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Metabolic profile and renal function of lambs fed with maniçoba hay replacement by spineless cactus. Perfil metabólico e função renal de ovinos alimentados com palma forrageira em substituição ao feno de maniçoba

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Abstract

The objective of this study was to evaluate the effect of the replacement of maniçoba hay with spineless cactus on the metabolic response and renal function of lambs. Blood and urine samples were collected 55 days after introduction of the tested diets. No blood parameters were affected and the urinary concentration of urea, creatinine, and potassium was influenced by diet with decreasing linear behavior. The urinary glucose excretion index and the fractional urinary excretion of glucose increased linearly. The spineless cactus does not negatively impact in the metabolic profile and renal function of confined lambs, suggesting that it may replace up to 100% of the maniçoba hay.

Keywords: Adapted plants, blood parameters, forage cactus, kidneys, metabolism

Resumo

O objetivo deste trabalho foi avaliar o efeito da substituição do feno de maniçoba por palma forrageira na resposta metabólica e função renal de cordeiros. Amostras de sangue e urina foram coletadas 55 dias após a introdução das dietas testadas. Nenhum parâmetro sanguíneo foi afetado e as concentrações urinárias de ureia, creatinina e potássio foram influenciadas pelas dietas com comportamento linear decrescente. O índice de excreção urinária de glicose e a excreção urinária fracionada de glicose aumentaram linearmente. A palma forrageira não impacta negativamente o perfil metabólico e a função renal de cordeiros confinados, sugerindo que pode substituir até 100% do feno de maniçoba.

Palavras-chave: Cacto forrageiro, metabolismo, parâmetros sanguíneos, plantas adaptadas, rins

Introduction

The exploration of sheep for meat production in semi-arid regions has grown in recent years, with the adoption of simple technology such as the introduction of exotic and native plants like spineless cactus (*Napolea cochenillifera* Salm Dyck) and maniçoba (*Manihot pseudoglaziovii* Muell Arg.), which result in visible improvements in production systems. Considering the adaptation of the plants of the genus *Manihot* to semi-arid zones and their nutritional attributes, these fodder resources are important strategies in the production of ruminants (LIMA JÚNIOR et al., 2014; MACIEL et al., 2019a). The maniçoba is a plant native to the Caatinga biome, toxic when *in natura*, but it can be used as food for ruminants when conserved in the form of hay or silage (FRANÇA et al., 2010; SILVA et al., 2015).

The spineless cactus (*Napolea cochenillifera* Salm Dyck) is an important forage for livestock in Brazilian semi-arid regions. It has adaptive agronomic characteristics, such as being tolerant to hydric stress (SILVA et al., 2018), and being suitable as forage in diets for sheep (CARDOSO et al., 2019; MACIEL et al., 2019b). Spineless cactus, regardless of genus (*Opuntia* or *Nopalea*), has high levels of non-fibrous carbohydrates and water, constituting an important food resource for arid and semi-arid zones of the world (BATISTA et al., 2003; COSTA et al., 2009). The supply of spineless cactus with maniçoba hay could be a important alternative for strategic supplementation of lambs destined for meat production, in periods of less availability of fodder, thus increasing the efficiency of the herds in the northeastern semi-arid region.

The determination of blood metabolites to monitor energy, protein and mineral status in ruminants has been a valuable tool to establish the nutritional status of animals, reliably reflecting the flow (ingress and egress) and metabolization of nutrients in the animals tissues (GONZÁLEZ, 2000), as well as to evaluate the functioning of organs such as the liver and kidneys (PEIXOTO; OSÓRIO, 2007). Few scientific investigations on the renal function of sheep submitted to diets with spineless cactus were found in the literature (VIEIRA et al., 2008b; PORDEUS NETO et al., 2016), confirming the need for further studies on the subject matter. According to HENRIQUES et al. (2016), the study of renal function is relevant, mainly in the ovine species, due to the high incidence of diseases in the urinary system.

There is, therefore, a need to know the metabolic alterations, through the metabolic profile, that can occur in the body of the animal resulting from the use of alternative foods. Thus, the aim of this study was to evaluate the replacement of maniçoba hay (*Manihot pseudoglaziovii* Muell Arg.) with spineless cactus (*Nopalea cochenillifera* Salm Dyck) on the metabolic response and kidney function of lambs.

Materials and Methods

The study was approved by the Ethics Committee of the Federal Rural University of Pernambuco (License n° 078/2015) and was conducted at the Animal Science Department, located in the city of Recife, Pernambuco State, Brazil.

Thirty-two lambs without defined racial pattern, uncastrated males, were used, with a mean initial weight of 20.8 ± 2.9 kg and mean age of eight months. All animals were housed in individual stalls equipped with individual feeders and drinking fountains. In the pre-experimental period, each animal was vaccinated against clostridia and treated against endo and ectoparasites.

The animals were distributed in a randomised blocks design, using the initial weight as the criterion for formation of blocks, with two treatments and eight replications. The experimental period lasted 75 days, with 20 days of adaptation to the management system and experimental diet and 55 days of data and biological material collection. The chemical composition of the dietary ingredients is presented on Table 1.

Table1. Chemical composition of ingredients of the experimental diets (g/kg dry matter).

Ingredients				Chemic	al compo	sition									
ingredients	DM ^a	OM	MM	CP	EE	NDF	ADF	CT	NFC						
Ground corn	886	984	16	89	30	179	18	864	685						
Spineless cactus	92	881	119	36	15	265	100	830	565						
Soybean meal	880	926	74	493	36	164	49	397	234						
Maniçoba hay	901	938	62	100	22	567	351	816	249						
Mineral salt	1000	-	1000	-	-	-	-	-	-						
Limestone	1000	-	1000	-	-	-	-	-	-						
Urea	1000	-	-	2620		-	-	-	-						

^a g/kg natural matter. DM - Dry matter; OM - Organic matter; MM - Mineral matter; CP - Crude protein; EE - Ether extract; NDF - Neutral detergent fiber; ADF - Acid detergent fiber; CT - Total carbohydrates; NFC - Non-fibrous carbohydrates.

The experimental treatments represented different levels of maniçoba hay replacement by spineless cactus (0, 333, 667, and 1000 g/kg) (Table 2). The experimental diets were formulated to meet the requirements of a mean daily weight gain of 200 g, according to the NRC (2007), being offered in a complete mixture with 60% roughage and 40% concentrate, offered twice a day (8:00 a.m. and 3:00 p.m.), with 50% of the total provided at each meal. The intake of the dry matter and nutrients was calculated by the difference between the quantities of feed offered and the leftovers. The adjustment of intake was based on the intake of the previous day, always allowing leftovers of 20%.

Table 2. Proportion of ingredients and chemical composition of diets, as a function of the levels of Maniçoba hay replacement by spineless cactus.

Ingredients	Levels of replacement (g/kg)						
	0	333	667	1000			
Ground corn	290	270	215	185			
Soybean meal	85	105	160	190			
Spineless cactus	0	200	400	600			
Maniçoba hay	600	400	200	0			
Mineral salt	5	5	5	5			
Limestone	10	10	10	10			
Urea	10	10	10	10			
Diet composition (g/kg dry matter)							
Dry matter ^a	897	327	200	144			
Organic matter	927	914	899	886			
Mineral matter	63	76	90	104			
Crude protein	154	149	159	158			
Ether extract	25	24	22	21			
Neutral detergent fiber	406	345	284	223			
Acid detergent fiber	219	170	122	72			
Total carbohydrates	774	767	744	733			
Non-fibrous carbohydrates	368	422	460	510			

a g/kg natural matter.

Water was permanently available to the animals. The voluntary water intake of the animals was measured over 26 days, during the experimental period. The daily ingestion of water in g/day was measured by subtracting the evaporation losses from the weight of the water supplied. Ingestions were quantified as follows: voluntary water (VW), water contained in dietary food (WDF), and total water (TW).

The feed offered and leftovers per animal were sampled to collect the relevant data. After collection, the samples were placed in a forced circulation oven at 55 °C for 72 hours. They were ground in a Willey-type knife mill using a 1 mm mesh sieve screen for further laboratory analysis. The determination in the feeds and leftovers of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), and ash was performed according to (AOAC, 2000), and neutral detergent fiber (NDF) by the methodology described by VAN SOEST et al. (1991) adapted by DETMANN et al. (2012). For estimation of total carbohydrates (TC), the following equation was used, proposed by SNIFFEN et al. (1992), TC (%) = 100 - (%CP + %EE + % Ash). Non-fibrous carbohydrates (NFC) were calculated according to HALL (2000): NFC (%) = 100 - [(%CP - %CP derived from urea + %urea) + %NDFap + %EE + %ash].

Blood samples were collected 55 days after introduction of the tested diets, four hours after the morning feeding through jugular venipuncture in siliconized tubes; with anticoagulant (10% diethylene diamine tetra acetic acid - EDTA) to obtain plasma and without anticoagulant to obtain serum. All these tubes were centrifuged for 15 minutes at 500G. Serum and plasma aliquots were then conditioned in Eppendorf tubes and stored at a temperature of -20° C.

The serum biochemical indicators determined by kinetic methodology were: urea, aspartate transaminase (AST), gamma glutamyl transferase (GGT), and alkaline phosphatase (AP), and fructosamine. The following variables were measured through the colorimetric method: creatinine, total protein (PT), albumin (ALB), calcium (Ca), phosphorus (P), and chlorine (Cl). The globulin concentration (GBL) was determined by the difference between serum concentrations of PT and ALB. Plasma glucose was determined by the enzymatic method. In addition, sodium (Na), potassium (K), and ionized calcium (Ca⁺⁺) were analyzed. Blood biochemical determinations were performed in a LABMAX 240 automated biochemical analyzer using commercial kits (Labtest Diagnóstica[®] S.A.). Na and K were determined by flame photometry.

The urine samples were obtained through spontaneous urination of the animals, using a plastic colostomy pouch attached to the preputial region with adhesive glue. A fraction of the urine was centrifuged for 5 minutes at 500G, and aliquoted in to 2 mL (Eppendorf type) conical tubes, followed by storage at -20°C. The biochemical indicators determined in the urine were: creatinine, urea, glucose, total protein (PT), calcium (Ca), phosphorus (P), sodium (Na) potassium (K), and chlorine (Cl). The excretion index of these metabolites was obtained through the formulas described by GARRY et al. (1990) and LUNN et al. (1990).

The data were submitted to regression analysis using the General Linear Model (GLM) procedure of the statistical program SAS (version 9.4, SAS Institute Inc., Cary, NC, USA), adopting 5% as significance level.

Results

There was an influence of the replacement levels of maniçoba hay for spineless cactus in CP, EE and NDF intake, all variables decreased linearly (Table 3). The CP intake was observed to be high, ranging from 161.9 to 202.8 g/day.

The replacement of maniçoba hay by spineless cactus influenced (P<0.01) the WDF and VW intake, which showed a linear and quadratic behavior, with better adjustment to the linear increasing and decreasing model, respectively (Table 3). The WDF intake increased from 135.0 to 6126.0 g/day with hay replacement by spineless cactus in diets. The opposite happened with the DW, which decreased from 2698.0 to 365.5 g/day. The TW intake presented (P<0.01) increasing linear behavior.

Table 3. Dry matter, nutrients and water intake, as a function of the levels of Maniçoba hay replaced by spineless cactus in the diet of growing lambs.

	Lev	vels of replac	P value				
Parameters	0	333	667	1000	SEM*	Linear	Quad.
			Nutrient inta	ke (g/day)			
Dry matter	1134.8	1183.4	1146.0	1022.8	0.03	NS	NS
Crude protein	202.8	186.1	188.9	161.9	0.01	0.0379^{a}	NS
Ether extract	32.9	30.4	28.6	23.3	0.00	0.0005^{b}	NS
Neutral detergent fiber	396.7	363.6	296.5	235.5	0.01	<0.0001°	NS
			Water intak	ce (g/day)			
From feed	135.0	3152.0	5130.9	6126.0	0.43	<0.0001 ^d	< 0.000
Voluntary	2696.6	928.7	582.7	365.5	0.17	<0.0001e	< 0.000
Total	2831.6	4080.7	5713.6	6491.6	0.29	< 0.0001 ^f	NS

^{*} Standard error of the mean. ${}^aY = 202.84 - 0.358 \times (R^2 = 0.83); {}^bY = 33.37 - 0.0914 \times (R^2 = 0.94); {}^cY = 405.58 - 1.65 \times (R^2 = 0.98); {}^dY = 9.967 + 0.0948 \times -0.0009 \times (R^2 = 0.88); {}^eY = 649.31 + 59.733 \times (R^2 = 0.95); {}^fY = 2240.8 - 21.9 \times (R^2 = 0.80).$ *NS* not significant (P>0.05).

No effects were observed in any blood variables (P>0.05) from the level of substitution of maniçoba hay by spineless cactus (Table 4). The urinary concentration of urea, creatinine and K, was influenced by diet (P<0.05), with linear decreasing behavior (Table 4).

Table 4. Blood and urinary parameters, as a function of the levels of substitution of Maniçoba hay by spineless cactus in the diet of growing lambs.

Danamatana	Levels of replacement (g/kg)						P value	
Parameters	0	333	667	1000	SEM*	Linear	Quad	
			Blood					
Urea (mmol/L)	10.33	8.99	10.32	6.75	0.84	NS	NS	
Creatinine (µmol/L)	26.46	31.20	31.53	31.14	1.21	NS	NS	
Total protein (g/L)	62.90	66.03	68.47	64.30	1.20	NS	NS	
Albumins (g/L)	34.28	37.83	53.78	35.38	4.55	NS	NS	
Globulins (g/L)	28.62	28.21	32.69	28.96	1.04	NS	NS	
Plasma Glucose (mmol/L)	4.75	5.34	5.38	5.28	0.15	NS	NS	
Fructosamine (mmol/L)	215.76	212.74	235.67	202.96	6.86	NS	NS	
Aspartate transaminase (U/L)	90.37	89.70	87.17	83.24	1.61	NS	NS	
Gamma glutamyl transferase (U/L)	49.99	50.83	49.98	54.01	0.96	NS	NS	
Alkaline phosphatase (U/L)	875.50	894.63	873.76	1011.3	32.84	NS	NS	
Total calcium (mmol/L)	2.85	2.67	2.65	2.70	0.05	NS	NS	
Ionized calcium (mmol/L)	1.75	1.41	1.42	1.46	0.08	NS	NS	
Phosphorus (mmol/L)	2.39	2.52	2.61	2.39	0.05	NS	NS	
Total calcium:phosphorus	1.22	1.10	1.12	1.21	0.03	NS	NS	
Sodium (mmol/L)	144.88	136.63	138.25	136.25	2.01	NS	NS	
Potassium (mmol/L)	4.22	4.20	3.99	4.06	0.06	NS	NS	
Chlorine (mmol/L)	116.54	112.08	112.07	110.52	1.30	NS	NS	
			Urine					
Volume ^a (mL)	146.88	185.00	133.75	175.00	11.94	NS	NS	
Urea (mmol/L)	443.66	395.74	371.51	236.83	44.31	0.0222^{b}	NS	
Creatinine (mmol/L)	5.22	3.35	4.36	2.33	0.63	0.0043^{c}	NS	
Total protein (g/L)	52.15	33.46	43.61	24.29	6.05	NS	NS	
Glucose (mmol/L)	0.46	0.40	0.42	0.41	0.01	NS	NS	
Total calcium (mmol/L)	0.12	0.10	0.10	0.10	0.01	NS	NS	
Phosphorus (mmol/L)	0.16	0.10	0.11	0.06	0.02	NS	NS	
Sodium (mmol/L)	5.00	3.69	3.31	3.31	0.40	NS	NS	
Potassium (mmol/L)	70.88	54.63	54.38	39.25	6.46	$< 0.0001^{d}$	NS	
Chlorine (mmol/L)	40.03	51.60	77.79	61.45	7.99	NS	NS	

^{*} Standard error of the mean. ^a Sample spot; ^b Y = 458.33 - 1.9278x ($R^2 = 0.88$); ^c Y = 4.9564 - 0.0228x ($R^2 = 0.62$); ^d Y = 68.999 - 0.2843x ($R^2 = 0.90$). SEM standard error of the mean. NS not significant (P > 0.05).

The urinary excretion index of glucose (UEI Glucose), fractional urinary excretion (FUE), UEI Ca, UEI Cl, as well as FUE Cl (P<0.05), increased linearly (Table 5). The other parameters did not change due to the replacement.

Table 5. Urinary excretion rates of metabolites and minerals, endogenous creatinine clearance rate (ECrCR), and electrolyte free water resorption rate (T^eH_2O), as a function of the levels of Maniçoba hay replaced by spineless cactus in the diet of growing lambs.

J 1 J 1		P value						
Parameters	0	333	667	1000	SEM*	Linear	Quad.	
	Index							
UEI Urea (mmol/L)	1.159.80	1.647.40	1.439.80	1.471.30	10.86	NS	NS	
UEI Total protein (mmol/L)	130.62	136.60	138.43	134.00	1.69	NS	NS	
UEI Glucose (mmol/L)	1.38	1.69	1.53	2.30	0.20	0.0118^{a}	NS	
UEI Calcium (mmol/L)	0.35	0.42	0.40	0.58	0.05	0.0120^{b}	NS	
UEI Phosphorus (mmol/L)	0.48	0.30	0.39	0.35	0.04	NS	NS	
UEI Sodium (mmol/L)	15.54	18.88	12.50	17.54	1.39	NS	NS	
UEI Potassium (mmol/L)	196.20	242.59	151.95	224.28	19.73	NS	NS	
UEI Chlorine (mmol/L)	111.32	226.22	258.30	345.93	48.50	0.0009^{c}	NS	
	Fraction	nal Urinary Ex	cretion of Me	etabolites and	Minerals			
FUE Urea (%)	28.86	45.43	31.79	46.69	4.59	NS	NS	
FUE Total protein (%)	0.42	0.48	0.46	0.49	0.02	NS	NS	
FUE Glucose (%)	0.06	0.07	0.07	0.11	0.01	0.0501^{d}	NS	
FUE Calcium (%)	0.03	0.04	0.04	0.06	0.01	NS	NS	
FUE Phosphorus (%)	0.05	0.03	0.04	0.03	0.01	NS	NS	
FUE Sodium (%)	0.02	0.03	0.02	0.03	0.00	NS	NS	
FUE Potassium (%)	9.21	13.28	13.88	13.88	1.13	NS	NS	
FUE Chlorine (%)	0.12	0.46	0.54	0.77	0.14	0.0029^{e}	NS	
	Endogenous Creatinine Clearance Rate							
ECrCR (mL/min./kg)	10.96	8.29	6.54	5.48	1.20	0.0149 ^f	NS	
	Electrolyte Free Water Resorption Rate							
TeH ₂ O (mL/h)	70.00	81.82	58.66	53.90	6.24	NS	NS	

* Standard error of the mean. ${}^aY = 1.337 + 0.0078x$ ($R^2 = 0.69$); ${}^bY = 0.3375 + 0.002x$ ($R^2 = 0.75$); ${}^cY = 125.4 + 2.2008x$ ($R^2 = 0.96$); ${}^dY = 0.0551 + 0.0004x$ ($R^2 = 0.76$); ${}^eY = 0.169 + 0.0061x$ ($R^2 = 0.95$); ${}^fY = 10.54 - 0.0545x$ ($R^2 = 0.96$). SEM standard error of the mean. NS not significant (P>0.05)

The endogenous creatinine clearance rate, which is the first stage of urine formation, decreased linearly with replacement levels. The electrolyte free water reabsorption rate did not demonstrate effects of diet (P>0.05).

Discussion

The high CP intake is associated with DM intake, which was high in all diets. Considering the age (8 months) of the animals used and the requirement proposed by the NRC (2007) of 0.9 kg DM and 107.0 g/day of CP; the intakes obtained from DM and CP were higher than the requirements recommended by this committee for a gain of 200 g/day. The lower EE content of the spineless cactus was reflected in the EE intake. The reduction in NDF intake is a reflection of the higher NDF content in maniçoba hay. The NDF levels in the diet of this study did not limit intake, since the proportion of NDF in the DM actually consumed varied from 23.6 to 39.7% with the replacement of maniçoba hay by the spineless cactus, and influence of NDF on intake occurs at levels above 50%. When the concentration of NDF in the diet is below 50 to 60%, intake is limited by the energy demand in adult animals (physiological regulations).

The increase in the WDF intake with the replacement of the hay by the spineless cactus and the decrease in the DW occurred as a function of the DM of the experimental diets, which resulted in lower

DW by the animals that consumed diets containing spineless cactus. The VW intake decreased as a function of the high moisture content of the spineless cactus (90.8%) compared to the maniçoba hay (9.9%); the water from the diet supplied a good proportion of the water needs of the animals, reducing the VW intake (Table 3). The reduction in VW intake was 86.4% (2331.1 g/day). Other studies have also shown reductions in voluntary water intake in sheep with the inclusion of spineless cactus in the diet (BISPO et al. 2007; COSTA et al. 2012; CARDOSO et al. 2019).

Regarding the metabolite creatinine, although the diet had no effect on concentration in blood relative to replacement levels, the means (26.46 to 31.53 μ mol/L) were well below the reference values recommended by KANEKO et al. (2008): 106.08 to 167.96 μ mmol/L for sheep. ARAÚJO et al. (2012) also found values (64.25 to 73.75 μ mmol/L) below reference values in a study on the effect of replacing Tifton hay by castor hull in diets based on spineless cactus in sheep. The reduction in serum creatinine concentration in the spineless cactus-containing diets may be a consequence of increased water intake via the spineless cactus, causing an increase in body fluid volume and consequently causing hemodilution of creatinine, however does not explain the decrease in the concentration of this variable, including in the treatment with 0% spineless cactus.

The concentration of fructosamine (202.96 to 235.67 mmol/L) did not change in relation to substitution levels, but remained above the reference values according to KANEKO et al. (2008) (172 \pm 2.9 mmol/L). According to FILIPOVIC et al. (2011), fructosamines are continuously formed glycated serum proteins, resulting from the non-enzymatic, irreversibly insulin-independent binding between circulating glucose and proteins. This glycation is dependent on the serum concentration of glucose and proteins, especially albumin, during the previous two weeks. The result of this variable in the present study may be related to the hyperglycemia found in the groups, since normal values were observed for albumin (KANEKO et al., 2008).

The blood concentration of AP ranged from 873.76 to 1011.30 U/L, well above the normal values according to KANEKO et al. (2008) (68 to 387 U/L). Similar results were found by DANTAS et al. (2011) and by ARAÚJO et al. (2012). All the aforementioned authors evaluated diets based on spineless cactus. The spineless cactus is known to be rich in oxalate (REKIK et al., 2010), which reduces the availability of Ca and, consequently, stimulates bone resorption in an attempt to maintain serum levels of this mineral (GONZÁLEZ; SCHEFFER, 2003). Alkaline phosphatase concentrations may increase when bone cell activity increases or as a result of bone diseases (GONZÁLEZ; SILVA, 2008). The mean values of other blood variables (Table 4) remained within the values recommended in the literature (KANEKO et al., 2008).

The reduction in urine urea concentration as the level of spineless cactus in the diet increased is likely due to the decrease in the ruminal ammonia production (BISPO et al., 2007; VIEIRA et al., 2008a). This result corroborates with VIEIRA et al. (2008b), while studying the effects of feeding high levels of cactus cladodes on urinary output and urinary electrolyte excretion in goats, which also observed decreased urea concentration in the urine. The linear decrease in the creatinine and K urinary concentration may be a consequence of the higher water intake by the animals (Table 3). In addition, VIEIRA et al. (2008b) when collecting total urine, observed an increasing linear in the urinary volume of goats as a function of the inclusion of spineless cactus in the diet (370 up 770 g/kg of dry matter).

The UEI and FUE Glucose increased linearly, possibly presenting a relation to the water ingested through the diet, which increased as the spineless cactus replaced the maniçoba hay (Table 3). The UEI Ca, UEI Cl as well as FUE Cl increased linearly, probably due to the greater intake of these elements provided by the diets containing spineless cactus, causing the animals to excrete the

metabolites through urine. The other variables were not affected by levels of spineless cactus (Table 5).

The ECrCR decreased linearly most probably due to linear increase the WDF intake and linear decrease in DW intake. According to RIVAS et al. (1997), there is a tendency to increase ECrCR in response to body volume expansion, but it should be emphasized that voluntary water intake was reduced with the increase of spineless cactus in the diet, and animals did not need to ingest large quantities of water at one time.

Conclusions

Spineless cactus may replace maniçoba hay by up to 100% in the diet of confined lambs, without affecting in the energy, protein, and mineral metabolisms, besides enzymatic activity and renal function. Additionally, contributing significantly to the water needs of lambs, and the values found for alkaline phosphatase can be used as a reference in studies using spineless cactus and maniçoba hay in sheep feed.

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