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Poisoning by *Brachiaria* spp. in various lamb breeds at increasing levels of supplementation during growth[1]

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ABSTRACT.- Melo G.K.A., Ítavo C.C.B.F., Silva J.A. Ferelli K.L.S.M., Silva P.C.G., Pupin R.C. & Lemos R.A.A. 2019. **Poisoning by *Brachiaria* spp. in various lamb breeds at increasing levels of supplementation during growth.** *Pesquisa Veterinária Brasileira 39(12): 978-982.* Laboratório de Anatomia Patológica, Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Avenida Senador Felinto Muller 2443, Vila Ipiranga, Campo Grande, MS 79074-460, Brazil. E-mail: ricardo.lemos@ufms.br

The study evaluate the frequency of poisoning by *Brachiaria* spp. at the rearing, growth and termination stages in various lamb breeds at increasing supplementation levels. Forty-five lambs were used in the growth phase in pastures of *Brachiaria* spp. with a history of having induced poisoning. The lambs were distributed in nutritional treatments: lambs receiving mineral supplementation (MS), energy/protein supplementation (EPS) at 0.8% of body weight (bw), EPS at 1.6% bw and EPS at 2.4% bw. The lambs were allotted two flocks (F1 and F2) of 21 and 24 lambs each. Clinical signs of poisoning were observed in all treatments. All MS lambs died. The frequency of poisoning were highest in the 0.8% EPS and 1.6% EPS treatments. All lambs in the 2.4% EPS treatment recovered. F1 lambs had a higher frequency of poisoning than F2 lambs regardless of nutritional treatment. Morbidity rates for the F1 and F2 lambs were 52.3 and 16.7%, respectively. Supplementation was not sufficient to decrease the frequency of poisoning in lambs at the termination stage, which was dependent on the genetic origin of the lambs. EPS of 2.4% bw treatment, was an efficient nutritional strategy to minimize the effects of poisoning in lambs fed on *Brachiaria* spp.

INDEX TERMS: Poisoning, *Brachiaria* spp., lamb, supplementation, growth, hepatogenic photosensitization, toxic plants, small ruminants, plant poisoning.

RESUMO.- [**Intoxicação por *Brachiaria* spp. em várias raças de cordeiros com incremento nos níveis de suplementação durante o crescimento.**] O objetivo deste estudo foi avaliar a frequência da intoxicação por *Brachiaria* spp. nas fases de recria, crescimento e terminação em várias raças de cordeiros em níveis crescentes de suplementação. Quarenta e cinco cordeiros foram utilizados na fase de crescimento em pastagens de *Brachiaria* spp. com história de intoxicação, distribuídos em diferents tratamentos nutricionais: cordeiros recebendo suplementação mineral (SM), suplementação energética/protéica (SEP) a 0,8% do peso vivo (pv), SEP a 1,6% pv e SEP a 2,4% pv. Vinte e um cordeiros vieram do lote F1 e 24 cordeiros foram do lote F2. Sinais clínicos de intoxicação foram observados em todos os tratamentos. Todos os cordeiros com MS morreram. As frequências de intoxicação foram maiores nos tratamentos com 0,8% e 1,6% de SEP. Todos os cordeiros no tratamento com 2,4% de SEP se recuperaram. Cordeiros F1 apresentaram maior frequência de intoxicação que os cordeiros F2, independentemente do tratamento nutricional. As taxas de morbidade para os cordeiros F1 e F2 foram de 52,3 e 16,7%, respectivamente. A suplementação não foi suficiente para diminuir a freqüência de intoxicação em cordeiros na fase de terminação, dependente da origem genética dos cordeiros. O SEP de 2,4% pv, no entanto, foi eficiente para minimizar os efeitos da intoxicação em cordeiros alimentados com *Brachiaria* spp.

TERMOS DE INDEXAÇÃO: Intoxicação, *Brachiaria* spp., cordeiros, suplementação, crescimento, fotossensibilização hepatógena, plantas tóxicas, pequenos ruminantes.

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INTRODUCTION

Most cultivated pastures in Brazil contain grasses of the genus *Brachiaria* (Oliveira et al. 2014). The Brazilian Cerrado, a large savanna, has an estimated 60 million ha of cultivated pastures, 85% of which contain *Brachiaria* (Macedo 2005). Poisoning by these grasses, however, causes direct and indirect losses in sheep flocks (Mustafa et al. 2012, Porto et al. 2013). Outbreaks have been described in weaned lambs in the growth phase (Riet-Correa et al. 2011, Mustafa et al. 2012, Pupin et al. 2016) and in suckling lambs (Melo et al. 2018), demonstrating that these categories are more vulnerable than others, being susceptible to poisoning from the beginning of the consumption of the forage (Melo et al. 2018).

The influence of genetics on the sensitivity to poisoning (Pupin et al. 2016, Castro et al. 2018) indicates that the selection of animals genetically resistant to poisoning is an efficient means of prevention. This selection, however, is a slow process, because methodologies for the identification of resistant animals have not been defined. Alternatives such as controlled grazing (Castro et al. 2018) reduce the time of grazing and consequently the amount of grass eaten by sheep. This method, although efficient under experimental conditions, requires the cultivation of forages other than *Brachiaria* spp., limiting factor in properties that are constituted only by foragers of this genus.

Dietary supplementation is a promising and easily applied alternative for suckling lambs (Melo et al. 2018) but has not been evaluated in other categories of sheep.

The aim of this study was to evaluate the frequency of poisoning by *Brachiaria* spp. in various lamb breeds during the growth phase submitted to increasing levels of energy/protein supplementation (EPS).

MATERIALS AND METHODS

The experiment was approved by the "Universidade Federal de Mato Grosso do Sul" (UFMS) ethics committee on the use of animals, protocol no. 481/12. It was conducted at the experimental farm of the Faculty of Veterinary Medicine and Animal Science, (20°26'34.31"S, 54°50'27.86"W), UFMS.

We tested 45 lambs with a mean age of 80 days and a mean weight of 19.50kg from two flocks, F1 and F2. We tested 45 lambs with a mean age of 80 days and a mean weight of 19.50kg from two flocks, Flock 1 (F1) and Flock 2 (F2). The breed composition of the herds was as follows: F1 lambs were the product of crosses between mixed-breed ewes raised from birth on pastures of *Brachiaria* spp. with an Ile de France ram. F2 lambs were the product of crosses between naïve (not previously exposed to *Brachiaria*) Dorper x Santa Inês ewes with a White Dorper ram. Both rams that originated F1 and F2 herds were naïve and were adapted to the consumption of *Brachiaria* spp., as previously described (Melo et al. 2018).

The experiment comprised two trials. The first trial began on August 16 and ended on November 26, 2013, and the second trial began on July 11 and ended on September 19, 2014. The termination phase (weaning to slaughter) was used as the experimental period.

Weaned lambs were distributed in four treatments (Table 1) and began receiving different levels of EPS. The lambs were allocated in pens containing *Brachiaria* grasses with a history of poisoning. EPS was provided daily at 8:00 a.m., and lambs had free access to food, water and mineral supplementation (MS) regardless of treatment. The pens were mixed pastures of *Brachiaria humidicola*, *B. decumbens* and *B. brizantha*, with a predominance of *B. brizantha*. Twelve 0.4-ha pens (three replicates per treatment) were used. The animal load used per pen was determined by the supply of leaf dry matter - 10% of body weight (bw) -, with variable stocking, and control sheep were used to adjust the animal load when necessary.

The concentrations of protodioscin in *Brachiaria* spp. were determined from 10 samples collected from representative areas in each plot every 28 days using metal quadrats of 0.5×0.5m. The grasses were cut close to the soil (McMeniman 1997), and the leaves were subsequently separated. The leaves were dried in a forced-ventilation oven at 55°C for 96h and ground in a mill with 1-mm sieves. The concentration of protodioscin was determined by the method described by Ganzera et al. (2001).

Lambs were weighed at the beginning and end of the experimental period, with fasting of solids for 16h, and weighed every 14 days during the experimental period. The experimental period ended when the animals in the group with the highest nutritional intake (2.4% EPS) reached 32kg bw.

Parasitological follow-up and anthelmintics treatment were performed every 14 days. Fecal samples were collected directly from the rectal ampulla for quantifying the number of eggs per gram (EPG) of feces (Gordon & Whitlock 1939 - modified), with a sensitivity of 1:25. Lambs with EPG counts ≥500 were dewormed with monepantel.

The lambs were monitored daily for signs of poisoning by *Brachiaria* spp. The expected lesions for determining poisoning in the lambs were restricted to specific sites of non-pigmented skin, mainly in the face and ears. Clinical signs consisted of edema, alopecia, erythema, formation and detachment of scabs, apathy, anorexia, jaundice, and weight loss (Mustafa et al. 2012, Porto et al. 2013, Pupin et al. 2016, Melo et al. 2018).

Lambs presenting clinical signs were separated from the flock and placed in shelters protected from solar radiation. They were fed with Tifton-85 hay (*Cynodon dactylon*), EPS, MS and water ad libitum. The lambs were reintroduced to their treatments when they had completely recovered. The lambs that died were necropsied, and fragments of various organs were sampled, fixed in 10% formalin, routinely processed and stained with hematoxylin-eosin for histological examination.

The experimental design consisted of randomized blocks based on the flock of origin and the distribution of lambs in the treatments

Table 1. Distribution of lambs by supplementation level

Treatment[a]	Number of animals (F1/F2)[b]	Description
MS	11 (4/7)	Lambs in continuous grazing, receiving MS
0.8% EPS	12 (5/7)	Lambs in continuous grazing, receiving MS and 0.8% body weight (bw[c]) EPS
1.6% EPS	10 (7/3)	Lambs in continuous grazing, receiving MS and 1.6% bw EPS
2.4% EPS	12 (5/7)	Lambs in continuous grazing, receiving MS and 2.4% bw EPS

[a] MS = mineral supplementation, EPS = energy/protein supplementation, [b] F1 = flock 1, F2 = flock 2, [c] bw = body weight.

(MS, 0.8% EPS, 1.6% EPS or 2.4% EPS). The frequency of poisoning was assessed by a chi-square test at $P<0.05$.

RESULTS

The concentrations of protodioscin (% dry matter) ranged from 1.04 to 2.17 (mean±SD: 1.59±1.33) in the experimental pens. Clinical signs of poisoning were observed at all levels of supplementation. The epidemiology, clinical evolution, genetic origin and nutritional treatment at the termination stage of the lambs submitted to pasture supplementation are described in Table 2.

The frequencies of poisoning were highest at the 0.8 and 1.6% EPS supplementation levels. Five lambs in the 0.8% EPS treatment became ill but recovered. Four lambs in the 1.6% EPS treatment presented clinical signs; three survived and one died. Three lambs in each of the MS and 2.4% EPS treatments became ill. All three lambs in the MS treatment died, and all three lambs in the 2.4% EPS treatment recovered (Table 2).

The frequency of poisoning did not differ significantly ($P>0.05$) between the supplementation levels (Table 3). The frequency of poisoning, however, differed significantly ($P<0.05$) between the two flocks. The morbidity rates of F1 and F2 lambs were 52.3% (11/21) and 16.7% (4/24), respectively.

Clinical, necropsy and histopathological findings differed in intensity but were similar between treatments. The main clinical signs included hepatogenic photosensitization, with lesions restricted to specific sites of non-pigmented skin, mainly on the face, and symptoms consisted of edema, alopecia, erythema, formation and detachment of scabs, apathy, anorexia, and weight loss. The necropsy findings were similar among the treatments and consisted of alopecia and crust formation around the eyes and ears, with scar retraction of the ears. Livers had rounded edges and an evident lobular pattern and were diffusely light brown. The kidneys were blackened, and the cut pelvis was yellowish. Histological lesions were present in the liver and the skin of the ears and eyelids.

DISCUSSION

The concentrations of protodioscin (1.59±1.33%) were similar to those previously considered toxic for lambs. Concentrations ranging from 0.74 to 2.56% have been reported in weaned lambs (Mustafa et al. 2012, Pupin et al. 2016) and from 0.62 to 1.15% in suckling lambs (Melo et al. 2018).

Table 2. Epidemiology, genetic origin, clinical evolution and history of *Brachiaria* spp. poisoning in the rearing stage of the lambs

Treatment[a]	Flock	Age[b] (d)	OCS[c] (d)	Month of poisoning	Clinical evolution (d)		History of poisoning
					Recovery	Death	
MS	F1	115	20	July		12	Yes
MS	F2	88	11	July		25	Yes
MS	F1	105	18	July		93	Yes
0.8% EPS	F2	123	24	August	43		Yes
0.8% EPS	F2	123	24	August	43		No
0.8% EPS	F1	97	33	August	28		No
0.8% EPS	F1	126	21	July	23		Yes
0.8% EPS	F1	110	21	July	24		Yes
1.6% EPS	F1	113	31	October	14		Yes
1.6% EPS	F1	107	14	July		56	Yes
1.6% EPS	F1	117	28	August	44		No
1.6% EPS	F1	115	26	July	52		Yes
2.4% EPS	F1	109	62	November	13		Yes
2.4% EPS	F2	167	120	January	31		No
2.4% EPS	F1	68	12	July	15		Yes

[a] MS = mineral supplementation, 0.8% EPS = lambs receiving 0.8% bw EPS, 1.6% EPS = lambs receiving 1.6% bw EPS, 2.4% EPS = lambs receiving 2.4% of bw EPS, [b] age at onset of clinical signs, [c] onset of clinical signs after the start of the experimental period (after weaning), [d] bw = body weight.

Table 3. Frequency of poisoning of F1 and F2 lambs in the growth phase at increasing levels of supplementation

Treatment	Flock		*P*
	F1[a]	F2[b]	
	Positive cases (%)		
MS	50.0 (2/4)[a]	14.3 (1/7)[b]	0.8204
0.8% EPS	60.0 (3/5)[a]	28.6 (2/7)[b]	0.8220
1.6%EPS	57.1 (4/7)[a]	0.0 (0/3)[b]	0.8450
2.4% EPS	40.0(2/5)[a]	14.3 (1/7)[b]	0.8320
P	0.0202	0.0222	-

[a] Lambs of the F1 flock are SRD sheep raised on *Brachiaria* spp. pastures and an Ile de France breeder adapted to the consumption of *Brachiaria* spp., [b] lambs of the F2 flock are offspring of Santa Inês × Dorper sheep and a White Dorper ram adapted to the consumption of *Brachiaria* spp.; different lowercase letters within a row indicate significant differences by a χ^2 test ($P<0.05$).

The frequency of poisoning was higher in F1 than F2 regardless of the level of supplementation. A genetic component was thus likely responsible for the resistance or sensitivity of the animals to poisoning. The role of genetics in the sensitivity to poisoning has previously been described by Pupin et al. (2016), who reported a morbidity of 73.33% and varying degrees of sensitivity in offspring of susceptible parents, but lambs from resistant parents presented no clinical signs. Fifteen lambs presented clinical signs during the experimental period, 11 of which had already had signs of poisoning in the breeding phase, with recurrence in the termination phase. Of these 11, seven (63.63%) survived and four (36.36%) died. Four other lambs presented signs of poisoning only at the termination stage, and all survived. The sheep therefore had adapted to the consumption of *Brachiaria* spp. to different degrees. The mechanisms of this adaptation remain unknown, but changes may occur in the ruminal microbiota, altering the transformation of the toxin (Albernaz et al. 2010, Riet-Correa et al. 2011). The transfer of ruminal content of adult sheep adapted to grazing *B. brizantha* to susceptible lambs can reduce the morbidity rate relative to lambs that do not receive transfaunation (Castro et al. 2018).

The genetic origin of the lambs was a determinant for the occurrence of poisoning. Three of the four deaths were in F1. The severity of the clinical signs, however, differed among the levels of supplementation. Five, four and three animals at the 0.8, 1.6 and 2.4% EPS supplementation levels, respectively, presented clinical signs. Only one animal died in the 1.6% EPS treatment. Three animals in the MS treatment became ill, all of which subsequently died. EPS minimized the effects of poisoning, influencing mortality rates at different levels of supplementation.

In another study, EPS efficiently prevented the effects of poisoning in suckling lambs reared on *Brachiaria* spp. and supplemented with creep feeding, with only four of 34 lambs presenting clinical signs of poisoning, but 17 of 34 lambs reared under the same grazing conditions but receiving only MS presented clinical signs (Melo et al. 2018).

We did not determine the mode of action of the supplement but presumed that the ruminal microbiota had been modified by the ingestion of the concentrated feed (Miles et al. 1991), altering the sequence of chemical changes of lithogenic steroid saponins in the rumen and consequently damaging liver tissue (Miles et al. 1994, Meagher et al. 1996, Albernaz et al. 2010). The macro- and microscopic lesions were characteristic of poisoning by *Brachiaria* forages (Mustafa et al. 2012, Porto et al. 2013, Pupin et al. 2016, Melo et al. 2018).

CONCLUSION

Increasing levels of EPS were not sufficient to decrease the frequency of poisoning, which depended on the genetic origin of the lambs. EPS of 2.4% bw, however, was an efficient nutritional strategy to minimize the effects of poisoning in lambs fed on *Brachiaria* spp.

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Statement of animal rights.- All procedures performed with the animals of the present experiment were in accordance with the ethical standards established by the Ethics Committee on the Use of Animals (CEUA) of the "Universidade Federal de Mato Grosso do Sul" (UFMS).

Conflict of interest.-The authors declare that there are no conflicts of interest

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