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Oral administration of Sauce llorón extract to growing lambs to control gastrointestinal nematodes and *Moniezia* spp.

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ABSTRACT

Objective: To explore anthelmintic effects of oral administration of aqueous extract of Sauce llorón (*Salix babylonica*; SB) against gastrointestinal nematodes and *Moniezia* spp.
Methods: Sixteen Pelibuey male lambs of 3–4 months of age and (23.7 ± 3.3) kg body weight were used in a completely randomized design to be fed a total mixed ration (Control; SB0), or Control plus SB extract using 20 (SB20), 40 (SB40) and 60 (SB60) mL/lamb/day for 45 days. Lambs had a natural gastrointestinal nematodes and *Moniezia* spp. infection and had never been treated with chemical anthelmintic drugs. Individual faecal samples were collected for ova counting using McMaster procedure after 0, 7, 14, 21, 30 and 45 days post extract administration.
Results: No extract dose × day interactions for both gastrointestinal nematodes and *Moniezia* spp. egg count were found. Administration of SB extract had a higher effect (quadratic effect, $P = 0.006\ 4$) at dose of 20 mL SB/lamb/day for gastrointestinal nematode eggs during the first 21 days; however, the dose of SB40 tended (linear effect, $P = 0.089\ 7$) to be more effective than the others for *Moniezia* spp. egg during the first 7 days. Sampling day had a linear ($P = 0.043\ 6$) effect on *Moniezia* spp. egg count.
Conclusions: The aqueous extract of SB could be more effective against nematodes at 20 and at 40 mL/lamb/day for *Moniezia* spp. The use of the SB extract could represent a promising alternative to synthetic anthelmintics for the treatment of gastrointestinal nematodes and *Moniezia* spp. in small ruminants from organic and conventional production systems.

1. Introduction

Nematode parasitic diseases have been classified as a major health and welfare problems in small ruminants. It is a major cause of sheep and goat mortality in tropical Mexico[1] and other tropical countries[2,3] where climatic factors favor the development of parasitic infection[4]. This action leads to serious economic loss for small holder farmers[5], delay in achieving target animal weights, the increase in feed requirements, reduced quality of carcass, and predisposition to other diseases[6]. It would be expected that reductions in the level of parasitism would be followed by improvement on the performance of parasitized hosts[7]. The husbandry system in which livestock are raised could affect the exposure to nematode parasites. In situations where Mexican farmers are almost entirely dependent on grazing, exposure to nematode larvae is continuous throughout the year[8].

A lot of money are annually used to combat helminth parasites in livestock[2]. To date, the repeated use of chemical anthelmintic drugs is a usual method for gastrointestinal parasitism control. However, chemical anthelmintic drugs have

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several disadvantages, including lack of availability in some areas, inconsistent quality in some countries, prohibitive cost, as well as environmental contamination[9]. Furthermore, regular and misuse of chemical anthelmintics have resulted in nematode resistance[10], along with the risk of contamination of animal products; a problem which is most serious in sheep and goats in the tropics and developing countries. Routine use of chemical anthelmintics has also reduced the development of natural immunity against helminthes[11]. This has compiled to search for alternatives on helminth control methods[3,12,13].

One of the alternative methods is the use of ethnoveterinary medicine (*i.e.*, phytotherapy) using traditional herbs with anthelmintics activity[13–15]. It is a new, safe, convenient and environmentally friendly product with reduced potential for the development of nematode resistance[6]. However, ethnoveterinary knowledge and plant-based anthelmintics were the mainstays of anthelmintic treatment, and are still widely used in many traditional societies[16]. The potential benefits of ethnoveterinary livestock anthelmintics are clear, as the latter societies often depend on livestock, and live in areas where synthetic anthelmintics are unavailable, unaffordable, and/or of poor quality. However, the demonstration of ovicidal, larvicidal and adulticidal activities of traditional medicinal plants extracts and determination of therapeutic doses remain in the preliminary stages.

Mejía-Hernández *et al.*[15] tested the anthelmintic effects of *Salix babylonica* L. (*S. babylonica* L.) (*i.e.,* SB) and *Leucaena leucocephala* Lam. water extracts at level of 30 mL/lamb/day for a 63 d trial in growing lambs and concluded that both extracts could be promising alternatives to conventional chemical anthelmintics for the control of gastrointestinal parasites in small ruminants. Therefore, this work aimed to evaluate the efficacy of Sauce llorón (*S. babylonica* L.) aqueous extract against gastrointestinal nematodes and *Moniezia* spp. in growing Pelibuey lambs of tropical regions in Mexico.

2. Materials and methods

2.1. Lamb's management, treatments and feeding

Sixteen Pelibuey male lambs with 3–4 months of age and (23.7 ± 3.3) kg live body weight, after weaning, were used in a completely randomized design to study the anthelmintic effects of oral administration of aqueous extract of *S. babylonica* (*i.e.,* SB) against gastrointestinal nematode and *Moniezia* spp. Selected lambs had a natural gastrointestinal nematodes and *Moniezia* spp. infection and had never been treated with any chemical anthelmintic drugs or traditional herbs with anthelmintic activity. Lambs were individually housed in pens of 1.24 m × 0.82 m. After 2 weeks of adaptation for consuming a total mixed ration (TMR) composed of [g/kg dry matter (DM) basis] alfalfa hay 150, sorghum grain 530, soybean meal 220, molasses 20, fish meal 35, salt 20 mineral and vitamin premix, 25 (containing per kg of mineral premix: 19.60 g/kg calcium, 22.10 g/kg sulfur, 4 mg/kg cobalt, 15.93 mg/kg iodine, 15.49 mg/kg selenium; 860.73 mg/kg copper, 224.07 mg/kg zinc). The TMR had the following nutritional composition (g/kg DM): organic matter 912.4, crude protein 173.6, neutral detergent fiber 131.0, acid detergent fiber 80.3 and hemicelluloses 51.0. The SB extract content for secondary metabolites was determined according to Cowan[17].

The TMR used was the same that was previously fed to lambs of the experiment done at the same farm by Salem *et al.*[18]. Lambs were fed the same TMR with the addition of 0, 20, 40 and 60 mL SB extract/lamb/day for Control (SB0), SB20, SB40 and SB60, respectively. Extract was orally administered daily at 7:00 h before morning feeding to each lamb for 45 days of the experimental period. Lambs were fed at 7:00, 13:00 and 17:00 h with a TMR that was formulated to meet all of their nutrient requirements[19]. Feed and water intake was recorded daily during the experimental period.

2.2. Parasitological test

The egg count was performed using the same methods described before in Mejía-Hernández *et al.*[15]. Briefly, faecal samples from each lamb, within each experimental group, were collected rectally before morning feeding. Ova were counted using McMaster procedure[20]. The egg count was performed after 0 (pre-extract administration), 7, 14, 21, 30 and 45 days post extract administration. Faecal samples were evaluated for the presence of worm eggs by a salt flotation technique[21], where the eggs were counted by the McMaster method. Faecal pellets were collected and weighed, and 60 mL of saturated salt solution added per gram of faeces. The pellets were broken up using a mechanical stirrer, and then strained in a sieve with an aperture of 250 μm. Ten milliliters of the strained solution was used for determination of faecal egg counts using a 2 chamber McMaster slide with a limit of detection of 200 eggs/g faeces. Identification of nematodes and *Moniezia* spp. eggs in the faeces were achieved according to the standard methods of MAFF[21]. Faeces contents of individual animal's samples were brought up to 500 mL saturated salt solution. Five aliquots of faecal content (one gram of fresh faeces) from each lamb were used to identify the worm egg species of nematodes and *Moniezia* spp. in the sub-sample by counting using a stereoscope (40×). Faecal cultures were prepared for each experimental period as 5 replicates of pooled samples from each lamb as described by Terrill *et al.*[22] to allow counting and identification of parasite nematode larvae to species. Mean egg counts of nematodes and *Moniezia* spp. from each lamb, within each experimental treatment, were used for statistical comparisons among experimental groups.

2.3. Proximate analysis of TMR and secondary metabolites assay

Samples of diet were analyzed for DM, ash, crude protein according to the Association of Official Analytical Chemists[23]. Neutral detergent fiber and acid detergent fiber contents were analyzed using the ANKOM F-57 filter bags in an Ankom^{200} Fiber Analyzer unit (Ankom Technolgy, Macedon, NY USA) according to Van Soest *et al.*[24]. The neutral detergent fiber was assayed using α-amylase (Sigma A-3403 Sigma–Aldrich® Co., Louis MO, USA) but with sodium sulfite in the neutral detergent fiber and expressed without residual ash. Hemicellulose content was calculated from the difference between neutral detergent fiber and acid detergent fiber.

S. babylonica extract was weekly prepared (5 L) as previously described in Salem *et al.*[25]. Briefly, fresh leaves were