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Research paper

Supplementation with dry *Mimosa caesalpiniifolia* leaves can reduce the *Haemonchus contortus* worm burden of goats

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ABSTRACT

Gastrointestinal nematodes (GINs) cause considerable economic losses in grazing goat herds. At present, GIN control cannot rely on conventional anthelmintic (AH) drugs because parasites have developed resistance against such drugs. Thus, alternative control methods are being sought to reduce the dependence on AH. Many tannin-rich plants exhibit AH activity and may be used as alternatives for GIN control. *Mimosa caesalpiniifolia* is a tannin-rich shrub consumed by small ruminants in Brazil. This study evaluated the *in vivo* AH effect of *M. caesalpiniifolia* leaf powder supplementation on GIN egg fecal excretion and worm burden in goats. Plant leaves were harvested, dried and ground to obtain a powder. Twenty-four castrated male goats, aged six to eight months, with a mean body weight of 15.0 ± 2.5 kg were used in the experiment. Animals were infected orally with 16,000 larvae comprising 50% *Haemonchus* spp., 41% *Trichostrongylus* spp. and 9% *Oesophagostomum* spp. Once the infection was patent, the goats were distributed into four groups of six animals. The control group received concentrate without condensed tannins (CTs) and did not receive any drench against GINs. The monepantel group received concentrate without CTs and were drenched once with monepantel. The other two groups received the *M. caesalpiniifolia* leaf powder in two periods of seven consecutive days (days 1–7 and 14–21), with one of the groups also receiving 10 g of polyethyleneglycol (PEG)/day. The animals were weighed weekly, and individual fecal eggs counts (FECs) were performed daily. After 28 days, the animals were humanly slaughtered, and the worm burden was estimated. Although live weight gain and FECs did not differ among the groups ($P > 0.05$), *post-mortem* worm counts showed a reduction in *Haemonchus contortus* adult worm burden (57.7%) in goats of the CT group compared to control goats ($P < 0.05$). The addition of PEG did not diminish AH activity in the CT + PEG group (66.9% reduction compared to the control). No AH effect against other GIN species was found. The result for the addition of PEG suggested that the observed AH activity was associated with plant secondary compounds, as opposed to CTs. As expected, no AH effect against *Oesophagostomum columbianum* was found for the monepantel group showed. Thus, feeding dry leaves of *M. caesalpiniifolia* represent a promising alternative for the control of GIN infections in goats.

1. Introduction

Gastrointestinal nematodes (GINs) constitute an important constraint for the production of grazing goats in tropical areas. *Haemonchus contortus* is the main agent responsible for the decreased productivity and high mortality of tropical goats, followed by *Trichostrongylus colubriformis* and *Oesophagostomum columbianum* (Costa et al., 2011). Control of GINs has been performed using synthetic anthelmintic with relative success during the last five decades (James et al., 2009). However, the rapid development and wide distribution of anthelmintic resistance in nematode populations in recent years is limiting GIN control (Kaplan, 2004). Thus, there is increasing interest for the development of alternative methods to reduce the dependence on synthetic anthelmintics (Torres-Acosta and Hoste, 2008).

Many tannin-rich plants have been studied as candidates with nutraceutical potential (Butter et al., 2000; Hoste et al., 2005). The

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condensed tannins (CTs) of these plants are considered responsible for the reduction in worm burden and/or for female fecundity, decreasing nematode egg excretion in the feces of the host and subsequently reducing pasture contamination, which leads to lower host re-infections (Hoste and Torres-Acosta, 2011; Martínez-Ortíz-de-Montellano et al., 2010; Lopes et al., 2016). CTs may also indirectly improve the productive performance and physiology of the host, causing a more effective immunologic response against GIN parasitism (Hoste et al., 2006, 2012). The anthelmintic (AH) activity of CT-rich plants appears to be influenced by the structural features of individual CTs and may vary with the location of production, the phenological stage of the plant and the plant species (Quijada et al., 2015; Aissa et al., 2016). Nonetheless, recent studies have reported that the CTs of some tropical plants are not responsible for the AH activity of tannin-rich plant materials (Vargas-Magaña et al., 2014; Chan-Pérez et al., 2016); moreover, the AH activity of certain plant is enhanced by blocking CTs (Hernández-Bolio et al., 2017a). Thus, it is important to study different CT-rich materials, particularly those that show potential as nutraceutical materials.

Mimosa caesalpiniifolia Benth, commonly known as "Sabiá" in the north of Brazil, is a tannin-rich plant characterized by rapid growth, high capacity for regeneration and drought resistance (Podadera et al., 2015). This shrub is readily consumed by ruminants and is used as a food supplement in the dry season in Brazil because of its high crude protein content, which varies between 18.8 and 28.6% (Vieira et al., 2005). Recently evaluated *in vitro*, this material demonstrated clear AH against *H. contortus* (Brito et al., 2017). Therefore, *M. caesalpiniifolia* is a tropical plant that may have a potential role as a nutraceutical, providing nutrients to goats while limiting the worm population of the animals consuming it (Hoste et al., 2005). The aim of the present study was to determine the *in vivo* anthelmintic effect of supplementation with *M. caesalpiniifolia* dry leaves against gastrointestinal nematodes in goats.

2. Material and methods

2.1. Plant material collection

Leaves of *M. caesalpiniifolia* were harvested in October 2012 in San Luis, Maranhão, Brazil (2°37′01″S and 44°16′19″W). Herbarium specimens were prepared, identified and deposited in the Herbarium Ático Seabra from Federal University of Maranhão, Brazil, under voucher number 101.

The plant material collected was dried under shade and ground using a mesh of 0.25 mm. Total phenols and total tannins were measured using the Folin-Ciocalteu method (Makkar et al., 1993, 2007; Makkar, 2000). The CT content was estimated using the HCl-butanol method (Porter et al., 1986). Total phenols and total tannins are expressed as tannic acid equivalents. CTs are expressed as leucocyanidin equivalents.

2.2. Experimental design

All procedures were approved through the Ethics Committee for the Animal Experimentation of the State University of Maranhão, Brazil, under number 015/2012.

Twenty-four Boer crossbred castrated male goats, aged six to eight months, of 15.0 ± 2.5 kg of body weight (BW) were obtained from a commercial farm. An initial fecal sample was obtained to determine the fecal egg count (FEC), as described by Gordon and Whitlock (1939). All animals were then treated with monepantel 5 mg/kg to eliminate natural GIN infection. The animals were maintained indoors during 15 days post-treatment. Once the animals were confirmed to be free of GIN infection by three negative FECs on three consecutive days, all the animals were infected (day 15) with a single oral dose of 16,000 larvae (L_3), consisting of 8000 *Haemonchus*, 6560 *Trichostrongylus* and 1440 *Oesophagostomum* worms (IFMA, Brazil). The goats were maintained inside concrete floor pens for 15 days for adaptation. The experiment was initiated (day 0) when the infection became patent, and the trial lasted for 28 days.

After the adaptation period, the goats were distributed into four groups of six animals balanced according to their initial FEC and BW. The control group received a concentrate feed without CT. The monepantel group received concentrate feed without CT and a single drench on day 0 (5 mg/kg BW of monepantel). The CT group received concentrate feed and *M. caesalpiniifolia* leaf powder. The CT + polyethyleneglycol (PEG) group received the same tannin-rich diet plus 10 g of PEG 4000 powder (Isofar, RJ, Brazil) per day mixed with the concentrate.

Goats consuming the *M. caesalpiniifolia* leaves (CT and CT + PEG groups) ingested 64.3 mg of CT/kg BW per day for seven consecutive days (days 1–7); during the third week, the same animals ingested 128.7 mg of CT/kg BW per day (days 14–21).

All animals were maintained indoors in individual pens and were fed a concentrated feed (68% corn, 26% wheat bran, 2% soybean, 2% calcareous and 2% mineral) representing 3% of the respective BW. Tifton grass hay (*Cynodon* sp.), water and mineral supplements specific for goats were provided *ad libitum*.

2.3. Ante mortem data

Animals were weighed weekly in the morning before feeding. During the experimental period, fecal samples were collected daily from the rectum of experimental goats to estimate individual FECs (Gordon and Whitlock, 1939).

Fecal samples were used once a week to produce a composite fecal culture for each group. The cultures were produced using the Roberts and O'Sullivan method as described by Ueno and Goncalves (1998). Infective larvae were harvested after five days and identified based on morphological characteristics according to Van Wyk and Mayhew (2013).

2.4. Post mortem data

At the end of the 28-day experimental period, the goats were fasted for 24 h prior to slaughter. During that time, they had *ad libitum* access to water. The animals were humanly slaughtered and their viscera immediately removed. The contents of and washings obtained from the abomasum and small and large intestines were preserved in separate individual containers that were clearly identified. The contents were analyzed to collect nematodes, and the parasites were separated with a stereoscopic microscope. All nematodes were mounted on slides with Hoyer's medium. Identification of nematodes was performed according to the morphological characters of Ueno and Goncalves (1998).

Worm burdens included all adult GINs. Geometric means were calculated for each experimental group following the formula used to determine efficacy in each treated group compared to the control group.

AH efficacy in the groups consuming *M. caesalpiniifolia* leaves (CT and CT + PEG groups) was estimated by comparing the respective worm burdens with that of the control group using the equation described by Wood et al. (1995):

$$\%\text{efficacy} = \frac{\text{control group geometric mean} - \text{treated group geometric mean}}{\text{control group geometric mean}} \times 100$$

2.5. Statistical analysis

Data on FECs and worm burdens were analyzed under logarithmic transformation log (x). These variables and body weight were then