Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2022v43n6p2425

Histologic changes in liver tissue of sheep fed spineless cactus resistant to *Dactylopius opuntiae*

Alterações histológicas no tecido hepático de ovinos alimentados com palma forrageira resistente a *Dactylopius opuntiae*

Francisca Mônica Couras Dias[1]; Robson Magno Liberal Véras[2]; Levi Auto Lopes[3]; Tomás Guilherme Pereira da Silva[4]; Francisco Fernando Ramos de Carvalho[5]; Ângela Maria Vieira Batista[5]; Daniel Barros Cardoso[6]*; Francisco de Assis Leite Souza[7]; Janaina de Lima Silva[8]

Highlights

Sheep fed OEM spineless cactus-based diet presented higher oxalates intake.
The use spineless cactus increased the liver weight.
Diets with or without spineless cactus cause liver parenchyma damage.

Abstract

This study aimed to assess the effects of diets containing spineless cactus genotypes resistant to carmine cochineal insect (*Dactylopius opuntiae*) on the liver condition of sheep. Thirty-six non-castrated Santa Inês male sheep (six months of age and average initial body weight of 22.0 ± 2.9 kg) were assigned to a completely randomized design, with three treatments and 12 replicates. The animals were fed a diet with Tifton hay as exclusive roughage (control) and two more diets in which the hay was partially replaced by 'Miúda' or 'Orelha de Elefante Mexicana' (OEM.) spineless cactus. The animals were randomly slaughtered at 86 days after 16-h solid fast. The use of spineless cactus, regardless of genotype, increased the liver

[1] Master's Student of the Animal Science and Pastures Postgraduate Program, Universidade Federal do Agreste de Pernambuco, UFAPE, Garanhuns, PE, Brazil. E-mail: monica_couras@hotmail.com
[2] Prof. Dr., UFAPE, Garanhuns, PE, Brazil. E-mail: robson.veras@ufape.edu.br
[3] PhD Student of the Integrated Doctoral in Animal Science Program, UFRPE, Recife, PE, Brazil. E-mail: levi_auto@hotmail.com
[4] Researcher, BFP/FACEPE, Department of Animal Science, UFRPE, Recife, PE, Brazil. E-mail: tomas-g@hotmail.com
[5] Profs. Drs., Department of Animal Animal Science, UFRPE, Recife, PE, Brazil. E-mail: ffrcarvalho@gmail.com, angelamvbatista@gmail.com
[6] Researcher, PNPD/Capes, Animal Science and Pastures Postgraduate Program, UFAPE, Garanhuns, PE, Brazil. E-mail: danbarrc@hotmail.com
[7] Prof. Dr., Department of Animal Morphology and Physiology, UFRPE, Dois Irmãos, Recife, PE, Brazil. E-mail: chicoleite@hotmail.com
[8] Profa Dra, Multidisciplinary Center of Barra, Universidade Federal do Oeste da Bahia, UFOB, Barra, BA, Brazil. E-mail: janaina.lima@ufob.edu.br
* Author for correspondence

Received: Mar. 06, 2022 - Approved: Nov. 20, 2022

weight and caused inflammatory processes and necrosis on the liver parenchyma. Spineless cactus in diets for sheep confined for 86 days, despite causing liver tissue damage, such as inflammation and death process of hepatocytes, does not compromise weight gain.
Key words: *Cactaceae.* Digestive system. Histopathology. Oxalates. Small ruminants.

Resumo

Este estudo teve como objetivo avaliar os efeitos de dietas contendo genótipos de palma forrageira resistentes ao inseto cochonilha do carmim (*Dactylopius opuntiae*) sobre a condição hepática de ovinos. Trinta e seis ovinos machos Santa Inês não castrados (seis meses de idade e peso corporal inicial médio de 22,0 ± 2,9 kg) foram distribuídos em delineamento inteiramente casualizado, com três tratamentos e 12 repetições. Os animais foram alimentados com uma dieta com feno de Tifton como volumoso exclusivo (controle) e mais duas dietas em que o feno foi parcialmente substituído por palma forrageira 'Miúda' ou 'Orelha de Elefante Mexicana' (OEM.). Os animais foram abatidos aleatoriamente aos 86 dias após jejum de sólidos por 16 horas. O uso de palma forrageira, independente do genótipo, aumentou o peso do fígado e causou processos inflamatórios e necrose no parênquima hepático. A palma forrageira em dietas para ovinos confinados por 86 dias, apesar de causar danos ao tecido hepático, como inflamação e processo de morte dos hepatócitos, não compromete o ganho de peso.
Palavras-chave: *Cactaceae.* Histopatologia. Oxalatos. Pequenos ruminantes. Sistema digestório.

Introduction

The spineless cactus (*Opuntia* and *Nopalea*) is one of the most important forages in the world. In Brazil, it is considered strategic due to the climatic conditions of the Northeast region. They are used for several purposes, such as human and animal feeding, energy production, medicine, cosmetics, and the chemical and food industry (Dubeux et al., 2013).

The main spineless cactus genotype is the 'Miúda' (*Nopalea cochenillifera* Salm Dyck), which is resistant to *Dactylopius opuntiae* and has high palatability by ruminants, although it is less resistant to dry (C. C. F. Silva & Santos, 2006). However, 'Orelha de Elefante Mexicana' (OEM.; *Opuntia stricta* [Haw]. Haw.) is less demanding in nutrients and more tolerant to water stress conditions, besides requires higher dry matter production by unit area than 'Miúda' (Santos et al., 2018).

The spineless cactus also has antinutritional components such as oxalate (ranging from 1.80 to 4.90 mg/kg of dry matter – DM) (T. G. P. Silva, 2020; T. G. P. Silva et al., 2021) and affect the energy metabolism of the intestine and liver, may cause histological changes (S. M. C. Silva, 2017; Barboza et al., 2019; Lima et al., 2019; T. G. P. Silva, 2020; T. G. P. Silva et al., 2021). The liver uses part of the energy intake of the whole body, so its metabolism is influenced by the different animal workloads (Haas, 2014).

Biochemical analyses, radiography, and ultrasonography are the main complementary tests to discover liver diseases in domestic animals (Radostits et al., 2002). They are efficient to detect organ changes and severity grading, but not to provide specific diagnoses and do not consider the clinician limitation to meet the real nature of the liver disease. Thus, the histological examination of liver tissue through biopsy is essential to determine a precise diagnostic and prognostic (Bunch et al., 1985), besides allow a broader view of liver disease so that the access to lesion morphology can be determined (Tostes & Bandarra, 2002).

T. G. P. Silva et al. (2021), when investigating the liver condition of goats fed spineless cactus, through biochemical indicators in the blood and histopathological evaluation, reported marked tissue damage and a decrease only in the cholesterol serum concentration. Thus, we hypothesized that spineless cactus-based diets alter the liver status of sheep. Consequently, the effects of diets containing spineless cactus genotypes resistant to carmine cochineal insect (*Dactylopius opuntiae*) on the liver status of sheep, through histopathology, were evaluated in this study.

Material and Methods

The experiment was carried out at the Animal Science Department of the Federal Rural University of Pernambuco (UFRPE), Recife, Pernambuco State, Brazil. All procedures were conducted and approved by the Committee of Ethics in the Use of Animals (CEUA) of the UFRPE (License Nº 142/2018).

Thirty-six non-castrated Santa Inês male sheep, with initial body weight (BW) 22.0 ± 2.9 kg, were assigned to a completely randomized design, with three treatments and 12 replicates. Sheep were housed in individual pens equipped with feeding and drinking troughs. They were identified, weighed, vaccinated against clostridia, and dewormed. The experiment lasted 86 days, with the first 30 days to the diet's adaptation, followed by 56 days for collecting samples and data.

Diets were formulated to meet the nutritional requirements of lambs weighing 25.0 kg and to achieve gains of approximately 200 g day^{-1} according to National Research Council [NRC] (2007). The roughage fraction was composed of Tifton-85 grass hay (*Cynodon* spp.), 'Orelha de Elefante Mexicana' – OEM (*Opuntia stricta* [Haw.] Haw.), and 'Miúda' (*Nopalea cochenillifera* Salm Dyck). The concentrate fraction of diets had ground corn, soybean meal, urea, and mineral premix. Three different component-fed solid diets (treatments) were evaluated: 1) diet with Tifton-85 hay (control), 2) diet with Tifton-85 hay partially (75.0% of dry matter - DM) replaced by 'Miúda' spineless cactus and, 3) diet with Tifton-85 hay partially (75.0% DM) replaced by OEM spineless cactus (Table 1).

Table 1
Ingredients and chemical composition of the diets (g/kg DM)

	Treatments (g/kg)		
Ingredients	Control	'Miúda'	'OEM'1
Tifton-85 hay	600	150	150
'Miúda' spineless cactus	0	450	0
'OEM'[1] spineless cactus	0	0	450
Ground corn	270	271	273
Soybean meal	110	100	100
Urea	5.00	14.0	12.0
Mineral mix[2]	15.0	15.0	15.0
Chemical composition (g/kg DM)			
Dry matter[3]	891	235	190
Ash	76.0	95.8	105
Crude protein	142	142	143
Ether extract	25.6	21.5	23.4
NDFap[4]	456	267	243
Non-fiber carbohydrates	300	473	486
Total digestible nutrients	648	709	633
Oxalates	4.00	3.60	4.90

[1] 'OEM' = Orelha de Elefante Mexicana spineless cactus. [2] Nutrients per kg of product = calcium (140 g), phosphorus (70 g), magnesium (1320 g), iron (2200 mg), cobalt (140 mg), manganese (3690 mg), zinc (4700 mg), iodine (61 mg), selenium (45 mg), sulphur (12 g), sodium (148 g) and fluorine (700 mg). [3] g/kg natural matter. [4] Neutral detergent fiber assayed with a heat stable amylase and corrected for ash and nitrogenous compounds.

Water and diets were provided ad libitum. The diets were offered as a total mixed ration (TMR) divided into two meals at 8h00 and 15h00, allowing 15.0% leftovers. Feeds and leftovers were weighed to measure diet intake, with the adjustment of the amount every two days. Feces samples were collected directly from the animal's rectums in the 8th week of the experimental period, for five consecutive days, at five alternate times (0, 2, 4, 6, and 8 hours).

Samples of feed, leftovers and feces were analyzed to determine the concentrations of DM (method 934.01), mineral matter (MM) (method 942.05), ether extract (EE) (method 920.39), and crude protein (CP) (method 990.13), according to the Association of Official Analytical Chemists [AOAC] (1990). The concentrations of neutral detergent fiber (NDF) and acid detergent fiber (ADF), and non-fibrous carbohydrates (NFC) were analyzed according to Van Soest et al. (1991), adapted by Detmann et al. (2012) and Hall (2000), respectively. The total digestible nutrients (TDN) were calculated according to Weiss (1999): TDN (%) = CPD% + NDFapD% + NFCD% + (EED% * 2.25). Total oxalates of samples of feed and leftovers were analyzed according to Moir (1953).

The DM, CP, and TDN intakes, initial BW (kg), and final BW (kg) used in the results and discussion of the present study were obtained by Lopes et al. (2020). After 86 days of the feedlot, the animals were randomized slaughtered following the recommendations of the current legislation (Instrução Normativa n° 3, 2000) and after fasting for 16 h were weighed to obtain body weight at slaughter (BWS). After the slaughter, the liver of each animal was collected and weighed on a digital scale. To the DM of the liver tissue and histopathological analysis, eight fragments about 2.00 cm in length and 0.50 cm from the different lobes were obtained. Four fragments were placed in identified plastic pots and stored at -20 °C. Subsequently, the samples were thawed to room temperature and 2.00 g allocated in glass Petri dishes for drying (105 °C, 24 h) (Marques et al., 2011). The other half of the liver fragments were immediately immersed in 10.0% formaldehyde sodium phosphate buffer solution (0.1 M and pH 7.2).

After 48 h of fixation, the fragments were transferred to 70.0% ethyl alcohol and subsequently dehydrated in increasing concentrations of ethyl alcohol, diaphanized in xylol, oven-impregnated with liquid paraffin (58 °C), and embedded in paraffin. The blocks were cut by a microtome set to 5-μm and the sections were stained by Hematoxylin-Eosin (H.E.), following the protocol of the Department of Animal Morphology and Physiology at UFRPE. Images capture was performed with the aid of the LAZ EZ version 4.1.0 program and using a Leica® DM500 optical microscope coupled to the camera and connected to a computer. The histopathological changes observed in the liver were analyzed for the degree of intensity and distribution (Table 2).

Table 2
Lesion score for histopathological evaluation of the liver of sheep according to the distribution and degree of intensity

Category	Score	Meaning
Intensity	0	Normal
	1	Mild
	2	Moderate
	3	Severe distribution
Distribution	0	Normal
	1	Focal
	2	Locally extensive
	3	Multifocal
	4	Multifocal to coalescing
	5	Diffuse

Source: T. G. P. Silva et al. (2021).

The experimental design was completely randomized, using the animal initial BW as a covariate. The productive data were analyzed by analysis of variance and the averages compared by Tukey test (*P* < 0.05) statistical package Statistical Analysis System [SAS] (1999). Histopathological changes were analyzed by frequency distribution and statistical inference, using the Kruskal-Wallis test (*P* < 0.05) (SAS, 1999).

Results and Discussion

Feeding spineless cactus-based diets, regardless of genotype, resulted in higher fresh liver changes concerning the control diet (Table 3).

Table 3
Productive parameters of sheep fed spineless cactus-based diets

Intake (g day^{-1})	Treatments			SEM[2]	P-value
	Control	'Miúda'	'OEM'1		
Dry matter *	1129b	1291a	1172ab		
Crude protein*	170a	192a	168b	4.00	0.010
TDN[3]*	728b	916a	740b	23.0	0.000
Oxalates#	4.98b	4.68b	6.21a	0.27	0.001
Productive data					
Initial body weight (kg)*	22.4	22.6	22.8	-	-
Final body weight (kg)*	35.0	36.7	36.5	0.70	0.491
Fresh liver (kg)	0.51b	0.68a	0.66a	0.03	0.001
Fresh liver (g kg^{-1} BW$^{0.75}$)[4]	34.3b	46.6a	44.1a	1.88	0.001
Dry liver (g kg^{-1} NM)[5]	344	305	320	13.9	0.196

[1] 'OEM' = 'Orelha de Elefante Mexicana' spineless cactus. [2] SEM = Standard error of the mean. [3] Total digestible nutrients. [4] BW0.75 = metabolic body weight. [5] NM, natural matter. * Values from Lopes et al. (2020). # Values from T. G. P. Silva (2020). Averages followed by different letters in rows differ statistically by Tukey test (5%).

It was verified inflammatory processes and necrosis in the liver parenchyma of sheep fed any spineless cactus genotype (Table 4). The animals fed the 'Miúda' spineless cactus-based diet showed higher congestion (83.3%) concerning that fed 'OEM' spineless cactus and control diet (Table 4). Both 'Miúda' spineless cactus-based diet and control diet caused hepatitis (especially lymphocytic infiltrates). The supply of 'OEM' spineless cactus and control-based diets results in 83.3% liver parenchyma necrosis (Table 4).

Table 4

Relative (%) and absolute frequency of histopathological changes in the hepatic parenchyma of sheep fed with spineless cactus-based diets

Changes	Treatments		
	Control n=6	'Miúda' n=6	'OEM'1 n=6
Congestion	66.6 (4/6)	83.3 (5/6)	66.7 (4/6)
Hepatocyte degeneration	0.00 (0/6)	33.3 (2/6)	16.7 (1/6)
Steatosis	0.00 (0/6)	16.7 (1/6)	0.00 (0/6)
Sinusoidal Narrowing	0.00 (0/6)	0.00 (0/6)	16.7 (1/6)
Hepatitis	66.7 (4/6)	66.7 (4/6)	50.0 (3/6)
Kupffer cells Hypertrophy	50.0 (3/6)	16.7 (1/6)	16.7 (1/6)
Hepatocyte hypertrophy	0.00 (0/6)	0.00 (0/6)	16.7 (1/6)
Necrosis	100 (6/6)	83.3 (5/6)	100 (6/6)

[1] 'OEM' = 'Orelha de Elefante Mexicana' spineless cactus.

According to Stalker and Hayes (2007), this response occurs in different ways. Kupffer cells have distinct properties allowing inflammatory insults from the portal circulation, and the liver is a regulatory center that influences pro-inflammatory insults and inflammatory mediators. The hepatocytes can degenerate due to toxic lesions, infectious or inflammatory processes, nutritional deficiency, or severe metabolic disorders such as hypoxia (Kelly, 1993). Therefore, it is not possible to know if the source of the inflammatory insults is resulting from the diets offered or from preexisting insults. So, it is necessary to take a liver biopsy before the animal's entrance into the experiment, to investigate the animal origin and plants of the region.

The higher intake of oxalates in sheep fed with 'OEM' spineless cactus (100%) compared to 'Miúda' (83.3%) could explain the predominance of necrosis in the liver parenchyma (Table 3). According to Cruz et al. (2001), oxalate poisoning can cause hypocalcemia, renal failure, shallow breathing, bradycardia, affecting the hepatic system. T. G. P. Silva et al. (2021) also found similar results in goats fed 'OEM' spineless cactus-based diets.

The dietary oxalate levels found (4.00 to 4.90 g kg^{-1} DM) is below the toxic level (13.0 to 18.0 g kg^{-1} DM), according to Blaney et al. (1982) for sheep. T. G. P. Silva et al. (2021), evaluating the highest intake of total oxalates by goats fed 'OEM' spineless cactus (5.77 g kg^{-1} DM), observed intense pericentral lobular coagulative necrosis, due to the hydrocyanic acid (HCN) and total oxalates in the spineless cactus. S. M. C. Silva (2017) found similar results in sheep fed spineless cactus-based diets.

The presence of oxalates in the spineless cactus builds insoluble salts with Ca (calcium) and may affect the ingestion and digestion of sheep (Ben Salem et al., 2002). Nutritional deficiencies, kidney damage, and central nervous system damage, as a result of oxalate poisoning, can lead to the death of some species of domestic animals. It is suggested that the incidence of hepatic necrosis of sheep fed control diet may have been caused by the presence of other anti-nutritional factors in this plant, such as HCN. Juffo et al. (2012) observed that Tifton-grass (*Cynodon dactylon*) is a species involved in HCN poisoning. The necrosis of hepatocytes is probably due to the low oxygenation caused by HCN, which has an affinity of iron, decreasing the availability to binding oxygen in erythrocytes, breaking the respiratory chain, and causing histotoxic anoxia (Rufino, 2014).

Evaluating the focal distribution congestion and discrete intensity, there was no effect after offering the experimental diets, but diffuse necrosis with accentuated intensity in the liver parenchyma was observed (Tables 5 and 6), without affecting BW (Table 3). Considering the predominance of severe and diffuse coagulative necrosis (Tables 5 and 6) in animals fed 'Miúda' and 'OEM' spineless cactus-based diets, it is suggested that the liver responds to various injuries and the presence of inflammatory lymphocyte processes, including toxic lesions (Cullen, 2009). Gonzalez and Silva (2006) observed that the liver, kidneys, intestines, pancreas, bones, and the placenta have higher concentrations of alkaline phosphatase enzyme (AP) in cell membranes, present in biliary epithelial cells, and the canicular membranes of hepatocytes. These authors suggested that the serum AP can be originated by other isoforms, such as bone or renal origin, which makes it difficult to interpret the results. Diseases such as hepatic lipidosis and parenchymal inflammation also lead to obstruction of small bile canaliculi and indirect AP release.

Table 5

Medians of the distribution of histopathological changes in the liver parenchyma of sheep fed with spineless cactus-based diets

Changes	Treatments			*P*-value*
	Control n=6	'Miúda' n=6	'OEM'1 n=6	
Congestion	1.50	1.50	1.50	0.888
Hepatocyte degeneration	0	0	0	0.623
Steatosis	0	0	0	0.853
Sinusoidal narrowing	0	0	0	0.854
Hepatitis	3.00	2.00	0.50	0.697
Kupffer cells hypertrophy	1.50	0	0	0.446
Hepatocyte hypertrophy	0	0	0	0.854
Necrosis	3.50	5.00	5.00	0.474

[1] 'OEM' = 'Orelha de Elefante Mexicana' spineless cactus; * Kruskal-Wallis test at 5% of significance.

Table 6
Medians of the intensity of histopathological changes in the liver parenchyma of sheep fed with spineless cactus-based diets

Changes	Treatments			*P*-value*
	Control n=6	'Miúda' n=6	'OEM'1 n=6	
Congestion	0.50	1.00	1.00	0.816
Hepatocyte degeneration	0	0	0	0.623
Steatosis	0	0	0	0.853
Sinusoidal narrowing	0	0	0	0.854
Hepatitis	1.00	1.00	1.00	0.932
Kupffer cells hypertrophy	0.50	0	0	0.532
Hepatocyte hypertrophy	0	0	0	0.854
Necrosis	1.50	3.00	3.00	0.095

[1] 'OEM' = 'Orelha de Elefante Mexicana' spineless cactus; * Kruskal-Wallis test at 5% of significance.

The higher concentrations of AP enzyme are related to liver changes such as cholangitis or extrahepatic bile duct obstruction, biliary cirrhosis, and hepatocyte necrosis (Kaneko, 2008). S. M. C. Silva (2017) observed high activity of serum AP enzyme (519 ± 183 U L^{-1} and 562 ± 151 U L^{-1}) using 'Miúda' and 'OEM' spineless cactus-based diets. Kaneko (2008) suggested the average value of 68-387 U L^{-1} (178 ± 102 U L^{-1}) is in the normal range.

It is noteworthy that, despite the histopathological lesions observed in the liver of sheep that received diets containing the spineless cactus genotypes, there was no impairment in the weight gain of the animals (Table 3), which reinforces the possibility of using this cactus as strategic food for dry regions of the world.

Conclusion

The addition of the spineless cactus resistant to Dactylopius opuntiae in the diets causes damage to liver tissue, such as inflammation and death process of hepatocytes, but it does not compromise sheep weight gain.

Acknowledgments

This research was supported by Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) – Recife, PE, Brazil (Grant: APQ-0425-5.01/14), and by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

Association of Official Analytical Chemists (1990). *Official methods of analysis* (15nd ed.). AOAC.

Barboza, S. C. R., Oliveira, J. S., Carvalho, M. T., Lima, D. M., Jr., Lima, H. B., & Guerra, R. R. (2019). Ovines submitted to diets containing cassava foliage hay and spineless cactus forage: histological changes in the digestive and renal systems. *Tropical Animal Health and Production, 51*(6), 1689-1697. doi: 10.1007/s11250-019-01863-9

Ben Salem, H., Nefzaoui, A., & Bem Salem, L. (2002). *Opuntia ficus-indica F. inermis* and *Atriplex nummularia* L.: two complementary fodder shrubs for sheep and goats. *Acta Horticulturae, 581*(1), 333-341. doi: 10.17660/Acta Hortic.2002.581.39

Blaney, B. J., Gartner, R. J. W., & Head, T. A. (1982). The effects of oxalate in tropical grasses on calcium, phosphorus and magnesium availability to cattle. The *Journal of Agricultural Science, 99*(3), 533-539. doi: 10.1017/S0021859600031208

Bunch, S. E., Polak, D. M., & Hornbuckle, W. E. (1985). A modified laparoscopic approach for liver biopsy in dogs. *Journal of American Veterinary Medical Association, 187*(10), 1032-1035.

Cruz, C. M. O., Guerreiro, C. I. P. D., & Reis, T. A. F. C. (2001). *Substâncias tóxicas e anti-nutricionais dos alimentos para animais.* http://www.alpetratinia.net/consulting/Downloads/SubsToxicas.pdf

Cullen, J. M. (2009). Fígado, sistema biliar e pâncreas exócrino. In M. D. McGavin, & J. F. Zachary (Eds.), *Bases da patologia em veterinária* (4nd ed., pp. 393-462). Rio de Janeiro.

Detmann, E., Souza, M. A., Valadares, S. C., Queiroz, A. C., Berchielli, T. T., Saliba, E. O. S., E. O. S.; Cabral, L. S.; Pina, D. S.; Ladeira, M., & Azevêdo, J. A. G. (2012). *Métodos para análise de alimentos.* Instituto Nacional de Ciência e Tecnologia de Ciência Animal. Suprema.

Dubeux, J. C. B., Jr., Santos, M. V. F., Cavalcante, M., & Santos, D. C. (2013). Potencial da palma forrageira na América do Sul. *Cactusnet Newsletter, 13*(Esp.), 29-40.

Gonzalez, F. H. D., & Silva, S. C. (2006). Introdução à bioquímica clínica veterinária (2nd ed.). Editora da Universidade Federal do Rio Grande do Sul.

Haas, L. S. (2014). *Histomorfometria hepática como método para predição de eficiência alimentar em bovinos de corte.* Trabalho de conclusão de curso de graduação, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. https://www.lume.ufrgs.br/handle/10183/104875

Hall, M. B. (2000). *Neutral detergent-soluble carbohydrates. Nutritional relevance and analysis.* University of Florida.

Instrução Normativa n° 3 (2000). Ministério da Agricultura, Pecuária e do Abastecimento (MAPA). Secretaria da Defesa Agropecuária (SDA). Departamento de Inspeção de Produtos de Origem Animal (DIPOA). Divisão de Normas Técnicas. Instrução Normativa n. 3, de 17 de janeiro de 2000. *Aprova o Regulamento Técnico de Métodos de Insensibilização para o Abate Humanitário de Animais de Açougue.* Lex: Diário Oficial da União de

24 de janeiro de 2000, Seção 1,14-16. Brasília.

Juffo, G. D., Pavarini, S. P., Wouters, F., Oliveira, L. G. S., Antoniassi, N. A. B., Cruz, C. E. F., & Driemeier, D. (2012). Intoxicação espontânea por *Sorghum sudanense* em bovinos leiteiros no Rio Grande do Sul. *Pesquisa Veterinária Brasileira, 32*(3), 217-220. doi: 10.1590/S0100-736X2012000300006

Kaneko, J. J., Harvey, J. W., & Bruss, M. L. (2008). *Clinical biochemistry of domestic animals* (6nd ed.). Academic Press.

Kelly, W. R. (1993). The liver and biliary system. In K. V. F. Jubb, P. C. Kennedy, & N. Palmer, *Pathology of domestic animals* (4nd ed., vol. 2, pp. 319-406). New York.

Lima, T. J., Costa, R. G., Medeiros, G. R., Medeiros, A. N., Ribeiro, N. L., Oliveira, J. S. O., Guerra, R. R. & Carvalho, F. F. R. (2019). Ruminal and morphometric parameters of the rumen and intestines of sheep fed with increasing levels of spineless cactus (*Nopalea cochenillifera* Salm-Dyck). *Tropical Animal Health and Production, 52*(2), 363-368. doi: 10.1007/s11250-018-1697-1

Lopes, L. A., Ferreira, M. A., Batista, A. M. V., Maciel, M. V., Andrade, R. B., Munhame, J. A., Silva, T. G. P., Cardoso, D. B., Véras, A.S.C., & Carvalho, F. F. R. (2020). Intake, digestibility, and performance of lambs fed spineless cactus cv. Orelha de Elefante Mexicana. *Asian-Australasian Journal of Animal Sciences, 33*(8), 1284-1291. doi: 10.5713/ajas.19.0328

Marques, A.V. S., Soares, P. C., Riet-Correa, F., Mota, I. O., Silva, T. L. A., Borba Neto, A. V., Soares, F. A. P., & Alencar, S. P. (2011). Teores séricos e hepáticos de cobre, ferro, molibdênio e zinco em ovinos e caprinos no estado de Pernambuco. *Pesquisa Veterinária Brasileira, 31*(5), 398-406. doi: 10.1590/S0100-736X2011000500006

Moir, K. W. (1953). The determination of oxalic acid in plants. *Queensland Journal of Agricultural and Animal Sciences, 10*(1), 1-3.

National Research Council (2007). *Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids* (7nd ed.). National Academic Press.

Radostits, O. M., Gay, C. C., Blood, D. C., & Hinchcliff, K. W. (2002). *Clínica veterinária: um tratado de doenças dos bovinos, ovinos, suínos, caprinos e equinos* (9nd ed.). Guanabara Koogan.

Rufino, M. N. (2014). *Sintomatologia clínica, alterações bioquímicas e teciduais observados após administração oral de acetona cianidrina no modelo murino.* Dissertação de mestrado, Universidade Católica de Dom Bosco, Campo Grande, MS, Brasil. https://docplayer.com.br/6731693-Sintomatologia-clinica-alteracoes-bioquimicas-e-teciduais-observados-apos-administracao-oral-de-acetona-cianidrina-no-modelo-murino.html

Santos, R. D., Neves, A. L. A., Santos, D. C., Pereira, L. G. R., Gonçalves, L. C., Ferreira, A. L., & Sollenberger, L. E. (2018). Divergence in nutrient concentration, in vitro degradation and gas production potential of spineless cactus genotypes selected for insect resistance. *The Journal of Agricultural Science, 156*(3), 450-456. doi: 10.1017/S002185961800031X

Silva, C. C. F., & Santos, L. C. (2006). Palma forrageira (*Opuntia Fícus- Indica* Mill) como alternativa na alimentação de ruminantes. *Revista Electrónica de Veterinária, 8*(5), 1-11.

Silva, S. M. C. (2017). *Histopatologia e morfometria do fígado de ovinos alimentados com palma forrageira resistente à cochonilha do carmim.* Dissertação de mestrado, Universidade Federal Rural de Pernambuco, Recife, PE, Brasil. http://www.tede2.ufrpe.br:8080/tede2/handle/tede2/6955

Silva, T. G. P. (2020). *Alterações metabólicas e histológicas em caprinos e ovinos alimentados com dietas contendo palma forrageira.* Tese de doutorado, Universidade Federal Rural de Pernambuco, Recife, PE, Brasil. http://www.ppgz.ufrpe.br/sites/default/files/testes-dissertacoes/tese_tomas_guilherme_ppgz_ 2020_ sem_assinaturas.pdf

Silva, T. G. P., Batista, A. M. V., Guim, A., Silva, V. A., Jr., Carvalho, F. F. R., Barros, M. E. G., Sousa, D. R., & Silva, S. M. C. (2019). Histomorphometric changer of the fora-stomach of lambs fed with diets containing spineless cactus genotypes resistant to *Dactylopius* sp. *Tropical Animal Health and Production, 52*(3), 1299-1307. doi: 10.1007/s11250-019-02129-0

Silva, T. G. P., Munhame, J. A., Lopes, L. A., Souza, F. A. L., Guim, A., Carvalho, F. F. R., Soares, P. C., Barros, M. E. G., Arandas, J. K. G., & Batista, A. M. V. (2021). Liver status of goats fed with cactus cladodes genotypes resistant to *Dactylopius opuntiae. Small Ruminant Research, 198*(3), 106359. doi: 10. 1016/j.smallrumres.2021.106359

Stalker, M. J., & Hayes, M. A. (2007). Liver and biliary system. In M. G. Maxie, *Pathology of domestic animals* (5nd ed.). Sounders Elsevier.

Statistical Analysis System (1999). *User's guide, version 8.* SAS Institute Inc.

Tostes, R. A., & Bandarra, E. P. (2002). A biopsia hepática em cães. *Revista do Conselho Federal de Medicina Veterinária e Zootecnia, 8*(27), 35-41.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*(10), 3583-3597. doi: 10.3168/jds.S0022-0302(91)78551-2

Weiss, W. P. (1999). Energy prediction equations for ruminant feeds. *Proceedings of the Cornell Nutrition Conference Feed Manufactures,* Ithaca, NY, EUA, 61.