Effects of *Brachiaria brizantha* Hay Containing a Steroidal Saponin in Lambs

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Abstract

Several species of *Brachiaria* are important forages in tropical regions of Brazil and elsewhere worldwide. Some species of *Brachiaria* have been reported to cause hepatogenous photosensitization in ruminants. Initially, the disease was attributed to *Pithomyces chartarum* fungus, but recent studies suggest that the steroidal saponins present in the grasses have toxic principles responsible for the photosensitization. The objective of this study was to evaluate hepatic function and the performance of lambs fed with *B. brizantha* hay or sugar cane (*Saccharum officinarum* L.), using clinical examinations, laboratory tests, and macro and microscopic analysis of the liver. Twelve Saint Ines lambs were used. The animals were divided into two experimental groups: group hay (six lambs fed with *B. brizantha* hay, plus a concentrate feed) and group sugar cane (six lambs fed with roughage from sugar cane with added concentrate feed). The hay and sugar cane used to feed the lambs did not contain *Pithomyces chartarum* spores. The *B. brizantha* hay contained 0.86% protodioscin, and the animals received a daily protodioscin dose of 200 mg/kg BW. The clinical examination occurred every 7 days, the laboratory tests were done every 14 days, and the animals were weighed every 21 days. At the end of 93 days of feeding, the lambs were slaughtered, the macroscopic analysis of the organs was carried out, and the liver fragments were collected for histological analysis. The lambs were clinically healthy during the whole period, except at the beginning of the experiment when some animals had pneumonia. The only biochemistry alteration suggestive of hepatic damage was an increase ($P < 0.05$) of the GGT values in both groups. No animal fed with *B. brizantha* hay showed any macroscopic alterations in the liver. Histological analysis of the liver revealed preserved hepatocytes and the presence of light multifocal infiltration of mononuclear inflammatory cells in the hepatic parenchyma and also in the portal space, indicating mild cholangitis in both groups. Degeneration changes suggestive of hepatic steatosis were observed in four animals fed with sugar cane. Feeding lambs with *B. brizantha* hay promoted similar performance as feeding animals with sugar cane. We conclude that feeding *B. brizantha* hay containing the concentration of steroidal saponins used in this study to lambs does not cause toxicity.

Keywords: *Brachiaria brizantha*, cholangitis, gamma glutamyltransferase, GGT, hepatogenous photosensitization, protodioscin, saponin

Introduction

In recent years the sheep industry in Brazil has grown significantly compared with the number of cattle, probably due to the numerous advantages offered such as the possibility to produce animals in

small holdings, lower individual animal forage intake, ease of handling, and the production of quality products such as milk, meat, and leather (Oliveira 2001).

Livestock losses can be attributed to the decrease in performance as a result of infections, poisoning, genetic and nutritional diseases, or deaths. In the Brazilian cattle industry, as well as in many other countries, a significant cause of loss is by the ingestion of poisonous plants. It is estimated that 9 to 16 percent of cattle deaths in Brazil is from poisonous plants (Assis et al. 2010).

Weight loss or the inability to gain weight in livestock may be due to clinical and subclinical diseases that affect the animal's health, which can sometimes lead to death. Among these are diseases that cause liver damage, such as hepatogenous photosensitivity, which may occur from the toxin produced by *Pithomyces chartarum* fungus (Di Menna et al. 2009) or may be caused by steroidal saponins often found in forage from the genus *Brachiaria*. Saponins have been associated with the deposition of crystalloid material in the biliary system (Brum et al. 2007).

At present, the progression from ingestion of steroidal saponins in *Brachiaria* spp. to pathogenesis and hepatic lesions in ruminants is unclear. The objective of this research was to conduct a controlled study by feeding a known amount of the steroidal saponin protodioscin in *Brachiaria brizantha* hay to lambs and to evaluate clinical signs of toxicity in conjunction with macro and histological analysis of potential liver lesions.

Materials and Methods

Twelve 104-day-old Santa Ines lambs were used. The animals were divided into two groups: the *Brachiaria* Group (BG)—six lambs fed with hay from *Brachiaria brizantha* Hochst ex A. Rich.) Stapf. cv. Marandu and the control Sugar Cane Group (SCG)—six lambs fed with roughage made from ground sugar cane (*Saccharum officinarum L.*) stems and leaves, *in natura*.

The experiment was conducted over 93 days at the experimental facility for ruminants at the Veterinary School at the Federal University of Goiás, Brazil, located in the city of Goiânia, from June to September 2008. This animal study was conducted under veterinary supervision under animal ethics protocols established by the Brazilian Society of Laboratory Animal Science (SBCAL, formerly Brazilian College of Animal Experimentation/ COBEA).

Concentrate feed (finely ground corn, soybean, and cottonseed) was used to balance the nutritional requirements of the two groups of animals and to minimize the nutritional differences between the two diets. The amount of total feed provided was limited to 4 percent of body weight. The proportion of roughage and concentrate was 62:38, and this amount was recalculated after every weighing of the animals.

For saponin analysis, samples were collected of fresh plant, freshly dried hay, and hay after storage for 3 months. Samples were dried in a forced air oven at 65°C for 72 hours, ground to pass a 2 mm screen in a Wiley mill, and stored in airtight bags until analysis. Saponin extraction and analysis were conducted at the Biological Institute, São Paulo, Brazil. The extraction was made in duplicate. Each sample (1 g) was extracted 3 times in 50% acetonitrile and water (v/v), using volumes of 8 mL, 5 mL, and 3 mL under agitation with a sonic agitator for 30 min, 30 min, and 20 min, respectively. Next the extracts were transferred to a test tube and centrifuged for 30 minutes at 5,000 rpm at 13ºC. The extracted solution was completed to 10 mL for analysis using HPLC/ELSD (high-performance liquid chromatograph/evaporative light scattering).

Saponin was quantified using a modification of the technique of Ganzera et al. (2001). The analysis was done with a Shimadzu HPLC model LC-10AD coupled to the detector; the evaporative light scattering from the Shimadzu model ELSD-LTs was used for quantitative analysis of protodioscin. The column used was a Shim-Pack CLC-ODS (4.6 x150 mm, 5.0 µm), using a gradient system with acetonitrile (B)/water (A), starting at 20% B, 5 min; 35% B, 12.5 min; and 20% B, 5 min; flow 1 mL/min, injected with 10 µL, using a 10 µL loop.

The only saponin detected in *B. brizantha* was protodioscin (Brum et al. 2009). The BG received 62 percent of their daily ration from *B. brizantha* hay at 4 percent of their body weight, which corresponded to a daily average protodioscin dose of 200 mg/kg BW for the 93-day experiment. There were no detectable levels of protodioscin in sugar cane.

The animals involved in this research had not been previously fed with *Brachiaria brizantha* forage or with sugar cane; neither the hay nor the sugar cane contained *Pithomyces chartarum* spores.

Animals were weighed every 21 days after an overnight fast. After slaughter, the carcass was weighed to determine the yield. The liver was weighed separately. Clinical evaluations were made weekly according to the protocol adapted from Rosenberger (1983).

Blood was sampled every 14 days using jugular venipuncture beginning on day zero to day 70 except for the final collection, which occurred on day 93 at the end of the study. Samples were handled by standard methods for serum and whole blood. The tests performed were hemogram, fibrinogen, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), total, conjugated and non-conjugated bilirubin, total protein, urea, creatinine, total cholesterol, glucose, and protein separation by electrophoresis. The results of red blood cells (RBC) were compared with those of Ferreira (2002), white blood cells (WBC) with the values described by Jain (1993) for sheep, and biochemistry and electrophoresis, respectively, with Kaneko et al. (2008) and Green et al. (1982).

Macroscopic examination of animals during slaughter was done by evaluating the lungs, lymph nodes, liver, gallbladder, bladder, kidney, pre-stomach compartments (rumen, reticulum, and omasum), abomasum, and intestines (small and large). Liver samples were collected (left lobe) for microscopic analysis.

The liver samples were fixed in 10% buffered formalin and, after 48 hours, stored in 70% ethanol. Subsequently, they were processed according to routine laboratory standards and stained with hematoxylin and eosin. We conducted a descriptive analysis of the lesions observed in the parenchyma and portal areas.

Statistical analysis between the two treatment groups for various blood variables was done using the non-parametric Mann-Whitney U test at significant levels of 5 percent.

Results

The level of protodioscin in the fresh plant was 1.87 percent; however, both the freshly-made *Brachiaria* hay and hay after storage had protodioscin concentrations of 0.86 percent.

The lambs exhibited normal behavior and food intake throughout the experiment, except for a reduction in food intake near the end of the study when the rainy season began. Three lambs in the SCG and one in the BG had pneumonia at the beginning of the experiment and were immediately treated with florfenicol and flunixin meglumine. No animal showed any further signs of any other diseases.

The clinical evaluations did not reveal any differences between treated animals, nor were there differences in RBC, hemoglobin, hematocrit, WBC, or fibrinogen (table 1). The initial and final weight of the BG lambs was 28 kg (±3.1) and 35.5 kg (±5.1), respectively, whereas the initial weight and final weight for the SCG lambs was 27 kg (±2.4) and 37 kg (±3.7), respectively ($P > 0.05$). Carcass yield (dressing %) was similar between the groups (BG = 46.14±6.55% and SCG = 46.17±1.41%), as was the weight of the liver (BG = 0.55±0.12 kg and SCG = 0.57±0.11 kg).The macroscopic evaluations of the BG animals showed that the liver, gallbladder, and bile were normal; one animal had pale spots on the cortical surface of the kidneys and one animal showed an area of rumen papillae atrophy and hyperemia. In the SCG, one animal showed an increased liver lobular pattern and another showed a pale liver; one animal showed lesions suggesting interstitial nephritis; and another animal showed focal areas of ulcers and scars 1 cm in diameter in the ruminal mucosa. The gallbladder and bile of all animals were within normal limits.

The evaluation of lungs in both groups proved to be consistent with the clinical evaluation. The lungs of animals suffering from pneumonia had areas of consolidation, hyperemia, emphysema, and in some cases, abscesses.

Histological evaluation of both groups revealed preserved hepatocytes and the presence of light multifocal mononuclear cell infiltration in the liver parenchyma and portal space. In the SCG, four animals were found to have micro and macrovacuolar degeneration suggesting hepatic steatosis, restricted to zones 1 and 2 of the hepatic acinus. None of the groups showed the presence of foamy macrophages, bile pigments, or crystals. The presence of mononuclear infiltrate in the portal space, usually around the bile ducts, suggested mild cholangitis.

Discussion

The naive lambs never exposed to *Brachiaria* forage were chosen because these animals were considered to be most susceptible to poisoning (Riet-Correa and Mendez 2007). However, lambs in the BG fed with *B. brizantha* hay showed no clinical signs of poisoning, demonstrating that, under these experimental conditions, the protodioscin dose of 200mg/kg BW was not toxic. In another study, forage levels of protodioscin above 0.3 percent were enough to poison sheep of the same breed (Castro et al. 2011). Other studies report that, in spite of an increase in some liver enzymes from 42.8 to 100 percent in sheep experimentally intoxicated by *Brachiaria* spp., these same animals did not show

Table 1. Average and standard deviations for AST, ALP, GGT, conjugated bilirubin (CB), unconjugated bilirubin (UCB), total bilirubin (TB), glucose, total cholesterol, total protein, albumin, alpha 1, alpha 2, beta and gamma globulins, albumin/globulin (A/G), urea and creatinine, for *Brachiaria*-fed (BG) lambs and lambs fed sugar cane (SCG) over 93 days

Biochemistry Test	Group	A/SD	D0	D14	D28	D42	D56	D70	D93	Ref
AST U/L	BG	A	131.00*	85.00	99.17	100.00	93.00	102.50*	98.17	60-280[1]
		SD	16.69	15.17	7.36	24.51	6.93	18.45	3.76	
	SCG	A	101.33	85.00	94.83	106.00	88.67	84.17	96.67	
		SD	14.76	17.33	21.70	32.22	8.85	4.22	13.68	
ALP U/L	BG	A	130.17	78.67	62.17	60.67	137.00	165.67	156.50	68-387[1]
		SD	46.58	14.95	19.08	34.15	62.50	64.08	77.17	
	SCG	A	126.67	85.83	57.50	71.50	194.17	176.67	147.00	
		SD	42.26	37.10	21.04	42.28	89.28	29.86	26.45	
GGT U/L	BG	A	52.65	60.33	48.85	29.08*	52.18	49.97	81.85	20-52[1]
		SD	14.37	22.37	13.78	6.65	9.77	13.41	26.01	
	SCG	A	64.54	69.07	57.27	48.57	54.28	51.45	72.68	
		SD	26.68	18.17	12.80	7.24	14.70	13.46	21.17	
CB µmol/L	BG	A	0.74	0.83	0.91	1.20	0.88	1.71	1.77	0.0-4.61[1]
		SD	0.21	0.41	0.55	0.96	0.74	1.70	1.92	
	SCG	A	0.68	1.08	1.17	0.74	0.74	1.11	1.08	
		SD	0.11	0.92	1.04	0.34	0.26	1.24	0.86	
UCB µmol/L	BG	A	4.67	3.88	5.73	2.57*	3.68	2.65	3.62	0.0-2.05[1]
		SD	1.88	2.36	2.42	1.04	0.95	1.92	0.95	
	SCG	A	5.02	4.53	4.87	4.59	4.45	3.59	3.65	
		SD	1.29	2.08	1.66	0.91	0.47	1.35	1.81	
TB µmol/L	BG	A	5.42	4.70	6.64	3.76*	4.56	4.36	5.39	1.71-8.55[1]
		SD	2.04	1.96	2.40	0.96	0.85	1.68	1.39	
	SCG	A	5.70	5.61	6.04	5.33	5.19	4.70	4.73	
		SD	1.31	1.81	1.38	0.90	0.35	0.89	1.29	
Glucose mmol/L	BG	A	3.90	3.60	3.98	2.79	3.18	3.53	3.88	2.78-4.44[1]
		SD	0.53	0.15	0.43	1.61	0.45	0.38	0.60	
	SCG	A	3.58	3.87	4.23	4.73	3.31	3.14	3.92	
		SD	1.94	0.36	0.30	1.29	0.13	0.35	0.81	
Cholesterol (total) mmol/L	BG	A	1.71	1.23*	1.19	1.25*	1.24*	1.50*	1.09*	1.35-1.97[1]
		SD	0.31	0.09	0.59	0.25	0.17	0.25	0.07	
	SCG	A	1.32	0.88	1.13	0.60	0.71	0.67	0.63	
		SD	0.30	0.31	0.34	0.23	0.22	0.44	0.12	
Total Protein g/L	BG	A	70.75*	80.67	81.50	61.50	72.00	71.83*	78.00*	60-79[1]
		SD	2.36	6.19	16.67	8.69	10.55	6.46	10.71	
	SCG	A	62.67	76.17	66.50	54.17	65.00	65.00	63.00	
		SD	4.61	2.56	3.94	4.71	5.48	2.19	6.32	
Albumin g/L	BG	A	37.00	40.00	41.00	27.00	39.00	39.00	44.00	24-30[1]
		SD	3.00	3.00	8.00	2.00	6.00	3.00	7.00	
	SCG	A	34.00	39.00	33.00	29.00	36.00	37.00	36.00	
		SD	2.00	4.00	4.00	4.00	3.00	3.00	3.00	
Alpha 1 g/L	BG	A	8.00	8.00	8.00	5.00	5.00	5.00	6.00	2.7-4.1[2]
		SD	1.00	1.00	2.00	1.00	1.00	1.00	1.00	
	SCG	A	6.00	7.00	6.00	4.00	5.00	5.00	4.00	
		SD	1.00	1.00	1.00	0.00	1.00	0.00	1.00	
Alpha 2 g/L	BG	A	10.00	11.00	10.00	9.00	8.00	9.00	9.00	4.9-5.8[2]
		SD	1.00	1.00	2.00	1.00	1.00	2.00	1.00	
	SCG	A	9.00	10.00	10.00	7.00	8.00	8.00	8.00	
		SD	0.00	1.00	2.00	1.00	1.00	1.00	1.00	
Beta g/L	BG	A	5.00	5.00	4.00	4.00	3.00	3.00	3.00	4.0-14.0[1]
		SD	1.00	1.00	1.00	2.00	1.00	0.00	1.00	
	SCG	A	3.00	4.00	4.00	2.00	3.00	3.00	3.00	
		SD	3.00	1.00	1.00	1.00	0.00	1.00	1.00	

Biochemistry Test	Group	A/SD	D0	D14	D28	D42	D56	D70	D93	Ref
Gamma g/L	BG	A	12.00	17.00	18.00	16.00	17.00	16.00	16.00	14.4-20.7[2]
		SD	2.00	4.00	6.00	6.00	4.00	3.00	3.00	
	SCG	A	12.00	15.00	15.00	12.00	14.00	12.00	12.00	
		SD	4.00	3.00	3.00	2.00	2.00	2.00	2.00	
A/G	BG	A	11.00	10.00	10.00	8.00	12.00	12.00	13.00	4.2-7.6[1]
		SD	2.00	1.00	1.00	2.00	1.00	1.00	1.00	
	SCG	A	12.00	11.00	10.00	12.00	12.00	14.00	14.00	
		SD	2.00	2.00	3.00	3.00	1.00	2.00	0.00	
Urea mmol/L	BG	A	7.75	6.63	8.36	6.90*	7.19	7.35	9.15	2.86-7.14[1]
		SD	1.13	1.27	3.76	1.75	1.01	2.47	3.28	
	SCG	A	7.49	5.88	6.96	10.81	7.55	10.20	8.77	
		SD	1.59	1.94	2.11	1.73	1.08	2.76	2.74	
Creatinine µmol/L	BG	A	82.51	91.35	86.93	71.90	101.66	81.03	76.61	106-168[1]
		SD	6.04	12.08	6.65	33.95	4.84	6.65	16.46	
	SCG	A	81.77	85.45	82.51	67.77	95.77	75.14	69.25	
		SD	9.17	9.13	12.08	7.22	11.75	18.33	8.69	

A/SD = average and standard deviation. Ref = Reference. D=day of the study.
[1] Kaneko et al. (2008)
[2] Green et al. (1982)
*Statistical analysis was done using the Mann-Whitney U Test.
Asterisks indicate that the treatment groups differ at $P < 0.05$ during the same period.

clinical signs of hepatogenous photosensitization (Cruz et al. 2001, Saturnino et al. 2010, Castro et al. 2011).

In this study, there were no differences between the *Brachiaria*-fed lambs and the control lambs in terms of body weight, nor were there differences for carcass yield and liver weight. In contrast, other work has shown that sheep given steroidal saponins in *Panicum miliaceum* forage, while maintaining a normal appetite, did lose some body weight, and this loss progressed to cachexia (Badiei et al. 2009).

Consistent with the pneumonia that we observed at the beginning of the study, the initial leucocyte counting and electrophoresis revealed an acute inflammatory process that gradually decreased until it was chronic at the end of the experiment. The treatments did not influence the inflammatory response because both groups showed the same trends. Saturnino et al. (2010) evaluated the toxicity to Santa Ines sheep from *B. decumbens* forage in a feedlot and also reported cases of pneumonia.

In this study, AST values were within physiological limits for both groups. When studying hepatogenous photosensitization in sheep fed with *Brachiaria* spp., Castro et al. (2011), Saturnino et al. (2010), Mendonça et al. (2008), and Brum et al. (2007) also found values of AST very close to the reference limit. AST is an enzyme that has two isoenzymes, one mitochondrial and one cytoplasmic. Together they determine the integrity of the hepatocyte, but AST is only elevated in acute liver injury, immediately returning to the reference limits (Kaneko et al. 2008).

The increased values of serum GGT activity in both groups suggested lesions in the biliary epithelium, which were confirmed in the histological analysis. We noted the presence of mononuclear cell infiltration in portal spaces that featured mild cholangitis. The elevation of GGT has been a recurrent finding in sheep poisoned by *Brachiaria* spp. in the absence of *P. chartarum* spores (Lemos et al. 1996, Brum et al. 2007, Mendonça et al. 2008, Albernaz et al. 2010, Assumaidaee et al. 2010, Saturnino et al. 2010, Castro et al. 2011). Interestingly, Cruz et al. (2011) administered fractionated extracts of *B. decumbens* and observed no changes in serum GGT activity and no clinical signs; however, their animals showed experimentally induced cholangiohepatopathy. According to Blackshaw (1978) and Pearson (1993), GGT shows sensitivity and specificity and almost always is high in cases of chronic liver disease. Increased GGT is directly related to the death of sheep poisoned by *B. decumbens* and can be used as a parameter to predict the onset of clinical signs and death of sheep that ingest this forage (Saturnino et al. 2010).

The high levels of unconjugated bilirubin and albumin reported here were not attributed to *Brachiaria* forage because these variables were elevated in both groups. The primary cause of hyperalbuminemia is dehydration (Russell and

Roussel 2007), but the animals showed no signs of dehydration.

The serum urea values were above the reference limits in most periods in both groups. The high levels of urea in this study can be attributed to the diets fed to both groups. The possibility of elevated levels of urea because of renal failure was discarded by analyzing creatinine, which was at or below reference levels. Castro et al. (2011), evaluating the same breed of sheep fed with *B. brizantha* and *B. decumbens*, found increased levels of urea and creatinine.

In several studies of sheep intoxicated by *Brachiaria* spp., histological findings in the liver have shown multifocal areas of pallor, hepatomegaly, printing on the surface of the ribs, hepatic lobular pattern, jaundiced and distended gallbladder, dark colored bile, and increased density (Lemos et al. 1996, Cruz et al. 2001, Driemeier et al. 2002, Brum et al. 2007, Mendonça et al. 2008, Albernaz et al. 2010, Assumaidaee et al. 2010, Saturnino et al. 2010, Castro et al. 2011). Even so, none of these lesions were found in the BG animals in our study, and Castro et al. (2011) also reported no histopathological changes in sheep poisoned by *Brachiaria* spp.

In the present study, the presence of multifocal mononuclear infiltrates in the parenchyma and portal areas (mild cholangitis) showed that the structure of hepatocytes and bile duct were preserved. Several studies have described the presence of mononuclear infiltrates around the portal space but were followed by other findings characteristic of intoxication, such as infiltrates of foamy macrophages and lymphocytes in the parenchyma and portal spaces, bile duct proliferation accompanied by epithelial degeneration, necrosis and hyperplasia, multifocal areas of cholangitis with accumulation of bile pigments, presence of crystals within the bile ducts and macrophages, diffuse swelling and vacuolization of hepatocytes (Lemos et al. 1996, Cruz et al. 2001, Driemeier et al. 2002, Brum et al. 2007, Mendonça et al. 2008, Albernaz et al. 2010, Assumaidaee et al. 2010, Saturnino et al. 2010, Castro et al. 2011).

Degenerative changes only present in the SCG can be attributed to the high availability of carbohydrates from the sugar cane at amounts substantially higher than in *Brachiaria* hay, which could have caused a lipid overload in the liver causing hepatic steatosis (Santos 1986). Future studies should evaluate the effect of sugar cane on liver function in sheep.

We conclude that lambs fed with *Brachiaria brizantha* hay containing 0.86 percent of protodioscin showed no decrease in performance and did not show clinical signs of poisoning. Other comparable studies will need to be conducted using known types and concentrations of steroidal saponins to clarify the etiology of hepatogenous photosensitization in ruminants consuming *Brachiaria* spp. forage.

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References

Albernaz, T.T., J.A.S. Silveira, N.S. Silva, et al. 2010. Fotossensibilização em ovinos associada à ingestão de *Brachiaria brizantha* no estado do Pará. *Pesquisa Veterinária Brasileira* 30(9):741-748.

Assis, T. S., R.M.T. Medeiros, F. Riet-Correa, et al. 2010. Intoxicações por plantas diagnosticadas em ruminantes e equinos e estimativa das perdas econômicas na Paraíba. *Pesquisa Veterinária Brasileira* 30(1):13-20.

Assumaidaee, A.A., M. Zamri-Saad, S. Jasni, and M.M. Noordin. 2010. The role of oxidative stress in *Brachiaria decumbens* toxicity in sheep. *Pertanika Journal of Tropical Agricultural Science* 33(1):151-157.

Badiei, K., K. Mostaghni, S. Nazifi, et al. 2009. Experimental *Panicum miliaceum* poisoning in sheep. *Small Ruminant Research* 82:99-104.

Blackshaw, C. 1978. Serum gamma glutamyltransferase in the diagnosis of liver disease in cattle**.** *New Zealand Veterinary Journal* 26:25-26.

Brum, K.B., M. Haraguchi, R.A.A. Lemos, et al. 2007. Crystal-associated cholangiopathy in sheep grazing *Brachiaria decumbens* containing the saponin protodioscin. *Pesquisa Veterinária Brasileira* 27(1):39-42.

Brum, K.B., M. Haraguchi, M.B. Garutti, et al. 2009. Steroidal saponin concentrations in *Brachiaria decumbens* and *B. brizantha* at different developmental stages. *Ciencia Rural* 39(1):279-281.

Castro, M.B., H.L. Santos, Jr., V.S. Mustafa, et al. 2011. *Brachiaria* spp. poisoning in sheep in Brazil: experimental and epidemiological findings. *In* F. Riet-Correa, J. Pfister, A.L. Schild, T. Wierenga, eds., *Poisoning by Plants, Mycotoxins and Related Toxins*, pp. 110-117. CAB International, Wallingford, U.K.

Cruz, C., D. Driemeier, V.S. Pires, E.P. Schenkel. 2001. Experimentally induced cholangiopathy by dosing sheep

with fractionated extracts from *Brachiaria decumbens*. *Journal of Veterinary Diagnostic Investigation* 13:170-172.

Di Menna, M.E., B.L. Smith, and C.O. Miles. 2009 A history of facial eczema (pithomycotoxicosis) research. *New Zealand Journal of Agricultural Research* 52(4):345-376.

Driemeier, D., E.M. Colodel, A.L. Seitz, et al. 2002. Study of experimentally induced lesions in sheep by grazing *Brachiaria decumbens*. *Toxicon* 40:1027-1031.

Ferreira, A.F. 2002. Valores de referência do eritrograma e teores plasmáticos da proteína total e fibrinogênio de ovinos (*Ovis aries*, linnaeus, 1758) da raça Santa Inês, criados na mesorregião metropolitana de Recife: Influência dos fatores sexual e etário. Publication from the Universidade Federal Rural de Pernambuco, Recife, Brazil.

Ganzera, M., E. Bedir, and I.A. Khan. 2001. Determination of steroidal saponins in *Tribulus terrestris* by reversed-phase high-performance liquid chromatography and evaporative light scattering detection. *Journal of Pharmaceutical Science* 90:1752-1758.

Green, S.A., S.J. Jenkins, and P.A. Clark. 1982. A comparison of chemical and electrophoretic methods of serum protein determinations in clinically normal domestic animals of various ages. *Cornell Veterinarian* 72(4):416-426.

Jain, J.C. 1993. *Essentials of Veterinary Hematology*. Lea & Febiger, Philadelphia, PA.

Kaneko, J.J., J.W. Harvey, and M.L. Bruss. 2008. *Clinical Biochemistry of Domestic Animals*, 6th edition. Elsevier, San Diego, CA.

Lemos, R.A.A., L.C.L. Ferreira, S.M. Silva, et al. 1996. Fotossensibilização e colangiopatia associada a cristais em ovinos em pastagem com *Brachiaria decumbens*. *Ciencia Rural* 26(1):109-113.

Mendonça, F.S., L.M. Camargo, S.H. Freitas, et al. 2008. Aspectos clínicos e patológicos de um surto de fotossensibilização hepatógena em ovinos pela ingestão de *Brachiaria decumbens* (Gramineae) no município de Cuiabá, Mato Grosso. *Ciencia Animal Brasileira* 9(4):1034-1041.

Oliveira, C.A.A. 2001. Influência da temperatura e do tempo de estocagem nas avaliações dos níveis séricos das proteínas totais, albumina, uréia e creatinina em ovinos e caprinos. Universidade Federal Rural de Pernambuco, Recife, Brazil.

Pearson, E.G. 1993. Moléstias do sistema hepatobiliar. *In* B.P. Smith, ed., *Tratado de Medicina Interna de Grandes Animais*, pp. 839-872. Manole, São Paulo, Brazil.

Riet-Correa, F., and M.D.C. Mendez. 2007. Intoxicações por plantas e micotoxinas. *In* F. Riet-Correa, A.L. Schild, R.A.A. Lemos, and J.R.J. Borjes, eds., *Doenças de Ruminantes e Equídeos*, pp. 99-222. Pallotti, Santa Maria, Brazil.

Rosenberger, G. 1983. *Exame Clínico dos Bovinos*, 2nd edition. Guanabara Koogan, Rio de Janeiro, Brazil.

Russel, K.E., and A.J. Roussel. 2007. Evaluation of the ruminant serum chemistry profile. *Veterinary Clinics of North America-Food Animal Practice* 23:403-426.

Santos, J.A. 1986. *Patologia Especial dos Animais Domésticos (Mamíferos e Aves)*, 2nd edition. Guanabara, Rio de Janeiro, Brazil.

Saturnino, K.C., T.M. Mariani, M. Barbosa-Ferreira, et al. 2010. Intoxicação experimental por *Brachiaria decumbens* em ovinos confinados. *Pesquisa Veterinária Brasileira* 30(3):195-202.

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