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Poisoning by *Brachiaria brizantha* in flocks of naïve and experienced sheep

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ABSTRACT

The aim of this work was to study the effects of protodioscin ingestion in two different flocks of sheep: a flock of 23 crossbreed Mato Grosso do Sul Native sheep raised on *Brachiaria* spp. pastures from birth (experienced flock) in the state of Mato Grosso do Sul; and another flock (naïve flock) of 18 crossbred Dorper × Santa Inês sheep raised in the state of Paraná in *Paspalum notatum* and *Lolium multiflorum* pastures. The two flocks grazed together in a *Brachiaria brizantha* pasture during a 140-day period in the rainy season. At the beginning of the experiment and every 14 days thereafter, blood samples were collected for determination of serum activities of gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST), and for determination of the icterus index. On the same days, samples of young, mature and old *B. brizantha* leaves were collected for protodioscin quantification. Naïve sheep were more susceptible to poisoning by *B. brizantha* than experienced sheep. Six sheep in the naïve flock were poisoned, and two of these died. Two sheep in the experienced flock were poisoned, and one of them died. The mean activities of serum GGT and AST were significantly higher in the naïve flock, also evidencing a higher susceptibility to the poison. These results suggest that flocks of sheep include animals with different degrees of resistance to *Brachiaria* spp. poisoning and that culling the susceptible animals may considerably increase of the resistance of the flock. The clinical signs and the lesions were similar to those previously reported. However, in sheep with black coats, the main clinical sign was weight loss without photosensitization-mediated dermatitis. One sheep from the experienced flock presented cirrhosis, with clinical signs of exercise intolerance. The protodioscin concentration (% DM) ranged from 0.87% to 2.58% (mean ± SD: 1.64 ± 0.58) in young leaves, 1.16%–2.53% (1.67 ± 0.44) in mature leaves and 0.98%–2.07% (1.52 ± 0.37) in old leaves. A negative relationship was found between saponin concentration and total cumulative precipitation.

1. Introduction

Due to its high dry matter production, easy cultivation, good adaptation to different soils for year-round growth, resistance to drought and low maintenance costs,

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Brachiaria spp. are the best option for grazing ruminants raised under range conditions in the Brazilian midwestern region (Castro et al., 2011; Seiffert, 1980). Despite its importance as forage in Brazil, outbreaks of hepatic photosensitization caused by different *Brachiaria* species have been reported in cattle and sheep (Riet-Correa et al., 2011). Lambs are considered to be more susceptible than adults (Albernaz et al., 2010; Brum et al., 2007), and sheep are more susceptible than cattle (Riet-Correa et al., 2011). Protodioscin, which is a steroidal saponin, has been implicated as the cause of this disease (Brum et al., 2007).

Due to the importance of *Brachiaria* spp. in most Brazilian regions, it is very important to implement preventive and control measures for its toxicity. Two main measures have been proposed for the safe use of *Brachiaria* spp. in pastures: the use of less toxic species or varieties of *Brachiaria* and the use of resistant animals (Riet-Correa et al., 2011). The use of *Brachiaria brizantha* is a good alternative because this species is considered less toxic (Gracindo, 2009; Mustafa et al., 2012; Riet-Correa et al., 2011) and exhibits a protodioscin concentration lower than that of *Brachiaria decumbens* (Barbosa-Ferreira et al., 2011; Lima et al., 2012). However, outbreaks of poisoning by *B. brizantha* have been reported in sheep (Albernaz et al., 2010; Macêdo et al., 2008; Mustafa et al., 2012; Rissi et al., 2011). It has been demonstrated that there are differences in the resistance/susceptibility of sheep to *Brachiaria* poisoning. In a previous study conducted in *B. decumbens* pastures, it was demonstrated that naïve 4- to 6-month-old lambs that were raised in other pastures and introduced to *B. decumbens* pastures were more susceptible than were lambs of the same age raised in *Brachiaria* spp. pastures and introduced to the same pastures as the naïve sheep (Castro et al., 2011).

One of the aims of this study was to determine the effect of grazing in a *B. brizantha* pasture on two herds of sheep, one raised in a pasture of *Brachiaria* spp. in state of Mato Grosso do Sul (experienced flock) and the other raised in pasture of *Paspalum notatum* and *Lolium multiflorum* in the state of Paraná (naïve flock). The other aim was to determine the protodioscin concentrations in the young, mature and old leaves of this plant, and to establish their relationship with the occurrence of poisoning.

2. Materials and methods

The experiment was conducted on the farm (20°26′34.31″S 54°50′27.86″W) of the Federal University of Mato Grosso do Sul (UFMS) in the municipality of Terenos, state of Mato Grosso do Sul (MS), Brazil, using two different flocks of sheep. One flock (called experienced), raised in *Brachiaria* pastures from birth at the Faculty of Veterinary Medicine and Animal Science (FAMEZ) in the municipality of Campo Grande, MS, Brazil was formed by 23 female crossbreed Mato Grosso do Sul Native clinically healthy sheep (Numbers 1–23) that were introduced to the experimental pasture in January 2012. At the beginning of the experiment, this flock was composed of three 5-month-old sheep (N21, N22, N23), three 11-month-old sheep (N18, N19, N20) and 17 sheep over 16-months old (N1–N17). The mean body weight of the experienced flock was 44.05 ± 12.05 kg. The naïve flock consisted of 18 (Numbers 24–41) approximately 9-month-old female crossbred Dorper × Santa Inês sheep raised in pasture of *P. notatum* and *L. multiflorum* in the state of Paraná that had not previously ingested *Brachiaria* spp. and were introduced to the same pasture as the experienced flock in October 2012 with a mean body weight of 30.37 ± 3.40 kg. The experiments were approved by the Ethics Committee for Animal Experimentation (CEUA) of the UFMS (protocol number 400/2012).

Blood samples were collected from all of the sheep at the beginning (Day 0) of the experiment, when the naïve flock was placed in the *B. brizantha* pasture, and every 14 days for determination of the icterus index and serum activities of gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST). Samples were collected for 140 days (Days 0–140). The biochemical analyzes were conducted using commercial kits (*Labtest*, *Minas Gerais*, *Brazil*) and a BIOPLUS-200 semi-automatic biochemical analyzer. The potassium dichromate method was used to determine the icterus index, which represents the intensity of the plasma's yellowish color, which is caused by hyperbilirubinemia (Stockham and Scott, 2011). The reference values used for the icterus index were 2–5 U, according Kaneko et al. (1997). The reference values used for GGT and AST serum activities were determined in accordance with Stockham and Scott (2011), calculating the maximum reference value of the population as the mean pre-exposure serum activities of the naïve flock on day zero plus twice the standard deviation (SD) of 95% of the central values. Therefore, the maximum reference values used were 85.33 U/L for GGT and 155.04 U/L for AST.

On the days that blood was sampled, samples of *B. brizantha* were collected from 10 sites in the pasture. In each site, 40 young leaves (developing leaves and sprouting leaves), 40 mature leaves (totally expanded leaves) and 40 old leaves (senesced leaves) were collected, totaling 400 young leaves, 400 mature leaves and 400 old leaves on each sample obtained every 14 days. Samples were mixed, dried in the shade and split in two for performing the determinations of dry matter (DM), according to AOAC (2000) and quantification of saponin. For quantification of the saponin protodioscin, the samples were ground in a mill (2 mm mesh), and sent to the Chemical Laboratory of the UFMS. Protodioscin was quantified by high-performance liquid chromatography (HPLC) using evaporative light-scattering detection (ELSD) according to a method proposed by Ganzera et al. (2001). For dry matter analysis the samples were performed according to AOAC (2000) method 930.15.

Percutaneous liver biopsies for histological examination and blood samples for AST and GGT determinations were obtained from the sheep that showed signs of poisoning by *Brachiaria* spp. To reduce their risk of death, the poisoned sheep were separated from the flock, placed in a corral with shade and fed *Cynodon dactylon* (Tifton grass) hay, concentrated food, and a mineral supplement. After their complete clinical recovery and the reduction of the activities of their serum GGT and AST, these sheep were reintroduced to the *B. brizantha* pasture.

The FAMACHA© method was performed weekly as a strategy to control infection by the nematode *Haemonchus*