41 to 198.50 g/d; *P* < 0.001) and oxalate (from 3.19 to 11.51 g/d; *P* < 0.001; Table 3). The inclusion of spineless cactus in sheep diets also increased the intake of Ca (from 3.66 to 24.32 g/d; *P* < 0.001) and P (from 3.86 to 6.68 g/d; *P* < 0.001), but only Ca showed an increase in fecal (from 2.69 to 10.51 g/d; *P* = 0.006) and urinary excretion (from 0.004 to 0.011 g/d; *P* = 0.001). Inclusion also increased Ca absorption (from 0.97 to 13.81 g/d; *P* < 0.001) and P (from 1.20 to 3.22 g/d; *P* = 0.004), and Ca retention (from 0.97 to 13.79 g/d; *P* < 0.001) and P (From 1.20 to 3.22 g/d; *P* = 0.004; Table 3).

The intake of Mg (from 1.50 to 7.88 g/d; *P* < 0.001), as well as its fecal (from 1.30 to 2.95 g/d; *P* = 0.04) and urinary excretion (from 0.006 to 0.021 g/d; *P* < 0.001) increased with the inclusion of spineless cactus in the diet (Table 3). The inclusion of spineless cactus also increases the absorption (from 0.19 to 4.93 g/d; *P* < 0.001) and retention of Mg (from 0.18 to 4.91 g/d; *P* < 0.001).

The inclusion of spineless cactus in sheep diets increased K intake (from 11.97 to 31.90g/d; *P* < 0.001), as well as urinary K excretion (from 0.24 to 1.34g/d; *P* < 0.001), K absorption (from 11.04 to 31.81g/d; *P* < 0.001) and retention (from 10.81 to 30.47 g/d; *P* < 0.001), but decreased fecal excretion (*P* = 0.017), occurring reduction from 0.926 to 0.094g/d. As for Na, there was an increase in intake (from 1.62 to 2.43 g/d; *P* = 0.007), an increase in urinary excretion (from 0.08 to 0.44 g/d; *P* = 0.032) as well as an increase in its absorption (from 1.30 to 2.22 g/d; *P* = 0.003).

The inclusion of spineless cactus in sheep diets reduced the intake of drinking water (from 2.13 to 0.02kg/d; *P* < 0.001; Table 4) and increased water intake from the diet (from 0.11 to 5.45kg/d; *P* <0.001), as well as the total water intake (from 2.92 to 6.38kg; *P* < 0.001). The inclusion of spineless cactus in the sheep diet increased the excretion of water in feces (from 127 to 242g/d; *P* = 0.03; Table 4), in urine (from 354 to 1296g/d; *P* <0.001) and total (from 481 to 1538g/d; *P* < 0.001). On the other hand, the water balance was not affected ($P = 0.46$) by the inclusion of spineless cactus in the diets.

In assessing the dynamics of urinary excretion rates of metabolites and minerals, there was an increase in urinary volume (from 1133 to 4009mL/day; $P < 0.001$; Table 5) and in the rate of urine formation; from 0.79 to 2.78mL/min; $P<0.001$) with the inclusion of spineless cactus in the diets. On the other hand, the inclusion did not affect the urinary concentrations of Ca ($P = 0.87$), Mg ($P = 0.21$) and Na ($P = 0.46$) but increased the urinary concentration of K (from 2077.4 to 3499.8 mg/L; $P = 0.009$). Regarding the urinary concentration of P, there is a tendency for reduction with the inclusion of spineless cactus in the sheep diet (from 0.47 to 0.23mmol/L; $P = 0.051$).

The inclusion of spineless cactus in sheep diets did not affect the urinary excretion rate of creatinine, urea, and macrominerals ($P > 0.05$; Table 5), as well as ECr ($P = 0.65$). The inclusion of spineless cactus in the diets increased the fractional excretion rate of urea ($P = 0.02$), but it did not affect the fractional excretion rate of creatinine and macrominerals.

Table 3. Intake of dry matter, ash, and oxalate, and balance of sheep macrominerals fed with spineless cactus

Variable	Inclusion of spineless cactus (g/kg DM[1])					SEM[2]	*P*-value	
	Control	121	245	371	500		L[3]	Q[4]
				Intake, (g/dia)				
DM[1]	1250.7*	1487.3	1759.8	1671.8	1748.2	0.067	0.025	0.225
Ash	76.41	116.47	155.88	168.41	198.50	0.010	<.001	0.368
Oxalate	3.19*	5.56	8.67	9.27	11.51	0.307	<.001	0.338
				Calcium (Ca)				
Intake (g/day)	3.66*	9.77	15.47	22.36	24.32	0.512	<.001	0.210
Feces (g/day)	2.69*	6.26	10.03	10.13	10.51	0.470	0.006	0.215
Urine (g/day)	0.004*	0.005	0.006	0.007	0.011	0.0006	0.001	0.280
Absorption (g/day)	0.97*	3.51	5.44	12.24	13.81	0.794	0.001	0.997
withheld (g/day)	0.97*	3.51	5.43	12.23	13.79	1.202	0.001	0.997
				Phosphor (P)				
Intake (g/day)	3.86	5.30	6.07	6.45	6.68	0.187	<.001	0.084
Feces (g/day)	2.41	3.11	3.43	3.05	3.46	0.832	0.258	0.558
Urine (g/day)	0.002	0.003	0.003	0.002	0.003	0.0003	0.520	0.785
Absorption (g/day)	1.20	2.19	2.64	3.09	3.22	0.870	0.004	0.349
withheld (g/day)	1.20	2.19	2.64	2.89	3.22	0.869	0.004	0.349
				Magnesium (Mg)				
Intake (g/day)	1.50*	3.51	5.47	7.42	7.88	0.198	<.001	0.132
Feces (g/day)	1.30*	2.16	2.94	3.10	2.95	0.177	0.044	0.320
Urine (g/day)	0.006*	0.011	0.012	0.015	0.021	0.0009	<.001	0.767
Absorption (g/day)	0.19*	1.35	2.52	4.32	4.93	0.168	0.001	0.693
withheld (g/day)	0.18*	1.34	2.51	4.31	4.91	0.410	0.001	0.693
				Potassium (K)				
Intake (g/day)	11.97*	18.81	26.32	28.49	31.90	0.708	<.001	0.068
Feces (g/day)	0.926*	0.193	0.201	0.117	0.094	0.023	0.017	0.094
Urine (g/day)	0.24*	0.52	0.64	1.24	1.34	0.060	<.001	0.882
Absorption (g/day)	11.04*	18.62	26.12	30.06	31.81	0.942	<.001	0.053
withheld (g/day)	10.81*	18.09	25.48	28.82	30.47	1.720	<.001	0.054
				Sodium (Na)				
Intake (g/day)	1.62*	2.08	2.29	2.42	2.43	0.076	0.007	0.183
Feces (g/day)	0.32	0.28	0.28	0.28	0.21	0.022	0.136	0.673
Urine (g/day)	0.08	0.13	0.42	0.26	0.44	0.044	0.032	0.626
Absorption (g/day)	1.30*	1.80	2.01	2.15	2.22	0.071	0.003	0.202
withheld (g/day)	1.22	1.67	1.59	1.88	1.79	0.110	0.081	0.391

[1]Dry matter. [2]Standard error means. [3]Linear effect. [4]Quadratic effect. P<0.05.

Table 4. Intake, excretion, and water balance of sheep fed with spineless cactus

Variable	Inclusion of spineless cactus (g/kg DM[1])					SEM[2]	*P*-value	
	Control	121	245	371	500		L[3]	Q[4]
Intake water								
Beverage (kg/day)	2.13*	1.24	0.22	0.04	0.02	0.040	<.001	0.0001
Diet (kg/day)	0.11*	1.45	3.14	4.24	5.45	0.110	<.001	0.425
Metabolic (kg/day)	633.5*	738.4	866.9	814.2	839.9	28.97	0.039	0.188
Total intake (kg)	2.92*	3.49	4.30	5.16	6.38	0.160	<.001	0.270
Water excretion								
Feces (kg/day)	126.7*	144.6	204.5	211.3	241.9	14.70	0.028	0.823
Urine (kg/day)	354.4*	714.4	816.1	863.7	1296.1	56.00	0.001	0.874
Total excretion (kg/day)	481.1*	858.9	1020.6	1075.0	1538.1	48.83	<.001	0.918
Hydric balance (HB)								
HB (kg/day)	0.48	0.29	0.39	0.62	0.56	0.080	0.465	0.657

[1]Dry matter. [2]Standard error means. [3]Linear effect. [4]Quadratic effect. P<0.05.

Table 5. Urinary volume, mineral composition, and renal function of sheep fed with spineless cactus

Variable	Inclusion of spineless cactus (g/kg DM[1])					SEM[2]	*P*-value	
	Control	121	245	371	500		L[3]	Q[4]
Urinary volume (UrV) / Urine Formation Rate (UFR)								
UrV (mL/d)	1133	2263	2552	2657	4009	175.6	0.001	0.883
UFR (mL/min)	0.79	1.58	1.77	1.84	2.78	0.120	0.001	0.880
Urinary mineral concentration (mmol/L)								
Calcium	0.72	0.56	0.54	0.66	0.69	0.040	0.870	0.221
Phosphor	0.47	0.40	0.32	0.28	0.23	0.032	0.051	0.791
Magnesium	2.11	2.01	1.98	2.15	2.13	0.017	0.213	0.111
Potassium	53.27	60.98	64.44	104.94	89.74	714.9	0.009	0.794
Sodium	34.21	24.42	68.10	35.76	43.33	124.0	0.465	0.377
Urinary Excretion Index (mmol/L)								
Creatinine	22.61	21.80	22.41	21.09	21.43	0.352	0.242	0.891
Urea	1.22	1.46	1.89	1.69	1.55	0.085	0.196	0.093
Calcium	0.004	0.002	0.002	0.004	0.005	0.0004	0.401	0.208
Phosphor	0.0013	0.0015	0.0012	0.0016	0.0016	0.0001	0.593	0.861
Magnesium	0.018	0.008	0.009	0.014	0.015	0.002	0.993	0.298
Potassium	5.28	4.73	4.25	5.75	6.83	0.585	0.444	0.422
Sodium	54.34	80.45	90.48	78.56	71.23	6.304	0.515	0.130
Endogenous Creatinine Clearance Rate (ECr)								
ECr (mL/min/$BW^{0.75}$)	2.24	2.37	2.41	2.28	2.40	0.082	0.651	0.757
Fractional Excretion Rate (FEY) (%)								
Urea	45.91	46.25	62.91	62.79	65.53	3.555	0.023	0.612
Calcium	0.55	0.28	0.27	0.78	0.64	0.077	0.401	0.455
Phosphor	0.30	0.35	0.25	0.29	0.32	0.028	0.947	0.701
Magnesium	6.89	2.78	2.61	5.13	5.41	1.837	0.954	0.281
Potassium	4.80	3.98	2.92	4.95	5.75	0.482	0.546	0.284
Sodium	0.014	0.002	0.003	0.002	0.004	0.001	0.297	0.286

[1]Dry matter. [2]Standard error means. [3]Linear effect. [4]Quadratic effect. P<0.05.

The inclusion of spineless cactus in sheep diets did not influence plasma concentrations of glucose, UFA and serum cholesterol, triglycerides, and fructosamine ($P > 0.05$; Table 6). However, the insertion of the spineless cactus influenced the plasma concentration of blood βHB ($P = 0.04$), with quadratic behavior and having a maximum point of 0.44 mmol/L for the inclusion of 275g/kg of spineless cactus. The spineless cactus in sheep diets did not alter the serum protein profile, with no variation in serum concentrations of total protein, albumin, creatinine, urea, and uric acid ($P > 0.05$). The same occurred with the mineral profile, in which there was no variation in the serum concentrations of Ca, P, Na, and K, ($P > 0.05$), however, the serum Mg concentration showed an increasing trend (from 0.77 to 0.85mmol/L; *P* = 0.06). The inclusion of spineless cactus in sheep diets did not vary in the enzymatic activities of AST, GGT, and AP (*P* > 0.05; Table 6).

Table 6. Energy, protein, mineral, and enzymatic profiles of the blood of sheep fed with spineless cactus

Variable	Inclusion of spineless cactus (g/kg DM[1])					SEM[2]	*P*-value	
	Control	121	245	371	500		L[3]	Q[4]
Energy Profile (mmol/L)								
βHB[5]	0.38	0.42	0.45	0.43	0.41	0.017	0.276	0.041
UFA[6]	0.48	0.74	0.36	1.23	0.42	0.114	0.722	0.440
Glucose	2.84	2.72	2.82	2.96	2.75	0.072	0.928	0.779
Cholesterol	1.20	1.19	1.24	1.16	1.24	0.053	0.865	0.864
Triglycerides	0.17	0.15	0.18	0.15	0.17	0.008	0.652	0.851
Frutosamine	196.1	190.1	187.2	195.8	194.9	3.921	0.885	0.456
Protein Profile								
Taotal protein (g/L)	66.35	62.99	62.53	68.08	64.34	2.155	0.921	0.716
Albumin (g/L)	299.2	293.5	274.1	300.3	295.1	8.671	0.984	0.473
Creatinine (µmol/L)	67.63	61.17	61.31	63.26	60.78	2.257	0.401	0.549
Urea (mmol/L)	8.10	7.87	7.89	7.99	6.97	0.252	0.114	0.290
Uric acid (µmol/L)	68.41	111.24	110.64	191.54	71.38	0.273	0.718	0.106
Mineral profile								
Calcium (mmol/L)	2.18	2.20	2.14	2.04	2.13	0.065	0.577	0.823
Phosphor (mmol/L)	1.35	1.23	1.39	2.09	1.36	0.114	0.808	0.803
Magnesium (mmol/L)	0.77	0.79	0.85	0.93	0.85	0.034	0.060	0.268
Potassium (mEq/L)	3.22	3.27	3.52	3.53	3.37	0.064	0.258	0.250
Sodium (mEq/L)	146.0	143.4	139.3	144.5	138.2	2.756	0.440	0.940
Enzymatic activity (U/L)								
AST[7]	64.68	61.96	65.45	62.75	63.23	2.850	0.843	0.934
GGT[8]	43.89	48.18	44.80	48.03	46.02	1.856	0.682	0.608
AP[9]	139.1	145.2	170.8	154.2	151.3	12.80	0.543	0.346

[1]Dry matter; [2]Standard error means; [3]Linear effect; [4]Quadratic effect; P<0.05. [5]Beta hydroxybutyrate. [6]Unesterified fatty acids. [7]Aspartate aminotransferase. [8]Gamma-glutamyltransferase. [9]Alkaline phosphatase.

DISCUSSION

The inclusion of spineless cactus in the diet increased the macrominerals intake and affected the balance of macrominerals in sheep. Renal function was not impaired by the inclusion of cactus and blood metabolites were practically not influenced by the inclusion of spineless cactus in the diet.

The macrominerals intake possibly increased as a response to the DM intake increase. Findings of this study were also reported by Araujo *et.al* (2020**)** in a complementary study evaluating the intake, digestibility, and ruminal digesta of sheep. The intake of DM from diets rich in spineless cactus increased possibly due to its high palatability and rumen degradability, which favors the increase in the rate of ingestion by animals (Costa *et al*., 2016; Cardoso *et al*., 2019). The increase in DM intake, together with the high levels of ash and oxalates in the spineless cactus in the present study contributed to an increase in the intake of these components when included in the diets.

Spineless cactus is rich in a range of minerals, mainly Ca, Mg, and K (Mayer and Cushman, 2019), which makes it a potential food for the formation of kidney stones. The presence of oxalate can also form crystals of Ca oxalate, by capturing minerals at the renal level and thus triggering urolithiasis and renal failure (Barboza *et al*., 2019). Renal function can be measured in several ways, from the evaluation of urinary compounds to the analysis of blood metabolites. Therefore, the balance of minerals, renal function, and blood metabolites are influenced by the level of intake of food and water, which is necessary for their measurement (Maciel *et al*., 2016). In the present study, the inclusion of spineless cactus in the diets increased the flow (intake, excretion, absorption, and retention) of most of these macrominerals. Although the spineless cactus has low levels of P and Na, it is still higher than the levels found in the Buffel Hay used, consequently providing an increase in mineral intake with the inclusion of cactus.

The greatest excretion of Ca occurs in the feces, with very low urine losses due to the process of reabsorption in the kidneys in which parathyroid hormone increases the mechanisms of renal reabsorption of this mineral, reducing its urinary loss (Goff, 2018). The Ca present in diets with a higher level of spineless cactus is probably chelated with oxalate, which increased its fecal excretion, providing less Ca for absorption. The lower the availability of Ca in the intestinal lumen, the greater the efficiency in its absorption, by increasing the production of the active form of vitamin D in the kidneys, which helps in the intestinal absorption of Ca and P (Riet-Correa *et al*., 2008). For all treatments the intake of Ca was above the minimum requirements for sheep, varying from 3.66 to 24.32g/d, which according to the NRC, 2007 is 2.2g / day, for an intake of DM 1.10kg/d. The higher intake of Ca resulted from the different chemical compositions of the diets (from 2.76 to 13.87g of Ca/kg of DM), however, in all diets, the Ca supply was below the level considered toxic for sheep, which is 15g/kg of DM (NRC, 2007).

The presence of Mg in the composition of the spineless cactus increased its intake and absorption. Although spineless cactus has high levels of K and there is an antagonism between Mg and K (Martín-Tereso and Martens, 2014), the absorption of Mg was not compromised, possibly because of the levels of Mg in the diet also increased with the inclusion of the spineless cactus. When calculating the K and Mg intake ratio in the experimental diets, the values found in the diet containing the largest amount of spineless cactus (4.04) are close to the ideal ratio for sheep recommended by the NRC, 2007, which is 4.88 g/d. The increase in the absorption rate of K, possibly caused it to increase its urinary excretion so that there was a mineral balance.

There is a negative correlation between Na intake and K excretion, in which the lower the Na intake, the greater the K excretion. Thus, the increase in K intake with ingestion of spineless cactus, possibly promoted the release of the hormone aldosterone, increasing the activity of the Na, K- ATPase pump, stimulating the renal reabsorption of Na, and increasing the excretion of K by urine. Despite the increase in the renal reabsorption of Na, its urinary excretion increased, and this can be justified by the higher water intake of sheep fed on spineless cactus. This intake leads to excess body water, inhibiting the center of thirst and the pituitary gland, reducing the synthesis of antidiuretic hormone (ADH), allowing the kidneys to excrete excess water in the urine, and by excreting water, the kidney will also excrete some solutes, such as Na (Reece, 2006).

Due to the high moisture content of the spineless cactus, the drinking water intake was reduced (from 2.13 to 0.02kg/d), as the animals started to meet their requirements through the intake of water from the food. The reduction of involuntary intake of drinking water by small ruminants was also found by other authors (Abidi *et al*., 2009; Andrade-Montemayor *et al*., 2011), with a decrease in the intake of up to 2.11kg/d of water when the animals were fed with cactus. The increase in the production of metabolic water, with the inclusion of spineless cactus in the diets, can be explained by obtaining the water from the nutrient metabolism itself. Cactus, in general, has a low content of lipids and high amounts of carbohydrates, which when fermented in the rumen increasing the production of short-chain fatty acids (SCFA).

The increase in fecal and urinary excretion of water reflects the increase in the total intake of this nutrient with the inclusion of spineless cactus in the diets, also the presence of oxalate in the composition of the cactus can cause a laxative effect in the animal (Nefzaoui and Ben Salem, 2001). The Mg levels can also be related to the laxative effect of spineless cactus, since the Mg^{2+} ion is partially motivating, water retention in the intestinal lumen to maintain osmotic balance, causing the formation of soft stools and increasing the passage rate, increasing the production of soft stools, and increasing the fecal excretion of water. While the increase in urinary volume with the inclusion of spineless cactus is a way to maintain the water balance in the animal's body, since high water intake, promoted by increased intake of spineless cactus, inhibits the release of the hormone ADH, which is responsible for the reabsorption of water in the renal tubules, so the reduction in its secretion results in increased excretion of water via urine.

When the results of the balance of macrominerals were evaluated, we observed that with the increase in the intake of these nutrients, the excretion, retention, and absorption increased for most of them, however evaluating exclusively the urine concentration, there are situations in which the excretion was reduced. Urine showed a decrease in P concentration with the inclusion of spineless cactus in the diets, which may be related to hypophosphatemia, which promotes increased renal reabsorption of this mineral. The increase in the concentration of K in the urine occurred possibly due to the diuretic effect caused by the intake of spineless cactus, as well as due to the increased absorption of this mineral, the excess being excreted in the urine.

The rate of urinary excretion of the minerals Ca, P and Mg with the inclusion of spineless cactus in the diets did not vary, possibly because the main route of excretion of these minerals is the fecal route, with their excretion via urine being very low (Costa e Silva *et al*., 2015). The increase in the fractional excretion rate of urea in diets with spineless cactus is due to the greater volume of water in the urine, which increases the peritubular pressure, and increased rates of urinary flow, generally, are accompanied by an increase in the amount of urea excreted in the urine. This increase agrees with a similar result reported by other researchers working with fresh spineless cactus (Pordeus Neto *et al*., 2016).

The animals in this study presented an increase in the plasma concentration of UFA. Ruminants have a small supply of hepatic glycogen, with fat deposits being the main form of energy stored in the body. The mobilization of fats is done by the action of lipases. In the energy surplus, fats are stored, with the opposite occurring in cases of a deficit. Thus, high insulin secretion blocks the action of lipases, but glucagon, growth hormone, adrenaline, negative energy balance, stress, and fasting strongly stimulate lipases, which mobilize triglycerides and transform them into glycerol and free fatty acids, also called UFA. The latter are carried to hepatocytes to be oxidized or esterified (Xue *et al*., 2020). The UFA has a direct relationship with an energy deficit but is very susceptible to an increase in common stressful situations, due to the increase in catecholamines, in moments of collection of biological material for exams (González, 2000; Mikulková *et al*., 2020), and this condition was the probable reason why the animals in this study had a slight elevation of this metabolite in the energy profile in all animals, regardless of the level of spineless cactus in the diet.

Even with UFA formation indication, the level of oxidation and esterification in hepatocytes did not promote liver dysfunction in the animals in this work, since the activities of the enzymes evaluated in this work were within the normal range for the species, according to Kaneko *et al*. (2008). In situations where high oxidation of UFAs occurs in the animal organism, there is a decrease in the availability of glucose in the blood (Qaid and Abdelrahman, 2016), a fact that potentiates the discussion of the little influence of diet on the formation of UFAs, at normal levels, since there was also no significant variation in the plasma glucose concentration in these animals. Although the insertion of spineless cactus influenced the plasma concentration of βHB with quadratic behavior and with a maximum point of 0.44mmol/L for the inclusion of 275g/kg of spineless cactus, the respective concentration at all levels remained within normality, which did not influence energy metabolism. Another aspect to be considered is that high concentrations of UFAs and βHB depress appetite (Bisinotto *et al*., 2012) and, consequently, intake, something that was also not observed in the animals of this study, even receiving different levels of spineless cactus in the diet.

The inclusion of spineless cactus in the diet did not influence the serum cholesterol concentration, although its concentration is very close to the lower limit for the species. This profile may be related to the low lipid content in diets. The spineless cactus has a low lipid content, which can cause a decrease in the cholesterol of the animals when fed with high levels of the cactus. In the study reported by Araújo *et al*. (2012), the authors also found low values (1.21mmol/L) of cholesterol in sheep fed with spineless cactus. However, the intake of spineless cactus increases the production of SCFA, which represents the main source of energy for the animal, decreasing the use of lipids ingested in the diet as an energy source.

The concentration of urea in the blood is important to assess protein metabolic activity in animals (Puppel and Kuczyńska, 2016), which is directly related to the level of protein in the diet, as well as the relationship between energy and dietary protein and ruminal ammonia absorption. Higher values are indicative of excess protein in the diet or energy deficit (González, 2000; Kaneko *et al*., 2008). The diets were formulated to have a forage:concentrate ratio 65:35 based on DM and were formulated to be isoproteic and to meet the requirements of castrated male sheep, to gain 100g, according to the NRC, 2007, so that the observed variation was very little above the upper reference limit for this species (Kaneko *et al*., 2008). The protein and energy inputs of the diets were adequate for the animals and did not reflect negatively on the protein profile evaluated by the dynamics of the concentration of this metabolite in the animals. It should be noted that the concentration of serum albumin did not change significantly, and that this metabolite also reflects the protein content in diets, although the changes occur more slowly.

Serum urea elevation was not accompanied by the dynamics of serum creatinine concentrations, which remained low, which were probably due to physiological or dietary changes. Considering that its excretion is only carried out via the kidney since it is not reabsorbed or reused by the animal organism, the decreased values of this variable can be considered as an indication of an increase in the rate of renal filtration, because of the effective participation of ingestion of water as well as the presence of forage palm, which provides water ingestion via food (Araújo *et al*., 2012). Other researchers also observed a reduction in the serum creatinine concentration of sheep in response to the increase in the percentage of spineless cactus in the diet (Araújo *et al*., 2012; Soares *et al*., 2020). In the present study, the experimental diets were based on spineless cactus, and as is known, this food has high water content and a low dry matter content, which causes a diuretic effect, also related to its high content of K.

The influence of the concentration of spineless cactus in the diet promoted a decrease in the serum Ca concentration and this profile has been followed in other studies (Santos *et al*., 2009; Araújo *et al*., 2012; Soares *et al*., 2020). The serum concentration of Ca is related to the intake of Ca in the diet, as well as by the action of components capable of making this mineral unavailable, through interaction mechanisms (González, 2000). The decrease may have reflected the consequent insolubility suffered by the action of oxalates present in the spineless cactus. The blood level of Ca is quite constant, but it is influenced by the endocrine system involving vitamin D, parathyroid hormone, and calcitonin, which act to adjust to the amount of Ca absorbed from the diet (González, 2000). The spineless cactus has a high ash content, especially the Ca content. The levels of P and Na, however, are low, which results in an extremely high Ca:P ratio [8]. According to Ben Salem *et al*. (2005), the high percentage of oxalate present in the spineless cactus can cause a reduction in the bioavailability of Ca and explain the laxative effect that the spineless cactus promotes in animals, with a consequent decrease in serum Ca concentration and an increase in AP, although its activity enzyme was not influenced by the diet and its values were found to be normal.

When there is an increase in the formation of calcium oxalate chelates, decreasing the rate of Ca in the metabolism, it will affect the osmotic balance of the animal organism, consequently, stimulating bone resorption to maintain the serum levels of this mineral. Considering that the spineless cactus has high levels of Ca and low P, nutritionally the binding of oxalate to Ca to a certain extent may be a positive effect on the animal.

The spineless cactus has a high ash content, especially the Ca content. The levels of P and Na, however, are low, which results in an extremely high Ca:P ratio (Santos *et al*., 2009). The high percentage of oxalate present in the spineless cactus reduces the availability of Ca and explain the laxative effect of the cactus, and, consequently, the decrease in serum Ca levels and increased AP, as previously seen (Ben Salem *et al*., 2002). The enzymatic activities represented by AST, GGT, and AP remained within the limit considered normal for the species (Costa *et al*., 2016), noting that none of the levels of spineless cactus in the diet signaled impairment of liver and bone metabolism.

The findings indicate that, although the cactus evaluated herein presents an imbalance in the content of macrominerals, the sheep organism can bypass this situation through homeostatic mechanisms, up to the inclusion of 500 g/kg DM of spineless cactus. Therefore, the inclusion of higher levels of spineless cactus can overcome the body's homeostasis capacity, requiring precaution with this use. It is interesting to also evaluate in future studies the insertion of chlorine in the analyzes, considering that this mineral is essential for determining the body's electrolyte balance.

CONCLUSION

The spineless cactus evaluated (*Opuntia stricta* Haw) can be added to the diets of sheep up to 500g/kg DM. Its inclusion does not compromise kidney function or blood metabolites evaluated herein, thus demonstrating the ability of sheep to adapt to diets with different proportions of macrominerals.

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