

Metabolic profile and histopathology of kidneys and liver of lambs fed silages of forages adapted to a semi-arid environment

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Abstract

Thirty-two mixed-breed ram lambs (average age, 5.6 ± 0.4 months, and average live weight, 17.61 ± 2.63 kg) were used to evaluate the effect of diets containing silages of forages adapted to a semi-arid environment on the metabolic profile in serum and the histopathological assessment of liver and kidney tissues of the lambs. Lambs were allocated, in a completely randomised design, to four treatments (silage of old man saltbush (*Atriplex nummularia* Lind), buffelgrass (*Cenchrus ciliaris*), gliricidia (*Gliricidia sepium*) and 'pornunça' (*Manihot* sp.). The feedlot period was 49 days, and animals were slaughtered at an average weight of 27 ± 4.6 kg. On the last day of the experiment, blood was collected from the jugular vein of all animals to measure the protein, metabolic and energy profiles of the lambs. At slaughter, kidney and liver samples were collected for histopathological examination. The consuming of the diet containing buffelgrass silage resulted in a significantly higher activity of alanine-aminotransferase (30.14 IU/L) and the concentrations of urea (44.25 mg/dL), creatinine (0.94 mg/dL) and albumin (4.48 g/dL) in serum. Diets containing gliricidia silage resulted in significantly higher gamma-glutamyl transferase activity (92.0 IU/L), while the diets with pornunça silage resulted in higher serum levels of triglycerides (37.85 mg/dL). The diets had no effect on aspartate-aminotransferase enzyme activity and total protein, cholesterol and globulin concentrations, or the albumin : globulin ratio in serum. Mild congestion, necrosis and foci of mineralisation were observed in the kidneys of animals fed diets containing old man saltbush (50%) and pornunça (25%) silages, and mild fatty degeneration and mild mononuclear inflammatory infiltrate in their livers. In conclusion, diets containing silages of forages that are adapted to semi-arid environments may be used for feeding lambs, given the absence of dysfunctioning plasmatic levels of liver enzymes and energy and protein profiles. Additionally, kidney failure was not observed in lambs fed these diets during the feedlot period.

Keywords: blood parameters, buffelgrass, gliricidia, old man saltbush, pornunça

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Introduction

In Brazil, the evaluation of the metabolic profile through analysis and assessment of indicators of protein and energy profiles as well as of enzymes that are related to liver activity (aspartate aminotransferase [AST] and γ -glutamyl-transferase [GGT]) has made it possible to differentiate the nutritional status of sheep under various feeding regimens (Tabeleão *et al.*, 2008). This confirms the

importance of evaluating the metabolic profile as a way to monitor nutritional status and understand responses in the productive performance of lambs in different production systems. The viability of an animal production system is related directly to the dietary regimen to which these animals are subjected. However, the intensification of systems has led to increasingly frequent metabolic disorders, since this type of rearing system (feedlot) results in a metabolic change due to the greater productive demand. According to González (2000), reducing the production cycle predisposes animals to imbalances in the intake and output of nutrients, in their ability to metabolise these compounds and in the production levels achieved.

The use of forages preserved as silage is a common practice in sheep farming. However, little research has been done on the potential influence of silages produced with old man saltbush (Ben Salem *et al.*, 2010), buffelgrass (Il *et al.*, 2017), gliricidia (Oliveira *et al.*, 2018) and pornunça (Lucena *et al.*, 2018), which are adapted to the semi-arid environment, on the liver and kidney functions when offered in diets for small ruminants such as lambs. Such investigations are necessary, considering the possible toxic effects of plants caused by antinutritional factors, which affect animal health and production and, consequently, the economic and social condition of producers.

The forages studied here contain antinutritional components (cyanogenic glycosides, high fibre, phenolic compounds, and high oxalate content) that may have a negative effect on animal intake and performance and possibly cause disease. Evaluating these variables may help producers make important decisions such as adjustments in diets and feeding management. On this basis, the present study proposes to evaluate the effects of diets containing silages of various forage plants that are adapted to the semi-arid environment on the metabolic, protein and energy profiles and on the hepatic and renal histopathology of feedlot-finished lambs.

Materials and Methods

The experiment was carried out on the Experimental 'Caatinga' Biome Field, in the Metabolism Unit of Empresa Brasileira de Pesquisa Agropecuária (Embrapa), located in Petrolina, PE, Brazil, between September and November 2012. The experimental period was 59 days, which included 10 days for the animals to adapt to the management and diets. All experimental procedures were approved by the Ethics Committee in Animal Use (CEUCA) of the School of Veterinary Medicine at the Federal University of Bahia (approval no. 0005/2016).

Thirty-two uncastrated crossbred Santa Inês lambs (mean age 5.6 ± 0.4 months and mean live weight 17.61 ± 2.63 kg), were acquired from producers of the region and were allocated to four treatments in a randomised complete block design with eight replicates. The animals were fed diets that included silages of four forage plants as the roughage source – old man saltbush (*Atriplex nummularia* Lind), buffelgrass (*Cenchrus ciliaris*), gliricidia (*Gliricidia sepium*) and pornunça (*Manihot* sp.) – plus a concentrate containing maize, soybean meal, mineral mix, calcitic limestone, and ammonium chloride, with a roughage-to-concentrate ratio of 50 : 50 (Tables 1 and 2).

To produce the silages, old man saltbush, gliricidia and pornunça were harvested on the experimental field of Embrapa Semi-arid, using the plant shoots, represented by the tenderest leaves and stems, 1.5 m in height. The buffelgrass was harvested at the Agricultural Research Company of Paraíba State S.A. (EMEPA), using a backpack mower at 10 cm above the soil before the inflorescence period, approximately 60 cm in height. All forage materials were processed through a PP-35 forage harvester to an average particle size of approximately 2.0 cm and stored in 200-L plastic-drum silos at a compaction density of 600 kg/m³. The buffelgrass, in turn, was compacted at a density of 400 kg/m³ because of its high dry matter (DM) content (40%).

The feed was offered daily at 08:30 and 15:30, and the orts were collected and weighed to determine intake and to adjust the DM intake to allow 10% to 20% as orts in the trough. The lambs had free access to water and the drinking troughs were monitored every day to avoid a water deficit.

During the experimental period, feed and orts samples were collected, packed in labelled plastic bags and stored in a freezer at -20 °C. After thawing, samples of roughage, concentrate and orts were pre-dried in a forced-air oven at 55 °C for 72 h. Next, they were ground in Wiley knife mills with a 1-mm screen and stored in containers with lids for subsequent laboratory analyses.

The DM, mineral matter (MM), crude protein (CP) and ether extract (EE) contents of all samples of feeds and orts were determined in accordance with the methodologies described in AOAC (1990). In all samples, the concentrations of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were obtained as described by Van Soest *et al.* (1991). Neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN) and lignin contents were determined by treating the acid detergent fibre residue with 72% sulphuric acid, according to the procedures described by Silva & Queiroz (2002).

The NDF corrected for ash (a) and protein (p) (NDFap) was determined according to the methodology described by Mertens (2002) and Licitra *et al.* (1996). The concentration of total carbohydrates (TC) was estimated according to Sniffen *et al.* (1992) as follows:

$$TC (\%DM) = 100 - (\%CP + \% EE + \% ash)$$

Because of the presence of urea, ash- and protein-free non-fibrous carbohydrates (NFCap) and NFCap from the concentrate were estimated as proposed by Hall (2000):

$$NFCap = 100 - [(\%CP - \%CP \text{ from urea} + \%urea) + \%NDFap + \%EE + \%MM]$$

The total digestible nutrient (TDN) content was estimated by the formula proposed by Weiss *et al.* (1999):

$$TDN = DCP + 2.25 \times DEE + DNFC + DNDF$$

where DCP, DEE, DNFC, and DNDF are the digestible fractions of crude protein, ether extract, non-fibrous carbohydrates and neutral detergent fibre, respectively.

Table 1 Composition of experimental diets of lambs fed diets with silage from forage plants adapted to semi-arid conditions

Ingredients (%DM)	Centesimal composition of ingredients			
	Old man saltbush	Buffelgrass	Gliricidia	Pornunça
Silage	50.00	50.00	50.00	50.00
Ground maize	37.23	39.30	46.80	45.13
Soybean meal	8.96	7.00	0.00	1.29
Urea	0.86	0.70	0.00	0.42
Mineral mix	1.68	1.75	1.95	1.92
Calcitic limestone	0.45	0.45	0.40	0.42
Ammonium chloride	0.84	0.85	0.85	0.83
Chemical composition of diets				
Dry matter (DM)	61.7	66.7	55.2	57.6
Mineral material ¹	11.6	9.9	8.4	7.1
Crude protein ¹	15.5	15.0	14.0	16.1
Ether extract ¹	1.8	1.8	2.5	3.4
NDFap ¹	31.6	36.8	28.0	26.9
Neutral detergent fibre ¹	45.9	50.0	40.0	40.0
Acid detergent fibre ¹	22.2	25.1	21.9	23.3
iNDF ¹	25.1	18.6	18.1	15.4
NDIN ²	14.6	14.3	16.0	21.5
ADIN ²	11.8	12.3	12.8	15.2
Cellulose ¹	17.1	23.0	15.7	16.1
Hemicellulose ¹	23.7	24.9	18.1	16.7
Lignin ¹	7.4	3.0	7.2	10.4
Total carbohydrates ¹	71.1	73.3	75.1	73.4
NFCap ¹	39.5	36.5	47.1	46.5

¹ In % DM; ² In % total nitrogen

³ Provides per kg in active ingredients: calcium: 120 g; phosphorus: 87 g; sodium: 147 g; sulphur: 18 g; copper: 590 mg; cobalt: 40 mg; chromium: 20 mg; iron: 1800 mg; iodine: 80 mg; manganese: 1300 mg; selenium: 15 mg; zinc: 3800 mg; molybdenum: 10 mg; maximum fluorine: 870 mg; phosphorus (P) solubility in minimum 2% citric acid: 95%.

NDFap: ash- and protein-free neutral detergent fibre; iNDF: indigestible neutral detergent fibre; NDIN: neutral detergent insoluble nitrogen; ADIN: acid detergent insoluble nitrogen; NFCap: ash- and protein-free non-fibrous carbohydrates.

The data pertaining to the intake of the nutritional components were estimated as the difference between the total amounts each component in the feed supplied and in the orts (Table 2).

Table 2 Intake of dry matter and nutritional components by lambs fed silages of forages adapted to the semi-arid environment

Variable kg ¹	Silages				SEM	P-value
	Old man saltbush	Buffelgrass	Gliricidia	Pornunça		
DM	53.2 ^a	36.1 ^c	42.2 ^{bc}	48.4 ^{ab}	0.002	0.0002
OM	47.5 ^a	32.3 ^c	0.79 ^{bc}	0.92 ^{ab}	0.019	0.0002
CP	9.6 ^a	4.8 ^c	5.5 ^c	8.0 ^b	0.003	<0.0001
EE	0.98 ^b	0.49 ^c	0.88 ^{bc}	1.47 ^a	0.001	<0.0001
NDFap	12.7a ^b	13.7 ^a	10.0 ^b	11.1 ^b	0.008	0.0410
NFC	24.4 ^a	11.9 ^c	20.4 ^b	24.2 ^a	0.008	<0.0001
TDN	36.3 ^a	22.7 ^b	30.3 ^a	33.5 ^a	0.013	<0.0001

¹The experimental period was 49 days

P-value: significant at the 5% probability level

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDFap: neutral detergent fibre corrected for ash and protein; NFC: non-fibrous carbohydrates; TDN: total digestible nutrients. SEM: standard error of the mean

To evaluate the influence of silages on the hepatic function, blood samples were collected by puncturing the jugular vein of all animals on the last day of the experiment before slaughter. To this end, after local aseptic procedures, 10 mL of blood were collected in Vacutainer® tubes without anticoagulant. The samples were kept at room temperature until clot retraction. The material was then centrifuged at 3500 rpm for 15 min to obtain the blood serum of each animal. Serum samples were stored in labelled Eppendorf® tubes and preserved in a freezer at -20 °C for further analysis. Plasma activities of alanine-aminotransferase (ALT EC 2.6.1.2), aspartate-aminotransferase (AST EC 2.6.1.1) and gamma glutamyl-transferase (GGT EC 2.3.2.2) enzymes were measured by colorimetric analysis and read on a spectrophotometer at the wavelengths of 340 nm (ALT and AST) and 410 nm (GGT), with the temperature between 20 and 30 °C. Activity was expressed in IU/L.

Total serum protein levels were measured by the biuret method, and urea and serum albumin were evaluated by the bromocresol green method using Doles® quantification kits and a spectrophotometer at the wavelengths of 550 and 600 nm, respectively. In the analysis of total cholesterol and triglycerides, which were required for the evaluation of energy status, Doles quantification kits were also used. The enzymatic colorimetric technique was also employed, with readings taken by a semi-automatic biochemical analyser. Globulin concentrations were obtained by subtracting the individual values of total protein and serum albumin. The albumin/globulin ratio was calculated based on the individual albumin and globulin values.

On the last day of the experimental period, the lambs were slaughtered at a mean body weight of 27 ± 4.6 kg. Prior to slaughter, the animals were fasted from solids and kept on a water diet for 16 h, according to animal welfare norms.

The lambs were then transferred to the abattoir of Instituto Federal Pernambucano, located in Petrolina - PE, Brazil, where they were slaughtered according to the current regulations established by the Ministry of Agriculture and Supply: Agriculture Defence Department (Brasil, 2000). The animals were slaughtered after being stunned by electronarcosis, followed by bleeding via sectioning the jugular veins and carotid arteries. During the slaughter, after skinning and evisceration, fragments of kidney and liver were collected from all animals, packed in bottles and fixed with 10% neutral buffered formalin solution.

The fragments of kidneys and liver that were collected during slaughter were transferred to the Laboratory of Veterinary Pathology (LPV/HOSPMEV) at the Veterinary Medicine Hospital at the Federal University of Bahia (UFBA). Samples were processed using the paraffin-embedding technique. Histological sections of 4-µm thickness were obtained and stained with haematoxylin and eosin (HE) (Prophet *et al.*, 1992) for subsequent histopathological analysis to identify and evaluate anatomopathological alterations caused by the consumption of these silages.

The metabolic profile was analysed as a completely randomised design, using the following model:

$$Y_{ij} = \mu + T_i + e_{ij},$$

where Y_{ij} = observed value of the dependent variable; μ = overall mean; T_i = effect of treatment i ($i = 1-5$); and e_{ij} = experimental error.

The experimental data were evaluated by analysis of variance (ANOVA) and Tukey's test, adopting 0.05 as the critical level for the probability of type-I error, in SAS statistical software (SAS, 2009).

The animals were in the same age range (5.6 ± 0.4 months) and weight (17.61 ± 2.63 kg), indicating homogeneous distribution.

Microscopic changes in the liver and kidneys were determined as presence (+) or absence (-) (Table 4) and were described separately for the intensity and distribution of the lesions by two veterinary pathologists. The concordance between examiners was 0.87, as assessed by the kappa (k) concordance test (Viera & Garrett, 2005). The agreement was considered 'small' when k was less than 0.20, 'minimum' when k was between 0.21 and 0.4, 'regular' between 0.41 and 0.60, 'good' between 0.61 and 0.80, and 'optimum' when k was equal to or greater than 0.81.

Results

Serum ALT enzyme activity differed ($P < 0.05$) (Table 3). The lowest values (22.25 IU/L) were found in the lambs that had consumed a diet containing gliricidia silage, while the highest were detected in the lambs that were fed buffelgrass silage (30.14 IU/L), which did not differ statistically from those fed the silages of old man saltbush and gliricidia. The silages had no effect ($P > 0.05$) on AST enzyme activity.

Table 3 Serum levels of urea, total protein, albumin, globulin, and albumin/globulin ratio, energy profile and enzymatic activities of gamma-glutamyl transferase, alanine aminotransferase and aspartate-aminotransferase in lambs fed diets with different silages of forages adapted to the semi-arid environment

Variable	Silages				SEM	P-value
	Old man saltbush	Buffelgrass	Gliricidia	Pornunça		
Urea (mg/dL)	41.87 ^a	44.25 ^a	17.37 ^b	22.37 ^b	1.677	<0.001
Creatinine (mg/dL)	0.68 ^c	0.94 ^a	0.71 ^b	0.80 ^b	0.019	<0.001
TP (g/dL)	7.58	7.28	7.23	6.81	0.222	0.544
Albumin (g/dL)	4.23 ^a	4.48 ^a	3.89 ^b	3.76 ^b	12.92	0.041
Globulin (g/dL)	3.30	3.23	3.34	3.05	19.76	0.819
A/G	1.29	1.17	1.17	1.22	31.69	0.070
Cholesterol (g/dL)	33.12	35.25	35.75	38.87	1.31	0.332
Triglycerides (g/dL)	26.28 ^b	29.57 ^{ab}	29.50 ^{ab}	37.85 ^a	1.49	0.033
AST/TGO (IU/L)	93.66	105.40	85.75	108.14	3.822	0.092
ALT/TGP (IU/L)	28.28 ^{ab}	30.14 ^a	22.25 ^b	26.25 ^{ab}	0.835	0.016
GGT (IU/L)	74.33 ^{ab}	68.50 ^b	92.00 ^a	70.66 ^{ab}	3.366	0.024

TP: total protein; A/G: albumin/globulin; AST/TGO: aspartate-aminotransferase; ALT/TGP: alanine-aminotransferase; GGT: gamma glutamyl-transferase; mg/dL: milligrams per decilitre; g/dL: grams per decilitre. IU/L: international units per litre; P-value*: significant at 5% probability level; SEM: standard error of the mean

The diets also influenced ($P < 0.05$) the serum urea concentrations (Table 3). For this variable, higher values were observed in the animals fed buffelgrass (44.25 mg/dL) and old man saltbush (41.87 mg/dL) and lower values were found in those fed gliricidia (17.37 mg/dL) and pornunça (22.37 mg/dL). Gamma-glutamyl transferase activities (Table 3) varied according to the diets ($P < 0.05$), with higher results detected in the animals that consumed silages of gliricidia, old man saltbush and pornunça (92.00, 74.33, and 70.66 IU/L, respectively) and lower values in the lambs fed buffelgrass silage (68.50 IU/L), which did not differ statistically from those fed pornunça silage. The silages influenced ($P < 0.05$) the serum concentrations of creatinine, which were below the normal range of 1.2-1.9 mg/dL recommended by González & Silva (2006).

The diets did not affect ($P > 0.05$) the serum concentrations of total protein in the lambs (Table 3). However, the serum albumin concentration was influenced ($P < 0.05$) by the feed, with higher values found

in the animals fed diets containing old man saltbush (4.23 g/dL) and buffelgrass (4.48 g/dL) and lower values in the lambs that consumed diets containing gliricida (3.89 g/dL) and pornunça (3.76 g/dL). The silages had no effect ($P > 0.05$) on the serum levels of globulin, albumin:globulin ratio and blood cholesterol concentrations. All diets influenced the serum concentration of triglycerides (Table 3) ($P < 0.05$). For this variable, higher values were found in lambs fed pornunça (37.85 mg/dL), buffelgrass (29.571 mg/dL) and gliricidia (29.500 mg/dL) compared to old man saltbush silage (26.28 mg/dL), which in turn did not differ statistically from those fed the buffelgrass and gliricidia silages.

Microscopic analysis of the kidneys revealed mild congestion in the lambs which were fed silages of old man saltbush (50% of the animals) and pornunça (25% of the animals). The congestion was mostly in the medulla, focal areas of necrosis in the cortical area, and foci of tubular mineralisation in the corticomedullary region and medulla (Table 4). Focal mononuclear inflammation, consisting predominantly of lymphocytes and plasmocytes, was observed in the cortical region, with intensity ranging from mild to moderate, in all treatment groups. The liver histopathological analysis revealed only mild fatty degeneration (macrovesicular steatosis) and mild mononuclear inflammatory infiltrate; both alterations were located predominantly in the periportal region (Table 4).

Table 4 Main histopathological findings of kidneys and liver of lambs fed diets containing silages of forages adapted to the semi-arid environment

Histopathological finding	Silage			
	Old man saltbush	Buffelgrass	Gliricidia	Pornunça
Histopathological findings of kidney				
Congestion	+	-	-	+
Necrosis	+	-	-	+
Mineralisation	+	-	-	+
Mononuclear inflammation	+	+	+	+
Histopathological findings of liver				
Congestion	-	-	-	-
Fatty degeneration	+	+	+	+
Periportal mononuclear infiltrate	+	+	+	+

(+) present; (-) absent

Discussion

The obtained serum ALT enzyme activity values (26.73 IU/L) were within the reference limits for the ovine species as described by Pugh & Dum (2005) and established by Viana (2014), who reported variations of 20 - 52 IU/L. The serum ALT and AST enzyme activity values were within the normal range, suggesting that the diets did not compromise the liver structure. According to Latimer (2005), an increase in serum levels of these enzymes is related to hepatocellular diseases, since the degree of increase is directly proportional to the number of hepatocytes affected. Borburema *et al.* (2012) evaluated the effect of dietary regimen on the metabolic profile of Santa Inês lambs in the feedlot and observed that the animals which consumed less feed displayed lower GGT enzyme values. Data obtained in the present study corroborate the reports of these authors, considering the diets containing buffelgrass silage provided the lowest nutrient intake and, consequently, the lowest GGT values.

It is possible that the diets caused toxicity in the hepatic parenchyma, since, according to Kaneko *et al.* (2008) and Batista *et al.* (2009), values higher than the average reference for the ovine species (20-52 IU/L) may cause toxicity. However, Nunes *et al.* (2010) evaluated the hepatic condition of lambs that were fed diets containing palm kernel cake and observed above-average values for GGT enzymes, whereas the cholesterol, total protein and alanine-aminotransferase and aspartate-aminotransferase enzyme values remained within the stipulated limits for sheep. Peneluc *et al.* (2009) reported similar values for GGT (63.2-83.92 IU/L) in Santa Inês sheep grazing on grasslands on the north coast of Bahia State, Brazil. Those authors noted that the Santa Inês breed and animals in rearing systems in the north-east of Brazil showed

GGT enzyme values that were higher than those cited in the literature and higher than those observed in other countries. The increased serum GGT activity values found in the present study indicate biliary epithelial injury, which was confirmed in the histological analysis and which revealed the presence of periportal inflammatory infiltrate. This was possibly as a result of the obstruction of bile ducts caused by the deposition of saponin crystals and injury in the cholangioles, suggesting a subclinical presentation of the disease.

Serum concentrations of urea were within the normal range of 17.12-42.8 mg/dL described by Silveira (1988), Radostitis *et al.* (2002), Kaneko *et al.* (2008) and Andrade-Montemayor *et al.* (2009). This difference was thus caused by the diet containing old man saltbush silage, which did not have the highest CP content in its composition. Additionally, it provided the highest intake (0.195 kg/day) of this nutritional component and the highest TDN intake (0.741 kg/day), which is associated with higher DM intake (1.085 kg).

The results obtained for the diets containing buffelgrass silage could be explained in part by the deficient energy intake by the animals. In the liver, this energy increased the gluconeogenesis from amino acids, with a subsequent increase in the levels of serum ammonia, which is transformed into urea. In this way, there is an elevation in circulating urea levels in addition to energy expenditure to metabolise this urea in the liver (Huntington & Archibeque, 2000).

The variations in average creatinine excretion among the animals were possibly caused by the pathological conditions of the individuals, which may interfere with the glomerular filtration rate (Chen *et al.* (2004). Moreover, physiological conditions may change this parameter, since animals with different proportions of muscle and fat may excrete lower amounts of creatinine per unit of live weight. This might have influenced the low results obtained in the present study. The low creatinine values obtained here may be associated with the amount of muscle mass and with the slaughter weight, because the animals were slaughtered at seven months old, while their muscles were still developing, and with an average weight of 27.16 kg.

The average total serum protein concentrations in the lambs (7.22) (Table 3) were within the values described for the ovine species (Kaneko *et al.*, 2008), which may vary from 6.0 to 7.9 g/dL. Serum albumin concentration also showed acceptable values for sheep, ranging from 3.0 to 4.5 g/dL (Kaneko *et al.*, 1998). This result was possibly influenced by the greater DM intakes of the animals fed old man saltbush (53.165 kg) and pornunça (48.412 kg), which contributed to the higher CP intake (9.555 and 8.036 kg, respectively), as albumin is an indicator of the protein content in ruminant diets (Kaneko *et al.*, 1997).

The serum globulin values (3.23 g/dL) were in the range of 3.1 to 5.1 and 3.4 to 5.7 g/dL, which was stipulated for this animal species by Hearly & Falk (1974) and Kaneko *et al.* (1997), respectively. Overall, the albumin : globulin ratios in this study were within the regular variation for the ovine species, from 0.60 to 1.3 g/dL (Kaneko *et al.*, 1997). As described by Bacila (2003), this ratio may be used as an indicator of low susceptibility of lambs to infection. Lower values are usually caused by a humoral response to the stimulus of antigens that are harmful to the organism and by excessive globulin production (Tizard, 2000). Based on the results of the current study, it appears that the lambs were not affected by a disease that would cause excessive immunoglobulin production from gamma globulin production.

According to the present results, and in line with the findings of Kaneko *et al.* (2008), total cholesterol levels were below the reference values for the ovine species. Normal values range from 52 to 76 mg/dL, as described by Zambom *et al.* (2005) and Beynen *et al.* (2000), who reported that the EE content of a diet may influence plasma cholesterol and triglyceride concentrations. Homem Júnior *et al.* (2010) evaluated the effects of three diets and two feeding regimens in feedlot lambs and found a significant effect of the treatments on the blood cholesterol level. The lambs that consumed diets containing sunflower seeds or protected fat showed higher blood cholesterol levels than those fed the control diet, with a normal EE content of 2.6%. This was explained by the greater energy supply from these diets. Thus, the diets containing pornunça silage provided higher cholesterol and triglyceride rates because of the higher EE content of (4.6%) and intake (91.470 kg) from these diets. According to Zambom *et al.* (2005) the serum levels of these components are influenced directly by the EE content of the diets. Thus, the animals that received the diet containing the highest levels of EE (pornunça) showed a higher concentration of triglycerides compared to those fed the old man saltbush silage.

After post-mortem analysis in the slaughterhouse, no anomalies or alterations were found in the macroscopic analysis of liver, kidneys and other organs. Therefore, the diets utilised during the feedlot period did not compromise the carcasses or cause their condemnation. It is worth pointing out that there were no visible effects that would have prevented the sale of meat from these animals.

Data from the macroscopic analysis revealed mild congestion in the kidneys of animals fed old man saltbush silage. This result is probably because of the high NaCl content of the plant, as described in the literature, or, according to Clark & Clark (1978), because animals fed plants of the genus *Atriplex* may be affected by kidney damage, since this shrub contains high levels of oxalates.

The fatty degeneration observed in the hepatic parenchyma in most of the animals, irrespective of treatment group, was mild and diffuse. A possibility is that steatosis occurred prior to the start of the experiment, possibly resulting from a management activity (deworming), as all animals displayed these alterations. The mild periportal inflammation, in turn, may be attributed to infectious diseases and parasitic infestations prior to the experimental period and which had a chronic course.

The results of the present study for the histopathological parameters of hepatic and renal parenchymas of lambs fed pornunça silage could be attributed to the presence of glycoside after incomplete elimination in the silage. However, Onwuka *et al.* (1992) stated that small ruminants which are exposed to cyanide show prolonged sulphur deficiency and retarded growth, which was not observed in the current study, as the animals fed pornunça silage showed similar growth patterns to those that received the other diets.

Conclusions

Subclinical presentation of congestion, necrosis, and foci of mineralisation in the kidneys and fatty degeneration and mononuclear inflammatory infiltrate were noticed in the liver of sheep fed old man saltbush and pornunça silage diets. Furthermore, the serum levels of some blood metabolites which are related to protein, energy and enzyme profiles were affected by the diets.

Nevertheless, there were no macroscopic alterations in the liver or clinical symptomatology when the animals were fed diets containing silage of tropical forages. Therefore, despite the nutritional quality of these silages, their inclusion in sheep diets must be carefully planned to prevent serious damage to the health of these animals.

Authors' Contributions

FSC, GCG and ALRM participated in designing the study, laboratory analysis, and manuscript writing. Involvement in drafting and revising the manuscript for important intellectual content. EMS, GGPC and GGLA were involved in drafting and revising the manuscript for important intellectual content. JS and MLMLA were responsible for all aspects of the work, ensuring that issues related to the accuracy or completeness of any part were adequately investigated and resolved. AESL carried out data analysis and interpretation and was involved in the preparation and revision of the manuscript. RRA contributed to the acquisition, analysis and interpretation of data.

Conflict of Interest Declaration

The authors declare that they have no competing interests

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