Evaluation of the toxicity of *Brachiaria decumbens* in rabbits*

Tatiane Cargnin Faccin[1+], Rayane Chitolina Pupin[2], Paula Velozo Leal[2], Ariany Carvalho dos Santos[1], Stephanie Carrelo de Lima[2], Valquiria Barbosa Nantes Ferreira[3], Walmir Silva Garcez[4] and Ricardo Antônio Amaral de Lemos[5]

ABSTRACT. Faccin T.C., Pupin R.C., Leal P.V., Santos A.C., Lima S.C., Ferreira V.B.N, Garcez W.S. & Lemos R.A.A. **Evaluation of the toxicity of *Brachiaria decumbens* in rabbits.** [Avaliação do efeito tóxico de *Brachiaria decumbens* em coelhos.] *Revista Brasileira de Medicina Veterinária, 38(2):143-146, 2016.* Laboratório de Anatomia Patológica, Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Av. Senador Filinto Müller, 2443, Campo Grande, MS 79074-460, Brasil. E-mail: tatifaccin@hotmail.com

The objective of this study was to evaluate the sensitivity of rabbits to poisoning by *Brachiaria decumbens* and the possible use of this animal species as an experimental model for studies on poisoning by this forage in livestock. The experiment was conducted during the dry season with 12 rabbits. The treated group of nine animals was kept in a paddock of *B. decumbens,* and the control group with three rabbits was kept in cages and received cabbage (*Brassica oleracea*). Samples of young, mature, and old leaves and seeds of *B. decumbens* were collected for quantification of the saponin protodioscin. Euthanasia of rabbits from both groups was performed 30, 60 and 90 days after the introduction of the treated group to the pasture. The rabbits showed no clinical signs and no macroscopic or histological findings characteristic of *Brachiaria* spp. poisoning. Both groups gained weight during the experiment. The concentration of the protodioscin (% dry matter) ranged from 0.87% to 2.72% (mean ± standard deviation: 1.95 ± 0.50%) in young leaves, 0.93% to 2.74% (1.71 ± 0.42%) in mature leaves, 0.59% to 1.66% (1.10 ± 0.25%) in old leaves and 0.11% to 0.28% (0.20 ± 0.05%) in seeds. These results demonstrate that *B. decumbens* is palatable to rabbits and that rabbits are not susceptible to poisoning by *Brachiaria* spp. with saponin concentrations that are toxic for sheep.

KEY WORDS. *Brachiaria* spp., experimental model, poisonous plants, protodioscin.

RESUMO. O objetivo do presente trabalho foi avaliar a sensibilidade dos coelhos à intoxicação por *Brachiaria decumbens* e o possível emprego desta espécie animal como modelo experimental para o estudo da intoxicação por esta forrageira em animais de produção. O experimento foi realizado durante a época seca do ano com 12 coelhos. O grupo tratado, com nove animais foi mantido em piquete de *B. decumbens* e o grupo controle, com três coelhos, em gaiolas recebendo repolho (*Brassica oleracea*). Amostras de sementes e folhas novas, maduras e senescentes de *B. decumbens* foram coletadas para quantificação da saponina protodioscina. A eutanásia dos coelhos de ambos os grupos foi realizada

*Received on September 23, 2015.
Accepted for publication on January 11, 2016.

[1] Médica-veterinária. Programa de Pós-Graduação em Ciência Animal, Faculdade de Medicina Veterinária e Zootecnia (FAMEZ), Universidade Federal de Mato Grosso do Sul (UFMS), Av. Senador Filinto Müller, 2443, Campo Grande, MS 79074-460. E-mail: ariany carvalho.vet@gmail.com; [+]Author for correspondence, E-mail: tatifaccin@hotmail.com

[2] Médica-veterinária. Residentes, Programa de Residência Profissional em Medicina Veterinária, FAMEZ, UFMS, Av. Senador Filinto Müller, 2443, Campo Grande, MS 79074-460. E-mails: ray_pupin_@hotmail.com; paulavleal@hotmail.com; stephanie_k_lima@hotmail.com

[3] Química. DSc. , UFMS, Av. Senador Filinto Müller, 2443, Campo Grande, MS 79074-460. E-mail: valbnf@hotmail.com - bolsista DTI

[4] Farmacêutico Bioquímico, DSc. Departamento de Química, UFMS, Av. Senador Filinto Müller, 2443, Campo Grande, MS 79074-460. E-mail: walmir.garcez@ufms.br

[5] Médico Veterinário, DSc. FAMEZ, UFMS, Av. Senador Filinto Müller, 2443, Campo Grande, MS 79074-460. E-mail: ricardo.lemos@ufms.br

30, 60 e 90 dias após a introdução dos animais do grupo tratado na pastagem. Os coelhos não apresentaram sinais clínicos, nem achados macroscópicos ou histológicos característicos de intoxicação por *Brachiaria* spp. Os dois grupos ganharam peso durante o experimento. A concentração de protodioscina (% matéria seca) variou de 0,87% a 2,72% (média ± desvio padrão: 1,95 ± 0,50) nas folhas novas, de 0,93% a 2,74% (1,71 ± 0,42) nas folhas maduras, de 0,59% a 1,66% (1,10 ± 0,25) nas folhas senescentes e de 0,11% a 0,28% (0,20 ± 0,05) nas sementes. Estes resultados demonstram que *B. decumbens* é palatável para coelhos. Conclui-se que os coelhos não são susceptíveis à intoxicação por *Brachiaria* spp. com concentrações de saponinas que são tóxicas para ovinos.

PALAVRAS-CHAVE. *Brachiaria* spp., intoxicação por plantas, modelo experimental, protodioscina.

INTRODUCTION

Brachiaria spp. pastures are the most important for Brazilian cattle, because among the most experienced and adapted to tropical conditions in Brazil forage stand those belonging to this genus because it correspond to 40% of the area cultivated pastures in the country (Guimarães Filho & Ataíde Junior 2009). However, a limiting factor for its use is toxicity (Riet-Correa et al. 2011). Most outbreaks of *Brachiaria* poisoning occur in ruminants (cattle, sheep, goats and buffalo) (Riet-Correa et al. 2011, Oliveira et al. 2013) and occasionally in horses (Barbosa et al. 2006). The main clinical manifestation of poisoning is hepatic photosensitization, and cases of weight loss without dermatitis are observed with a lower frequency (Riet-Correa et al. 2011). Protodioscin, which is a steroidal saponin, has been implicated as the cause of this disease (Brum et al. 2007) and is the main saponin found in toxic *Brachiaria* spp. pastures in Brazil (Riet-Correa et al. 2011). Most outbreaks occur in *Brachiaria decumbens*, which is considered more toxic than other *Brachiaria* spp. (Mustafa et al. 2012, Riet-Correa et al. 2011).

The existence of animals resistant to intoxication has been demonstrated experimentally in sheep, and animals with these characteristics have been proposed for use as a prophylactic measure for poisoning. However, it is not clear whether this resistance is of genetic origin or whether it occurs through adaptation (Riet-Correa et al. 2011). Sheep have been successfully used as experimental models for intoxication (Driemeier et al. 2002) and have advantages compared to cattle: a lower cost to purchase and maintain, a higher number of parturitions per year, and a larger number of twin pregnancies. However, the search for other experimental models that increase these advantages may be important for the study of intoxication. Thus, rabbits are an important species for study due to their small size, short reproductive cycle and low maintenance. Rabbits have been used for this purpose in the study of poisoning by sporidesmin, which is also responsible for hepatic photosensitization in livestock (Thompson et al. 1983).

The objective of this study was to evaluate the sensitivity of rabbits to poisoning by *B. decumbens* and the possible use of this animal species as an experimental model for the study of poisoning by this forage in livestock.

MATERIALS AND METHODS

The experiments were approved by the Ethics Committee for Animal Experimentation (CEUA) of the UFMS (protocol number 400/2012). The experiment was conducted from June to September 2012 in the dry season, beginning with 12 approximately 25-day-old white rabbit (*Oryctolagus cuniculus*) weanlings that remained in cages, fed with feed and cabbage (*Brassica oleracea*) until reaching 45 days of age, when they were randomly separated into two groups.

The treated group of nine animals was kept in cages (1.0 x 1.0 x 2.0 m) in a paddock of *B. decumbens* established approximately six months earlier. The rabbits were observed twice a day, early morning and late afternoon. The cage was moved to another location in accordance with the availability of forage, so the criterion for the change of location of the cage was the total consumption of leaves. In other words, when there were only stalks, the cage was moved. The control group of three rabbits were maintained in cages and continued to receive cabbage. Both groups were fed com feed *ad libitum* and were exposed to the sun for four hours in the morning and four hours in the afternoon daily.

Samples of *B. decumbens* were collected from 10 sites in the pasture at the beginning of the experiment (D0) when rabbits were introduced to the pasture of *B. decumbens* and twice a week thereafter. At each site, 40 young leaves (developing leaves and sprouting leaves), 40 mature leaves (totally expanded leaves), 40 old leaves (senesced leaves) and seeds were collected. Samples were mixed, dried in the shade and split in two for the determination of dry matter (DM) and the quantification of saponin. For quantification of the saponin protodioscin, the samples were ground in a mill (2 mm mesh). Protodioscin was quantified using high-performance liquid chromatography (HPLC) with evaporative light-scattering detection (ELSD). The determination of DM and the quantification of saponin were conducted in a manner similar to that described by Faccin et al. (2014).

Data on the rainfall, during the experimental period, were obtained from the National Institute of Meteorology (INMET), Centro de Monitoramento de Tempo, do

Clima e dos Recursos Hídricos de Mato Grosso do Sul (Cemtec, MS), Agência de Desenvolvimento Agrário e Extensão Rural (Agraer).

Euthanasia was performed 30, 60 and 90 days (D30, D60, and D90) after the introduction of the rabbits to the pasture. Each 30 days, four rabbits were weighed and sacrificed (three from the treated group and one from the control group). Fragments of various tissues were collected, fixed using 10% formalin, processed for routine histopathology and stained with hematoxylin and eosin. The criterion used to consider a lesion to be characteristic of poisoning by *Brachiaria* was the presence of foamy macrophages in the liver sinusoids; crystals; and negative images of crystals within the hepatocytes, macrophages and bile ducts (Faccin et al. 2014).

RESULTS

During the experiment, the cages were moved twice a day because the rabbits ate the forage avidly. The rabbits showed no clinical signs and no macroscopic or histological findings characteristic of *Brachiaria* spp. poisoning. The weight of the rabbits in the control group was 1.3 kg at D30, 2.0 kg at D60 and 2.15 kg at D90. In the treated group, the average weight of the rabbits was 1.43 kg at D30, 2.06 kg at D60 and 2.31 kg at D90. The groups gained weight similarly during the experiment.

The concentration of the protodioscin (% DM) ranged from 0.87% to 2.72% (mean ± standard deviation: 1.95 ± 0.50%) in young leaves, 0.93% to 2.74% (1.71 ± 0.42%) in mature leaves, 0.59% to 1.66% (1.10 ± 0.25%) in old leaves and 0.11% to 0.28% (0.20 ± 0.05%) in seeds (Figure 1).

The experiment was conducted during the dry season with average daily rainfall (ADR) of 0 mm to 4.6 mm, and total cumulative precipitation (TCP) of 1.6 mm to 45.8 mm (Figure 1). There was no relationship between protodioscin concentration and ADR and TCP.

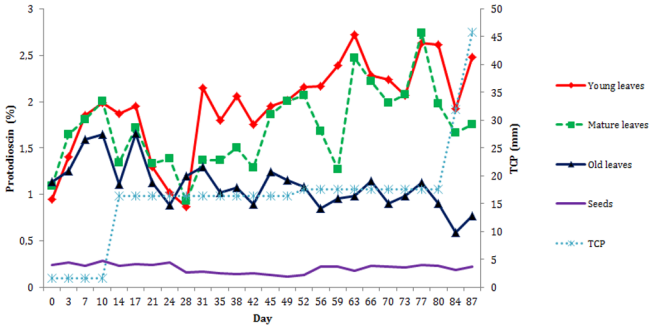

Figure 1. Protodioscin concentrations (% DM) in the seeds and in young, mature and old *B. decumbens* leaves, and total cumulative precipitation (TCP) during the experimental period (from June to September 2012).

DISCUSSION

Under the conditions of this study, the rabbits showed no clinical signs and no macroscopic or histological findings consistent with those observed in *Brachiaria* spp. poisoning in ruminants, whereas the levels of protodioscin found in pastures were similar to those determined with the same methodology in toxic pastures for sheep (Faccin et al. 2014). Therefore, our results suggest that rabbits are more resistant to poisoning than are sheep.

The greater resistance of the rabbits may be related to the mechanism of action of the saponins. The pathogenesis proposed for liver injury caused by saponins occurs through deposition of birefringent crystals in the bile ducts and hepatocytes, causing inflammation and obstruction of the biliary system, in addition to necrosis of the hepatocytes, resulting in jaundice, hepatitis, and photosensitization (Santos et al. 2008). In ruminants, the hydrolysis of these saponins occurs in the rumen (Meagher et al. 2001). Cases of hepatic photosensitization in *Brachiaria* have been described in horses (Barbosa et al. 2006), which, like the rabbits, are not ruminants. As the degradation of cellulose occurs in the equine large intestine (Allison 2006), hydrolysis of saponins has been suggested to occur at this location. Thus, rabbits, due to anatomical similarities between their digestive tracts and those of horses, could be a good experimental model for the study of the toxic effects of plants of the genus *Brachiaria*.

On the other hand, in a report of photosensitivity associated with the ingestion of *Brachiaria humidicola* in horses, protodioscin was not found in the pasture, and in the histological examination, no foamy macrophages were observed in the hepatic sinusoids and no negative images of these crystals were found in the bile ducts (Barbosa et al. 2006). These lesions are characteristic of poisoning by *Brachiaria* sp. in ruminants (Riet-Correa et al. 2011). Low-level protodioscin samples from *B. humidicola* have been described (Lee et al. 2011), suggesting that horses are more sensitive to poisoning, which can influence the characteristics of the histological lesions.

The period of exposure of rabbits to the toxic principle ranged from 30 to 90 days. In sheep that grazed *Brachiaria brizantha* with similar levels of protodioscin, the first clinical case occurred 35 days after the introduction of grazing animals, and new cases occurred until 137 days (Faccin et al. 2014). For most spontaneous reports of *Brachiaria* spp. poisoning in sheep, the period of permanence in the pasture until the appearance of clinical cases was 15 to 60 days (Brum et al. 2007, Albernaz et al. 2010),

but the occurrence of cases after 7 days of permanence has also been described (Albernaz et al. 2010).

The rabbits of the two groups gained weight similarly during the experiment, demonstrating that there was no negative effect of consumption of *Brachiaria* on weight gain. Weight loss without dermatitis is a clinical manifestation described for *Brachiaria* poisoning in cattle (Riet-Correa et al. 2011) and sheep (Faccin et al. 2014). No changes compatible with *Brachiaria* sp. poisoning were observed in the liver, intestine or lymph nodes in the present study.

The concentrations of the protodioscin observed in this study were similar to those observed in another study of grazing on *B. brizantha* conducted in the rainy season (Faccin et al. 2014). The concentration of the protodioscin in the seeds was very low compared to that in the leaves. In another study (Brum et al. 2009), the concentration of saponin in the seeds was absent in both *B. decumbens* and *B. brizantha*.

A limiting factor of the model used is that the rabbits were fed with feed in addition to the *B. decumbens*. Recent studies (Lemos 2014)[6] showed that lambs receiving concentrate supplementation were less susceptible to *Brachiaria* sp. poisoning than unsupplemented lambs. Thus, an ideal model should feed the rabbits exclusively with *Brachiaria* sp., but this procedure would most likely cause adverse effects, considering the nutritional requirements of rabbits.

CONCLUSIONS

The results show that *B. decumbens* is palatable to rabbits. For the method used, the rabbits demonstrate that they are not susceptible to *Brachiaria* poisoning with concentrations of saponins that are toxic to sheep.

Acknowledgements. This work was financially supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq #14/2011 - Projeto Universal Proc. 483211/2012-5) and the Instituto Nacional de Ciência e Tecnologia para o Controle das Intoxicações por Plantas (INCT/CNPq, Proc. 573534/2008-0).

REFERENCES

Albernaz T.T., Silveira J.A.S., Silva N.S., Oliveira C.H.S., Belo Reis A.S., Oliveira C.M.C., Duarte M.D. & Barbosa J.D. Fotossensibilização em ovinos associada à ingestão de *Brachiaria brizantha* no Estado do Pará. *Pesquisa Veterinária Brasileira,* 30:741-748, 2010.

Allison M.J. Microbiologia da digestão fermentativa no rúmen e no intestino grosso, p.438-449. In: Reece W.O. (Ed.), *Dukes, fisiologia dos animais domésticos*. 12ª ed. Guanabara Koogan, Rio de Janeiro, 2006.

Barbosa J.D., Oliveira C.M.C., Tokarnia C.H. & Peixoto P.V. Fotossensibilização hepatógena em eqüinos pela ingestão de *Brachiaria humidicola* (Graminae) no Estado do Pará. *Pesquisa Veterinária Brasileira,* 26:147-153, 2006.

Brum K.B., Haraguchi M., Lemos R.A.A., Riet-Correa F. & Fioravanti M.C.S. Crystal-associated cholangiopathy in sheep grazing *Brachiaria decumbens* containing the saponin protodioscin. *Pesquisa Veterinária Brasileira,* 27:39-42, 2007.

Brum K.B., Haraguchi M., Garutti M.B., Nóbrega F.N., Rosa B. & Fioravanti M.C.S. Steroidal saponin concentrations in *Brachiaria decumbens* and *B. brizantha* at different developmental stages. *Ciência Rural,* 39:279-281, 2009.

Driemeier D., Colodel E.M., Seitz A.L., Barros S.S. & Cruz C.E.F. Study of experimentally induced lesions in sheep by grazing *Brachiaria decumbens*. *Toxicon*, 40:1027-1031, 2002.

Faccin T.C., Riet-Correa F., Rodrigues F.S., Santos A.C., Melo G.K.A., Silva J.A., Ferreira R., Ítavo C.C.B.F. & Lemos R.A.A. Poisoning by *Brachiaria brizantha* in flocks of naïve and experienced sheep. *Toxicon,* 82:1-8, 2014.

Guimarães Filho C. & Ataíde Junior J.R. *Manejo básico de ovinos e caprinos: guia do educador*. Sebrae, Brasília. 2009. 146p.

Lee S.T., Mitchell R.B., Gardner D.R., Tokarnia C.H. & Riet-Correa F. Measurement of steroidal saponins in *Panicum* and *Brachiaria* Grasses in the USA and Brazil, p.142-147. In: Riet-Correa F., Pfister J., Schild A.L. & Wierenga T.L. (Eds), *Poisoning by Plants, Mycotoxins and Related Toxins*. CAB International, Wallingford, 2011.

Meagher L.P., Smith B.L. & Wilkins A.L. Metabolism of diosgenin-derived saponins: implications for hepatogenous photosensitization diseases in ruminants. *Animal Feed Science and Technology*, 91:157-170, 2001.

Mustafa V.S., Moscardini A.R.C., Borges J.R.J., Reckziegel G.C., Riet-Correa F. & Castro M.B. Intoxicação natural por *Brachiaria* spp. em ovinos no Brasil Central. *Pesquisa Veterinária Brasileira,* 32:1272-1280, 2012.

Oliveira C.H.S., Barbosa J.D., Oliveira C.M.C., Bastianetto E., Melo M.M., Haraguchi M., Freitas L.G.L., Silva M.X. & Leite R.S. Hepatic photosensitization in buffaloes intoxicated by *Brachiaria decumbens* in Minas Gerais state, Brazil. *Toxicon,* 73:121-129, 2013.

Riet-Correa B., Castro M.B., Lemos R.A., Riet-Correa G., Mustafa V. & Riet-Correa F. *Brachiaria* spp. poisoning of ruminants in Brazil. *Pesquisa Veterinária Brasileira,* 31:183-192, 2011.

Santos J.C.A., Riet-Correa F., Simões S.V.D. & Barros C.S.L. Patogênese, sinais clínicos e patologia das doenças causadas por plantas hepatotóxicas em ruminantes e eqüinos no Brasil. *Pesquisa Veterinária Brasileira,* 28:1-14, 2008.

Thompson K.G., Jones D.H., Sutherland R.J., Camp B.J. & Bowers D.E. Sporidesmin toxicity in rabbits: biochemical and morphological changes. *Journal Comparative Pathology,* 93:319-329, 1983.

[6]Ricardo Lemos, unpublished data, 2014.