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Blood biochemical parameters of lambs fed diets containing cactus cladodes

[*Parâmetros bioquímicos sanguíneos de cordeiros alimentados com dietas contendo palma forrageira*]

T.G.P. *Silva*[1], L.A. *Lopes*[1], F.F.R. de *Carvalho*[1], P.C. *Soares*[2], A. *Guim*[1],
V.A. *Silva Júnior*[2], Â.M.V. *Batista*[1]

[1]Departamento de Zootecnia, Universidade Federal Rural Pernambuco, Recife, PE, Brasil
[2]Departamento de Medicina Veterinária, Universidade Federal Rural Pernambuco, Recife, PE, Brasil

ABSTRACT

This study aimed to evaluate the effects of partial replacement (750g/kg) of Tifton hay by two cactus cladodes (*Nopalea* or *Opuntia*) on the metabolic profile of lambs. Thirty-six uncastrated male Santa Inês lambs (22.0 ± 2.9kg initial body weight) were distributed in a completely randomized design, with three treatments and 12 repetitions. The animals were fed a control diet (Tifton hay as exclusive roughage), Miúda cactus cladodes-based diet or Orelha de Elefante Mexicana (O.E.M.) cactus cladodes-based diet. Blood samples were collected one day before (baseline) and 45 days after the introduction of the tested diets. The Miúda cactus cladodes caused an increase (P=0.055) in the serum activity of the gamma-glutamyl transferase enzyme (53.66U/L) and in the blood content of glucose and fructosamine. The O.E.M. cactus cladodes caused lower (P=0.038) serum cholesterol content (41.33mg/dL). Regardless of the variety, there was a decrease (P=0.001) in the serum content of indirect bilirubin, urea, and sodium, and increase in the serum magnesium concentration. The partial replacement of the Tifton hay by Miúda or O.E.M. cactus cladodes in lamb feeding increases the enzyme activity, indicating liver and/or kidney changes, but does not cause relevant damage to energy, protein, and mineral metabolism.

Keywords: cactaceae, drylands, metabolic profile, nutrition, small ruminants

RESUMO

Este trabalho teve como objetivo avaliar os efeitos da substituição parcial (750g/kg) do feno de Tifton por duas variedades de palma forrageira (Nopalea ou Opuntia) no perfil metabólico de cordeiros. Trinta e seis cordeiros Santa Inês, machos, não castrados (22,0 ± 2,9kg de peso corporal inicial), foram distribuídos em delineamento inteiramente ao acaso, com três tratamentos e 12 repetições. Os animais foram alimentados com dieta controle (feno de Tifton como volumoso exclusivo), dieta à base de palma forrageira Miúda ou dieta à base de palma forrageira Orelha de Elefante Mexicana (OEM). Amostras de sangue foram coletadas um dia antes (baseline) e 45 dias após a introdução das dietas testadas. A palma Miúda causou aumento (P=0,055) na atividade sérica da enzima gamaglutamiltransferase (53,66U/L) e no teor sanguíneo de glicose e frutosamina. A palma OEM causou menor teor (P=0,038) de colesterol sérico (41,33mg/dL). Independentemente da variedade, houve diminuição (P=0,001) do teor sérico de bilirrubina indireta, ureia e sódio, e aumento na concentração sérica de magnésio. A substituição parcial do feno de Tifton por palma Miúda ou por OEM na alimentação de cordeiros aumenta a atividade enzimática, o que indica alterações hepáticas e/ou renais, mas não causa danos relevantes nos metabolismos energético, proteico e mineral.

Palavras-chave: cactaceae, pequenos ruminantes, perfil metabólico, nutrição, terras secas

INTRODUCTION

Cactus is an important fodder option for arid and semiarid zones of the world. According to Ben Salem *et al.* (2002), Magalhães *et al.* (2019) and Dubeux Jr. *et al.* (2021), cactus cladodes represent source of water, energy, and mineral elements for livestock, with a high palatability, even under limiting environmental conditions for other types of forage crops.

Corresponding author: tomasguilherme91@gmail.com
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Diets containing cactus cladodes, compared to diets with grass hay as the only roughage, have greater ratio between non-fibrous carbohydrates:neutral detergent fiber (Silva *et al.*, 2021a), due to the high levels of starch (Batista *et al*., 2003) and pectin (Pessoa *et al*., 2020) and low fiber content of this forage plant, representing an important energy source for animal feed (Rocha Filho *et al*., 2021).

Lambs fed cactus cladodes reduce voluntary water intake, as there is increase in water intake via diet (Pordeus Neto *et al*., 2016; Silva *et al.*, 2021a). Additionally, cacti have oxalates in their composition (Rekik *et al.*, 2010; Silva *et al.*, 2021a), substances that can bind with minerals contained in food and change the metabolism of these elements in the animal organism (Rahman *et al.*, 2013).

The study of biochemical markers in the blood is essential for understanding the possible effects that dietary components can cause in one or more organ systems and, consequently, in animal health and production. Several scientific investigations with sheep and goats fed diets containing cactus cladodes have been showing changes in blood indicators of energy, protein, mineral and enzyme metabolism (Dantas *et al.*, 2011; Cardoso *et al.*, 2019; Gouveia *et al.*, 2019; Maciel *et al.*, 2019; Silva *et al.*, 2021b).

The Miúda (*Nopalea cochenillifera* Salm Dyck) and Orelha de Elefante Mexicana (O.E.M.) cactus cladodes (*Opuntia stricta* [Haw]. Haw.) configure varieties resistant to carmine cochineal (*Dactylopius opuntiae*) (Vasconcelos *et al.*, 2009; Lopes *et al.*, 2010). On the other hand, O.E.M. has little time of use in animal feeding and there is little research to investigate the effects of this variety on the blood parameters of sheep, thus signaling the importance of evaluations in this sense.

Tifton hay (*Cynodon* spp.) is a grass that has been successful when associated with cactus cladodes (*Opuntia* and *Nopalea*) in the sheep growth performance (Lopes *et al.*, 2020; Cardoso *et al.*, 2021). However, Cardoso *et al.* (2019), when evaluating the serum biochemistry of lambs fed diets with increasing levels (0, 150, 300 and 450g/kg, dry matter (DM) basis) of Miúda spineless cactus, associated with Tifton hay, reported decreased levels of glucose, urea, calcium, phosphorus, and magnesium. Silva (2017) observed that lamb fed diets based on different varieties of cactus cladodes showed an increase in the serum activity of the enzyme alkaline phosphatase.

Thus, it was hypothesized that diets containing cactus cladodes cause damage to the metabolic profile of sheep. Therefore, this study aimed to evaluate the effects of partial replacement of Tifton hay by two cactus cladodes varieties on indicators of energy, protein, mineral metabolism, and enzymatic activity of Santa Inês lambs in feedlot.

MATERIAL AND METHODS

The management and care of animals were performed in accordance with the guidelines and recommendations of the Committee of Ethics in the Use of Animals (CEUA) at the Federal Rural University of Pernambuco (UFRPE), under license number 142/2018.

The experiment was carried at the Department of Animal Science, UFRPE, located in Recife, Pernambuco State, Brazil. This research is a part of a larger project with a methodology based on a previous study by Lopes *et al.* (2020). Thus, further information regarding collection, processing and chemical analysis of feed, leftovers and feces were reported by these authors.

Thirty-six uncastrated male Santa Inês lambs, with an initial body weight of 22.0±2.9kg and approximately six months old were distributed in a completely randomized design, with twelve lambs per treatment. The animals housed in individual stalls (1.0m x 1.8m), provided with a drinking fountain and feeder. The experimental period lasted 75 days, with the first 30 days for the adaptation of the animals to the diets and the management and the remaining 45 days for data and samples collection. In the pre-experimental period, all animals were identified, vaccinated against clostridia (Covexin 9®, São Paulo, SP, Brazil) and treated against ectoparasites and endoparasites with doramectin 1% (Dectomax®, Guarulhos, SP, Brazil).

The diets were formulated based on NRC (Nutrient…, 2007) recommendations for gains of approximately 200g/day. The ingredients used were Tifton hay (*Cynodon* spp.), Miúda cactus cladodes (*Nopalea cochenillifera* Salm Dyck), O.E.M. cactus cladodes (*Opuntia stricta* [Haw]. Haw.), ground corn, soybean meal, mineral mix, and urea (Table 1).

Table 1. Chemical composition of ingredients of experimental diets (g/kg dry matter)

	Ingredients						
Item	Tifton hay	Miúda	O.E.M.[a]	Ground corn	Soybean meal	Urea	Mineral mix
Dry matter[b]	895.5	123.6	97.2	877.1	882.7	990.0	990.0
Ash	83.9	129.4	149.0	12.3	70.3	-	-
Crude protein	86.0	40.0	55.0	85.0	487.0	2800.0	-
Ether extract	22.6	13.8	17.8	38.3	15.0	-	-
apNDF[c]	669.4	252.7	198.0	146.7	134.5	-	-
NFC[d]	138.0	563.9	580.0	717.6	293.0	-	-
Oxalates	3.80	2.91	5.77	5.59	1.88	-	-

[a] orelha de elefante mexicana cactus cladodes, [b] g/kg natural matter, [c] neutral detergent fiber assayed with a heat stable amylase and corrected for ash and nitrogenous compounds, [d] non-fibrous carbohydrates, (-) not determined.

The experimental treatments were a diet with Tifton hay as exclusive roughage (control), diet with partial replacement of Tifton hay by Miúda cactus cladodes, and diet with partial replacement of Tifton hay by O.E.M. cactus cladodes (Table 2).

Table 2. Ingredients proportion and chemical composition of the experimental diets

Ingredients (g/kg)	Diets		
	Control	Miúda	O.E.M[a]
Tifton hay	600	150	150
Miúda cactus cladodes	0	450	0
O.E.M.[a] cactus cladodes	0	0	450
Ground corn	270	271	273
Soybean meal	110	100	100
Urea	5.0	14.0	12.0
Mineral mix[†]	15.0	15.0	15.0
Diet composition (g/kg dry matter, unless stated)			
Dry matter[b]	890.8	234.8	190.3
Ash	76.0	95.8	104.7
Crude protein	142.1	141.8	143.2
Ether extract	25.6	21.5	23.4
apNDF[c]	456.1	267.4	243.1
Non-fibrous carbohydrates	300.2	473.4	485.8
Total digestible nutrients	648.2	709.8	632.7
Oxalates	4.0	3.6	4.9

[a] orelha de elefante mexicana cactus cladodes, [b] g/kg natural matter, [c] neutral detergent fiber assayed with a heat stable amylase and corrected for ash and nitrogenous compounds, [†] Nutrients/kg of product: Ca = 110g; Ca (max.) = 135g; P = 87g; S = 18g; Na = 147g; Mg = 20g; Co = 15mg; Cu = 590mg; Cr = 20mg; I = 50mg; Mn = 2000mg; Mo = 300mg; Se = 20mg; Zn = 3800mg; F = 870mg (max.); Fe = 1800mg.

The diets were provided *ad libitum* as a total mixed ration twice a day, at 08:00 am and 03:00 pm, and the animals had *ad libitum* access to water. The cactus plants were manually harvested when they were 2 years old. The processing of cactus cladodes was carried out immediately before each feeding in a forage machine (MC1n Laboremus®, Campina Grande, Brazil), and the mixture of ingredients was conducted manually in the feeders. The Tifton hay was acquired in local commerce and ground in a forage machine with an 8-mm sieve screen. The determination of total oxalates in feed and leftovers samples was performed according to methodology described by Moir (1953).

Blood samples were collected twice, the first collection being made one day before the introduction of the experimental diets (baseline) and the second 45 days after feeding with the tested diets. They were collected by venipuncture of the jugular, four hours after the morning feeding, in vacuum siliconized tubes (Vacutainer®) without and with anticoagulant (sodium fluoride/EDTA), to obtain serum and plasma, respectively. The samples were immediately placed in a container containing recyclable ice. Then, they were centrifuged at 1,600 x g for 15 minutes. The serum and plasma aliquots were then stored in 2mL polyethylene tubes previously identified and frozen in a freezer at -20ºC for further analysis.

The biomarkers analyzed in the blood were alanine aminotransferase enzyme activity (ALT), aspartate aminotransferase enzyme activity (AST), alkaline phosphatase enzyme activity (AP), gamma-glutamyl transferase enzyme activity (GGT), total and direct bilirubin, plasma glucose, cholesterol, triglycerides, fructosamine, urea, creatinine, uric acid, total proteins, albumin, total calcium (Ca_t), phosphorus (P), magnesium (Mg), sodium (Na) and potassium (K). The indirect bilirubin values were estimated by subtracting the total and direct bilirubin values. Globulin was determined by the difference between the values of total serum protein and albumin. The albumin:globulin ratio and the Ca_t:P ratio were calculated by dividing the values of these biochemical indicators. Ionized calcium (Ca_i) was estimated by calculation involving the values of serum Ca_t (mg/dL), total protein (g/dL) and albumin (g/dL), following the recommendation suggested by the manufacturer - Labtest® (Labtest Diagnóstica S.A., Brazil).

The experimental design was completely randomized, considering initial body weight (BW) as covariate. The data were analyzed using the GLM procedure in the software package Statistical Analysis System 9. 0 (Statistical…, 2009), according to the model below:

$$Y_{ij} = \mu + T_i + \beta(X_{ij} - X) + e_{ij}$$

Where Y_{ij} = the observed dependent variable; μ = general mean; T_i = effect of treatment; $\beta(X_{ij} - X)$ = covariate effect (initial BW); and e_{ij} = experimental error. The data were submitted to ANOVA and the Tukey's test, at 5% probability, was used to compare the averages between treatments. To compare the means of blood biochemical indicators between baseline collection and after 45 days, the paired Student's t-test was applied, using the Minitab software version 17 (2014).

RESULTS

Dry matter (g/day, %BW and g/kg $BW^{0.75}$), crude protein, total digestible nutrients, and oxalates intakes (g/day) are shown in Table 3.

Table 3. Nutrients and oxalates intake, and weight gain by sheep fed cactus cladodes-based diets

Item	Diets				
	Control	Miúda	O.E.M.[a]	SEM[b]	P-value
Dry matter (g/day)[*]	1129.00b	1290.70a	1172.20ab	30.0	0.020
Dry matter (% BW[c])[*]	3.92b	4.35a	3.94b	0.08	0.002
Dry matter (g/kg $BW^{0.75}$)[d]	90.77b	101.36a	91.98b	2.32	0.005
Crude protein (g/day)[*]	170.00a	192.00a	168.00b	4.00	0.010
TDN[5] (g/day)[*]	728.00b	916.00a	740.00b	23.00	0.001
Oxalates (g/day)[#]	4.98b	4.68b	6.21a	0.27	0.001
Average weight gain (g/day)[*]	225	252	245	0.01	0.605

[a] orelha de elefante mexicana cactus cladodes, [b] standard error of the mean, [c] body weight, [d] metabolic body weight; [e] total digestible nutrients, [*] values obtained by Lopes *et al*. (2020). [#] values obtained by Silva *et al.* (2022). Averages in rows followed by different letters are statistically different by the Tukey’s test at 5% probability.

The serum activity of the ALT enzyme did not differ between diets (Table 4), whose variation was from 20.58 to 24.71U/L, as well as there was no significant difference between initial and final collection (Table 5).

Table 4. Blood biochemistry of sheep fed cactus cladodes-based diets

Parameters	Diets				
	Control	Miúda	O.E.M.[a]	SEM[b]	P-value
		Enzymes			
ALT (U/L)[c]	24.71	20.58	20.74	1.73	0.190
AST (U/L)[d]	126.13	116.52	114.41	6.11	0.383
AP (U/L)[e]	509.06	561.39	492.04	49.54	0.576
GGT (U/L)[f]	43.30b	53.66a	51.00ab	2.96	0.055
		Metabolites			
Total bilirubin (mg/dL)	0.60a	0.36b	0.48ab	0.05	0.005
Direct bilirubin (mg/dL)	0.15	0.07	0.14	0.03	0.199
Indirect bilirubin (mg/dL)	0.45a	0.29b	0.34b	0.03	0.001
Plasma glucose (mg/dL)	67.05b	79.48a	68.77b	1.78	0.001
Cholesterol (mg/dL)	50.02a	44.78ab	41.33b	2.23	0.038
Triglycerides (mg/dL)	20.74	21.13	19.67	2.23	0.888
Fructosamine (µmol/L)	244.97b	259.95a	244.45b	3.38	0.003
Urea (mmol/L)	12.10a	8.58b	9.04b	0.55	0.001
Creatinine (μmol/L)	60.17	57.33	56.24	3.06	0.662
Uric acid (μmol/L)	4.69	5.77	5.06	0.58	0.413
Total protein (g/L)	68.18	67.78	68.13	1.47	0.977
Albumin (g/L)	25.10	25.12	24.50	0.56	0.679
Globulin (g/L)	43.08	42.66	43.63	1.17	0.837
Albumin:Globulin ratio	0.58	0.59	0.56	0.01	0.539
		Minerals			
Total calcium (mg/dL)	10.40	10.53	10.08	0.19	0.253
Ionized calcium (mg/dL)	6.24	6.32	6.07	0.10	0.238
Phosphorus (mg/dL)	8.54	7.75	7.78	0.43	0.359
Total calcium:Phosphorus ratio	1.22	1.36	1.29	0.08	0.649
Magnesium (mg/dL)	2.30c	3.21b	3.67a	0.11	0.001
Sodium (mEq/L)	189.08a	150.95b	155.70b	6.87	0.001
Potassium (mmol/L)	17.54	15.48	17.11	0.88	0.224

[a] orelha de elefante mexicana cactus cladodes, [b] standard error of the mean, [c] alanine aminotransferase, [d] aspartate aminotransferase, [e] alkaline phosphatase, [f] gamma-glutamyl transferase. Averages in rows followed by different letters are statistically different by the Tukey's test at 5% probability.

Regarding the AST values, these did not change between collections (Table 5), as well as differences were not noticed depending on the diets, with an average value of 119.02 U/L (Table 4).

Table 5. Enzymatic profile and serum concentration of bilirubin's of sheep before and after supply of cactus cladodes-based diets

	Diets		
	Control	Miúda	O.E.M.[a]
	Alanine aminotransferase (U/L)		
Baseline	20.60±0.86	21.43±1.65	20.27±1.32
45th day	24.71±1.02	20.58±1.81	20.74±1.72
P-value	0.174	0.366	0.765
	Aspartate aminotransferase (U/L)		
Baseline	123.13±5.28	130.00±10.2	112.78±6.98
45th day	126.13±3.52	116.52±6.1	114.41±5.35
P-value	0.479	0.210	0.851
	Alkaline phosphatase (U/L)		
Baseline	107.20±8.5b	91.00±8.6b	103.40±14.5b
45th day	509.06±28.1a	561.39±45.2a	492.04±63.2a
P-value	0.001	0.001	0.001
	Gamma-glutamyl transferase (U/L)		
Baseline	46.11±1.98	46.23±2.75b	41.66±3.47b
45th day	43.30±1.82	53.66±3.16a	51.00±3.36a
P-value	0.137	0.022	0.020
	Total bilirubin (mg/dL)		
Baseline	0.99±0.25	0.55±0.05a	1.05±0.16a
45th day	0.60±0.08	0.36±0.02b	0.48±0.03b
P-value	0.057	0.001	0.003
	Direct bilirubin (mg/dL)		
Baseline	0.30±0.03a	0.24±0.03a	0.35±0.05a
45th day	0.15±0.02b	0.07±0.01b	0.14±0.03b
P-value	0.001	0.001	0.001
	Indirect bilirubin (mg/dL)		
Baseline	0.69±0.08a	0.31±0.03	0.70±0.12a
45th day	0.45±0.02b	0.29±0.02	0.34±0.02b
P-value	0.009	0.203	0.021

[a] orelha de elefante mexicana cactus cladodes. Averages followed by different letters in the same column differ statistically by Student's t-test at 5% probability.

AP activity was similar between animals that consumed cactus cladodes and those that were submitted to the control diet. However, when evaluating the activity of this enzyme before and after the supply of experimental diets, it was noticed that there was an increase in values in animals of all groups (Table 5). The diet containing Miúda cactus cladodes led to an increase in serum GGT activity (53.66U/L) (Table 4). Additionally, when comparing the values between collections, it was found that the varieties of cactus cladodes increased the average activity of this enzyme (Table 5).

Serum levels of direct bilirubin were not influenced by diet (Table 4). For total bilirubins, there was a 40% decrease in the concentration of this biochemical marker in the treatment with Miúda cactus cladodes (0.36mg/dL) compared to the control diet (0.60mg/dL) (Table 4). When evaluating the two collections, it is noted that the cactus cladodes varieties reduced these metabolic components (Table 5). Cactus cladodes also caused a decrease in the levels of indirect bilirubin (Table 4), as well as a decrease between the baseline collection and the test collection in the control diets and with O.E.M. cactus cladodes (Table 5).

There was no significant variation between the serum concentration of triglycerides, creatinine, uric acid, total proteins, albumin, globulin, and albumin:globulin ratio due to the diets (Table 4). Plasma glucose levels were higher in animals that received Miúda cactus cladodes in the diet (79.48mg/dL) (Table 4), as well as an increase in circulating glucose after the supply of all tested diets (Table 6).

Regarding the influence of the cactus cladodes on the serum cholesterol content, the O.E.M. cactus cladodes caused a lower value of this biochemical indicator (41.33mg/dL) compared to the content found in the animals of the control group (Table 4). The serum concentration of triglycerides was higher 45 days after the introduction of the tested diets, without oscillating significantly as a function of the diets (Table 6), while the Miúda cactus cladodes increased the serum fructosamine value (Table 4 and 6).

Table 6. Indicators of energy and protein metabolism of sheep before and after supply of cactus cladodes-based diets

	Diets		
	Control	Miúda	O.E.M.[a]
		Plasma glucose (mg/dL)	
Baseline	50.33±1.78b	49.82±2.51b	46.57±1.36b
45th day	67.05±2.00a	79.78±1.48a	68.77±1.97a
P-value	0.001	0.001	0.001
		Cholesterol (mg/dL)	
Baseline	49.36±3.69a	63.65±4.31a	53.06±3.74b
45th day	50.02±3.35a	44.78±1.67b	41.33±1.52a
P-value	0.886	0.002	0.011
		Triglycerides (mg/dL)	
Baseline	11.06±0.46b	10.92±0.65b	10.56±0.94b
45th day	20.74±1.25a	21.13±2.43a	19.67±1.60a
P-value	0.001	0.003	0.001
		Fructosamine (µmol/L)	
Baseline	242.96±4.26	234.20±7.87b	247.59±6.23
45th day	244.97±2.29	259.95±3.06a	244.45±3.61
P-value	0.133	0.006	0.661
		Urea (mmol/L)	
Baseline	7.12±0.49b	7.01±0.28b	7.58±0.19
45th day	12.10±0.54a	8.58±0.39a	9.04±0.60
P-value	0.001	0.020	0.197
		Creatinine (µmol/L)	
Baseline	70.13±2.32a	67.70±3.84a	72.97±3.90a
45th day	60.17±1.73b	57.33±2.77b	56.24±3.81b
P-value	0.001	0.005	0.002
		Uric acid (µmol/L)	
Baseline	3.31±0.47b	2.81±0.86b	4.44±0.69
45th day	4.69±0.33a	5.77±0.60a	5.06±0.62
P-value	0.001	0.003	0.383
		Total protein (g/L)	
Baseline	69.38±1.65	68.34±1.23	71.25±1.04
45th day	68.18±1.18	67.78±0.94	68.13±1.77
P-value	0.480	0.607	0.157
		Albumin (g/L)	
Baseline	22.57±0.76b	22.27±0.69b	23.13±0.81
45th day	25.10±0.51a	25.12±0.43a	24.50±0.65
P-value	0.005	0.001	0.195
		Globulin (g/L)	
Baseline	46.27±1.67	46.07±1.35a	48.12±0.80a
45th day	43.08±1.03	42.66±0.76b	43.63±1.36b
P-value	0.088	0.011	0.007
		Albumin:Globulin ratio	
Baseline	0.49±0.01b	0.48±0.02b	0.48±0.02b
45th day	0.58±0.01a	0.59±0.01a	0.56±0.02a
P-value	0.001	0.001	0.005

[a] orelha de elefante mexicana cactus cladodes. Averages followed by different letters in the same column differ statistically by Student's t-test at 5% probability.

At the end of the experiment, lower serum urea levels were found in animals that received cactus cladodes, regardless of variety, compared to those that received the control diet (Table 4). When compared to baseline values, the animals that subsequently consumed the Miúda cactus cladodes and the control diet had higher serum urea levels, which was not obvious with the O.E.M. cactus cladodes (Table 6).

Diets with cactus cladodes altered the metabolism of Mg and Na, causing higher serum concentrations of Mg (P=0.001) and lower Na content (P=0.001) (Table 4). The same trend was observed when comparing the averages of these minerals between collections (Table 7).

Table 7. Serum minerals of sheep before and after supply of cactus cladodes-based diets

	Diets		
	Control	Miúda	O.E.M.[a]
	Total calcium (mg/dL)		
Baseline	7.72±0.18b	8.03±0.13b	8.00±0.15b
45th day	10.40±0.22a	10.53±0.14a	10.08±0.23a
P-value	0.001	0.001	0.001
	Ionized calcium (mg/dL)		
Baseline	4.72±0.10b	4.93±0.06b	4.84±0.08b
45th day	6.24±0.13a	6.32±0.08a	6.07±0.09a
P-value	0.001	0.001	0.001
	Phosphorus (mg/dL)		
Baseline	5.82±0.45b	4.92±0.31b	5.17±0.30b
45th day	8.54±0.61a	7.75±0.33a	7.78±0.33a
P-value	0.004	0.001	0.001
	Total calcium:Phosphorus ratio		
Baseline	1.33±0.12	1.63±0.12a	1.55±0.07a
45th day	1.22±0.11	1.36±0.06b	1.30±0.07b
P-value	0.442	0.015	0.006
	Magnesium (mg/dL)		
Baseline	2.70±0.04a	2.50±0.06b	2.68±0.07b
45th day	2.30±0.07b	3.21±0.11a	3.67±0.12a
P-value	0.001	0.001	0.001
	Sodium (mEq/L)		
Baseline	168.30±4.5	160.88±1.23a	170.66±2.95a
45th day	189.10±12.6	150.95±2.75b	155.70±1.41b
P-value	0.188	0.019	0.001
	Potassium (mmol/L)		
Baseline	7.84±1.84b	13.29±2.18	8.78±2.17b
45th day	17.54±0.81a	15.48±0.65	17.11±1.13a
P-value	0.003	0.390	0.011

[a] orelha de elefante mexicana cactus cladodes. Averages followed by different letters in the same column differ statistically by Student's t-test at 5% probability.

DISCUSSION

According to Kaneko *et al.* (2008), healthy sheep present activities of 26-34U/L and 60-280U/L for ALT and AST, respectively. In the present study, the serum activity of ALT was below the normal range and the enzymatic activity of AST fell within the physiological limits. Thus, it can be said that these enzymatic indicators do not signal hepatic damage, considering that only values above the reference range indicate possible liver cell degeneration.

The AP values determined before the introduction of the tested diets were within the normal range (Kaneko *et al.*, 2008), which did not occur after the intake of the control diets and with cactus (Table 5). Most of the serum AP is of hepatic origin, as this enzyme is found in the cells of the biliary epithelium and in the canicular membranes of hepatocytes (González and Silva, 2006). In this context, Silva *et al*. (2021b) observed significant lesions in the hepatic parenchyma of goats fed with the same cactus cladodes varieties used in this study. However, there is a possibility that the increase in AP may also occur for bone or kidney metabolic reasons. Considering the bone origin, it is highlighted that oxalate is an anion derived from organic acid that can bind with minerals forming crystals and reduce the availability of Ca and Mg, thus stimulating bone resorption in an attempt to maintain the serum levels of these minerals (González and Scheffer, 2003; Rahman *et al.*, 2013).

In the present study, all experimental diets showed concentrations of total oxalates in their composition (Table 2), which may have triggered hormonal actions to maintain serum calcium levels (Table 4), thus increasing the activity of AP, which represents an important indicator of bone mineral mobilization. On the other hand, oxalate levels contained in all diets can be considered safe (Table 2), considering that James and Butcher (1972) reported that diets containing up to 6% of soluble oxalates in DM did not cause acute poisoning in sheep, although they could cause mild hypocalcemia and decrease in serum Mg.

According to Tennant and Sharon (2008), even though GGT is present in the tissues of many organs (liver, kidneys, pancreas, small intestine, and bile duct epithelium), the elevation of its serum activity is observed primarily in cases of acute liver damage. This is the most specific enzyme for diagnosing cholestasis or bile duct obstruction (Gomes *et al.*, 2008). Cholestasis consists of the decrease or interruption of bile flow and during this process the bile cells overflow, releasing some enzymes that can be detected in the bloodstream.

Some clinical signs may show cholestasis, such as excess lipids in the feces (steatorrhea). In this research, differences were observed for the values of ether extract in feces (P=0.009), so that the sheep fed with Miúda cactus cladodes had 30.3g/kg DM, higher than those detected in the feces of the animals of the control group (27.4g/kg DM) and the treatment with O.E.M. cactus cladodes (23.3g/kg DM), which may have been caused by the decrease in the arrival of bile in the small intestine, thus affecting lipid digestion and increasing the concentration of this nutrient in the fecal material, reinforcing the idea that there may have been cholestasis.

After 45 days of supply of the experimental diets, the serum levels of direct bilirubin remained within the physiological limits for the sheep specie, however in the baseline collection the concentrations of this metabolite in the animals that were subsequently submitted to the control diets and with O.E.M. cactus cladodes were above the reference range (Kaneko *et al.*, 2008). According to Barini (2007), conjugated or direct bilirubin increases in cases of hepatocellular damage or even injury or obstruction of the bile ducts. For total bilirubins, the reduction caused using cactus cladodes led to values within the ideal margin.

All indirect bilirubin values, including that of the control diet, were recorded above the normal range. According to Lassen (2007), bilirubin may increase due to pre-hepatic, hepatic, or post-hepatic causes. Unconjugated or indirect bilirubin increases in cases of excessive erythrocyte destruction or by defects in the bilirubin transport mechanism within hepatocytes (Hendrix, 2005). Therefore, as there was no increase in these metabolites due to the presence of cactus cladodes in the diets, it can be inferred that the animals did not develop liver damage. Additionally, it is possible that the highest water intakes by animals that received cactus cladodes in the diet cause hemodilution of total and indirect bilirubins.

The superiority in the plasma glucose level in the animals that received Miúda cactus cladodes may be related to the higher concentration of propionic acid generated by this variety during rumen fermentation. According to Rocha Filho (2012) sheep fed diets based on Miúda cactus cladode presented fermentative profile with more propionate (greater gluconeogenic precursor) than those which consumed O.E.M. cactus cladodes (23.7 and 22.7mM/L of ruminal fluid, respectively). However, regardless of diet, the animals' blood glucose falls within the reference range for sheep (Kaneko *et al.*, 2008).

Comparing the effect of cactus cladodes varieties, the lowest cholesterol content in animals that received O.E.M. cactus cladodes can be justified by the lower intake of total digestible nutrients. Furthermore, the presence of saponins has been detected in the cactus cladodes (Féboli *et al*., 2016). According to Tadele (2015), saponins decrease the intestinal absorption of glucose and cholesterol, based on intraluminal physical-chemical interactions, which may have contributed to the reduction of serum cholesterol.

Additionally, cacti cladodes, when compared to other forage plants, have shown high levels of linoleic acid (omega-6), which is a precursor to arachidonic acid that has caused hypocholesterolemic effects (El-Mostafa *et al.*, 2014). The serum decrease in cholesterol may still be related to liver failure, since the liver is the main site of synthesis of this metabolite (Lassen, 2007). In this sense, Silva *et al*. (2021b) observed decrease in serum cholesterol levels and histopathological lesions in the liver of goats fed the same cactus cladodes genotypes used in the present study. According to Oh and Lim (2006), the glycoprotein (90kDa) isolated from *Opuntia ficus-indica* lowers plasma lipid content in mice. At the end of the experimental period, all averages were below the values considered normal (52-76mg/dL) (Kaneko *et al.*, 2008).

The highest average of the variable fructosamine was registered in the animals that received the diet containing Miúda cactus cladodes (259.95μmol/L) in comparison with those of the control treatment and with O.E.M. cactus cladodes (244.97 and 244.45 μmol/L, respectively) (Table 4), which is related to the higher glycemia observed in animals that consumed this diet. According to Gouveia *et al*. (2015), the fructosamine can be defined as a stable ketoamine, formed from the non-enzymatic reaction between glucose and protein amine groups, especially albumin and IgG; and its blood concentration is controlled by the balance between the synthesis and elimination of these protein and glucose compounds. Thus, since the concentrations of total proteins, albumin, and globulin were not modified by the diets (Table 4), the highest level of fructosamine in sheep fed with Miúda cactus cladodes is associated with a higher blood glucose concentration.

According to Kaneko *et al*. (2008), serum urea values in sheep must be between 2.86 and 7.14mmol/L. Thus, all serum urea averages were above the reference limits. On the other hand, it should be noted that the average blood concentrations of urea (8.58 and 9.04mmol/L, in the animals submitted to diets with Miúda cactus cladodes and O.E.M. cactus cladodes, respectively), are close to the value reported by Maciel *et al*. (2019), which was 9.51mmol/L, when offering diets with 500g/kg of Miúda cactus cladodes and 250g/kg of Tifton hay for sheep, similar percentages to the present study.

The concentration of circulating urea has a strong connection with the amount of protein consumed, with the absorption of rumen ammonia and with the energy-protein ratio of the diet. Urea biosynthesis in the liver occurs in amounts proportional to the concentration of ammonia produced in the rumen, so that the higher the dietary protein intake, the higher the blood urea levels (Harmeyer and Martens, 1980). In this perspective, there was a higher crude protein intake by the animals that received Miúda cactus cladodes (Table 3), but with a reduction in blood urea (Table 4). Thus, diets with cactus cladodes seem to have caused a more adequate synchronism between the availability of nitrogen and energy in the rumen, which resulted in lower blood urea content. Cardoso *et al*. (2019), with the inclusion of increasing levels of Miúda cactus cladodes in the sheep diet, reported a linear decrease in blood urea, associating this response to the more efficient synchronism between nitrogen and energy in the rumen environment.

In addition, it is likely that the higher rate of passage of diets containing cactus cladodes (Costa *et al*., 2016) decreased the transformation of dietary protein into ammonia and, subsequently, into urea, reducing the circulating content of this metabolite. Bispo *et al*. (2007) found a linear decrease in ruminal ammoniacal nitrogen concentration with the replacement of elephant grass hay by cactus cladodes in the sheep diet.

Due to the lack of significance between serum concentrations of creatinine, uric acid, total proteins, albumin, globulin, and albumin-globulin ratio due to diets and taking into account that the means of the variables of total proteins, albumin and globulin were found according to the normal parameters for the sheep species (Kaneko *et al.*, 2008) (Table 4), it can be said that all diets provided adequate protein supply and, possibly, did not alter the functional state of liver cells. In the case of creatinine, high levels of this metabolite in the blood reflects the rate of filtration in the kidneys and may signal kidney damage (González and Scheffer, 2002), behavior that was not observed in this study, thus indicating that there was no significant renal impairment.

In the collection after the introduction of the experimental diets, the serum concentrations of Ca_t and Ca_i (free) in the sheep of all groups were similar (Table 4) and were within the physiological range (Kaneko *et al.*, 2008), however, in the baseline collection, all means indicated hypocalcemia (Table 7). The measurement of Ca_i levels provides safer results when compared to Ca_t, as it represents the form of calcium that is biologically active and, therefore, physiologically more relevant (Kaneko *et al.*, 2008). According to Guedes *et al.* (2016), the Ca_i, which corresponds to values between 50 and 60% of the Ca_t, is crucial for the transmission of nerve impulses, muscle contractions, in addition to cell signalling. In the present study, Ca_i averages represented 60% of Ca_t (Table 4). The proportions of Ca_t:P in the blood remained narrow (Table 4) and close to the ratio in which these minerals are found in the bones, which is between 1:1 and 2:1 (Pugh, 2004).

The serum level of Mg was higher in animals fed with cactus cladodes varieties (Table 4 and 7) due to the high content of Mg in this feed, whose concentration ranges from 17.0 to 18.2g/kg DM (Batista *et al.*, 2003; Santos *et al.*, 2009). According to Kaneko *et al.* (2008), normal serum Mg levels vary from 2.2 to 2.8mg/dL, lower values than those observed in this study, which were 3.21 and 3.67mg/dL in sheep that received Miúda and O.E.M. cactus cladodes, respectively (Table 4). The increase in serum Mg in sheep was also reported by Maciel *et al.* (2019), when observing a concentration of 2.95 mg/dL of this element, using 500g/kg of cactus cladodes, based on DM.

Na concentrations were lower in treatments containing cactus cladodes (Table 4) and decreased between collections (Table 7) due to the higher level of this mineral in Tifton hay. Santos *et al.* (2009) reported Na contents of 0.700 and 0.300g/kg of DM in the Tifton hay and in the Gigante cactus cladodes (*Opuntia fícus-indica* Mill), respectively.

CONCLUSIONS

The partial replacement (750g/kg) of the Tifton hay by Miúda or Orelha de Elefante Mexicana cactus cladodes in lambs feeding increases the enzyme activity, indicating liver and/or kidney changes, but does not cause relevant damage in indicators of energy, protein, mineral metabolism, and weight gain, being these cacti a fodder resource option. However, that is possible that the metabolic changes may restrict its use, which demonstrates the need for long-term studies.

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