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Sheep adaptation management, and investigation of inherited resistance to prevent *Brachiaria* spp. poisoning

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ABSTRACT

Brachiaria spp. is the most used forage in extensive cattle production in the Central-Western region of Brazil. However, livestock fed with these grasses can develop hepatogenous intoxication due to the presence of steroidal saponins that promote photosensitivity, inefficiency of production in the herds and death. Grass toxicity depends on factors related to animal susceptibility or resistance and intrinsic plant factors, providing significant differences in the clinical manifestation and mortality of livestock. Three experiments were conducted to investigate the adaptation management and inherited resistance to prevent *Brachiaria* spp. poisoning in sheep. In the first experiment, the adaptive management of lambs was performed, controlling the grazing time in *Brachiaria* spp. pastures. Groups of sheep grazing previously in *Brachiaria decumbens* paddocks for 2 h daily or on alternate days for 60 days presented low hepatotoxic changes and had reduced the number of intoxicated lambs when compared to the control group during the challenge. In experiment 2, ruminal transfaunation from adapted adult sheep to non-adapted lambs to grazing on the grass reduced the hepatotoxicity, which suggests that *Brachiaria* spp. poisoning resistance could be transferred by ruminal fluid to non-adapted animals. In the third experiment, lambs from flocks raised in *Brachiaria* pastures showed less susceptibility to poisoning than lambs from flocks raised in non-toxic pastures, suggesting inherited resistance to the toxicosis. These results suggest that the control of grazing time combined with transfaunation can be employed to decrease the frequency of poisoning in susceptible flocks. However, a definitive solution in the long-term would be the selection of resistant sheep herds.

1. Introduction

Brachiaria spp. is considered the most common toxic plant in Central-Western Brazil (Furlan et al., 2012; Pessoa et al., 2013). Steroidal saponins contained in *Brachiaria* spp. pastures cause livestock production limitations due to liver toxicity, photosensitivity and death (Castro et al., 2011; Riet-Correa et al., 2011). Lambs are more susceptible to *Brachiaria* spp. poisoning, presenting higher mortality and lethality than other ruminants (Lemos et al., 1998; Santos et al., 2008; Riet-Correa et al., 2011; Mustafa et al., 2012).

Brachiaria spp. poisoning in the last 30 years in Brazil has drastically declined in cattle herds, probably due to the death of susceptible animals or the utilization of fewer toxic grasses in paddocks like *B. humidicola* and *B. brizantha* (Riet-Correa et al., 2011). The herd management method of preventing young animals from grazing on sprouting pastures, which are considered the most toxic grass stage, may have also contributed to poisoning reduction in bovine species (Castro et al., 2011; Riet-Correa et al., 2011).

In contrast, in the Midwest Region of Brazil, the poisoning of sheep by *Brachiaria* spp. has increased due to the importation of susceptible flocks from Brachiaria-free areas (Faccin et al., 2014). However, lambs from flocks raised on *Brachiaria* spp. pastures may be kept in toxic paddocks with no signs of hepatotoxicity, demonstrating the possibility of secure forage utilization by this species (Gracindo et al., 2014).

Knowledge of toxic plants around the world has progressed over the last 20 years, with a considerable improvement in the comprehension in active principles, mechanisms of action and poisoning prevention. However, mechanisms of adaptation to toxic principles and animal resistance to toxic plants still remain poorly understood. In this paper, three experiments were performed to investigate the adaptation management and inherited resistance of sheep to *Brachiaria* spp. poisoning.

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2. Materials and methods

The experiments were performed over 120 days at the Experimental Farm of the University of Brasília (S15°47′ W47°56′; elevation 1080m), Brasília, Federal District, Brazil.

Clinical examination of the lambs was performed and blood samples were collected weekly to determine the serum levels of gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST) (Mustafa et al., 2012; Porto et al., 2013; Gracindo et al., 2014). Maximum reference values (MRV) for the serum activities of GGT and AST were determined in each of the experiments, calculating the maximum reference value of the population as the mean pre-exposure serum activities of the flock on day zero plus twice the standard deviation (SD) of 95% of the central values (Stockham and Scott, 2008).

Intoxicated animals showing clinical signs were immediately withdrawn from the experiments to avoid suffering and aggravation of the clinical condition. All sheep that died were necropsied. Samples of organs and tissues were collected, fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin for histological examination. All animal procedures were approved by the Bioethics Committee (activity CEP 69-2013), and performed under veterinary supervision.

Samples of the pastures were harvested at 15-day intervals, air dried and ground in a mill (2 mm mesh) to measure saponin (protodioscin) concentrations by high-performance liquid chromatography (HPLC) using evaporative light-scattering detection (Ganzera et al., 2001; Oleszek, 2002). The number of spores of the fungus *Pithomyces chartarum* were determined in samples of fresh grass collected at the same intervals (Di Menna and Bailey, 1973).

The serum activity of GGT and AST and the protodioscin concentration in the pastures were subjected to repeated measurements analysis of variance (ANOVA) and Tukey's test for means comparison. In Experiment 3, the T-test was used to compare GGT and AST serum levels. In all experiments, the frequency of animals which presented higher serum GGT and AST values than MRV were submitted to analysis of frequencies by the Fisher exact test. All figures and analysis were conducted with GraphPad Prism 6.01 ® software.

2.1. Experiment 1 – adaptive management of lambs to induce resistance to *Brachiaria* spp. poisoning

Forty-five naïve (not adapted) crossbred Santa Ines male sheep, aged 4–5 months, from flocks that never grazed on *Brachiaria* spp. pastures (Castro et al., 2011; Riet-Correa et al., 2011; Gracindo et al., 2014), were randomly divided into five groups of nine animals (n = 9). Experiment 1 was conducted in two phases: adaptation and challenge.

In the adaptation phase, four groups of animals were submitted to different grazing management protocols in paddocks: group 1: BD2 h – 2 h grazing daily in *Brachiaria decumbens* cv. Basilisk; group 2: BDalt – grazing in alternate days in *Brachiaria decumbens* cv. Basilisk (24 h of interval); group 3: BBc – grazing continuously in *Brachiaria brizantha* cv. Marandu and group 4: BHc – grazing continuously in *B. humidicola*. All of the animals received fresh water *ad libitum* and were supplemented with 1% of bodyweight with commercial feed. According to the adaptation protocol, the animals were placed in stalls and kept with commercial concentrate feed and coastcross hay (*Cynodon* spp.) during the intervals.

Afterwards, all groups of lambs were challenged by continuously grazing in sprouting *B. decumbens* paddocks for 60 days, when the pasture is considered more toxic (Riet-Correa et al., 2011; Lima et al., 2012; Gracindo et al., 2014), to evaluate the efficacy of the adaptation management protocols. The control group (CG) was introduced into the paddock only during the challenge phase.

2.2. Experiment 2 – ruminal transfaunation application to prevent *Brachiaria* spp. intoxication of sheep

The experiment was divided in two phases and conducted with 24 naïve male sheep (similar animals to those used in experiment 1) distributed randomly in three groups of eight animals (n = 8).

In the initial phase, two groups of lambs were kept for 60 days in a paddock formed exclusively with *Brachiaria brizantha* cv. Marandu and submitted to transfaunation protocols: The single transfaunation group (ST) received only one treatment as soon as they were introduced into the pasture. The repeated transfaunation group (RT) received ruminal fluid three times a week with one day of interval during the entire first phase.

The ruminal content was collected and the fluid was extracted from 12 adult sheep previously with ruminal cannulation, adapted for at least three years to graze in *Brachiaria* spp. (Duarte et al., 2013). The transfaunation procedure consisted of the transfer of 800 ml of fresh rumen fluid from adult adapted sheep to naïve lambs through an esophageal tube immediately after collection (Albernaz et al., 2010; Duarte et al., 2013).

In the challenge phase, the transfaunated groups and the control group (CG), which received no treatment, were submitted to continuous grazing in a sprouting *B. decumbens* paddock for 60 days.

2.3. Experiment 3 – investigation of inherited resistance to prevent *Brachiaria* spp. poisoning

Eighteen mixed-breed Santa Inês breeding females in late pregnancy were selected from two different herds to compose the following groups: the group of adapted females (n = 9) was composed of animals kept for at least 3 years in *Brachiaria* spp. paddocks with no signs or outbreaks of grass poisoning; and the non-adapted group (n = 9) was composed of sheep from flocks that grazed in *Cynodum* spp. paddocks and never had contact with *Brachiaria* spp. grass (Castro et al., 2011; Riet-Correa et al., 2011; Gracindo et al., 2014). Both groups of females were kept confined in separate stalls receiving *Cynodum* spp. hay and commercial feed until the birth of the lambs and remained together until weaning at three months of age. The eighteen lambs born in stalls composed the adapted lambs group (AL, n = 9) and non-adapted lambs (NL, n = 9). The animals remained confined until 4 months of age, receiving *Cynodum* spp. hay and commercial ration until the beginning of the experimental protocol. The adapted and non-adapted lambs were subjected to grazing in sprouting *B. decumbens* paddock for 60 days to determine their susceptibility to grass poisoning.

3. Results

The mean of grass protodioscin concentration (%) on dry matter in *B. decumbens* samples at the adaptation phase (Experiment 1) was 0.62 ± 0.18 and 1.18 ± 0.10 at the challenge phase of all three experiments. In *B. brizantha* pastures, the protodiocin level was 0.74% ± 0.23, and 0.11% ± 0.01 in the paddock of *B. humidicola*. The *Pithomyces chartarum* spore count was less than 5000 spore/g in *Brachiaria decumbens* paddocks and absent in other pastures samples during all of the experiments.

The mean serum activity of GGT and AST, and the number of sheep that presented higher serum levels of GGT and AST than the maximum reference value (MRV) in each experiment are shown in Table 1.

3.1. Experiment 1 – adaptive management of lambs to induce resistance to *Brachiaria* spp. poisoning

Three lambs from the control group presented mild jaundice, lethargy, weight loss, hyperemia in the ocular mucosa and high serum levels of GGT and AST during grazing in *B. decumbens* pasture. These animals were removed from the paddock; however, two of them died