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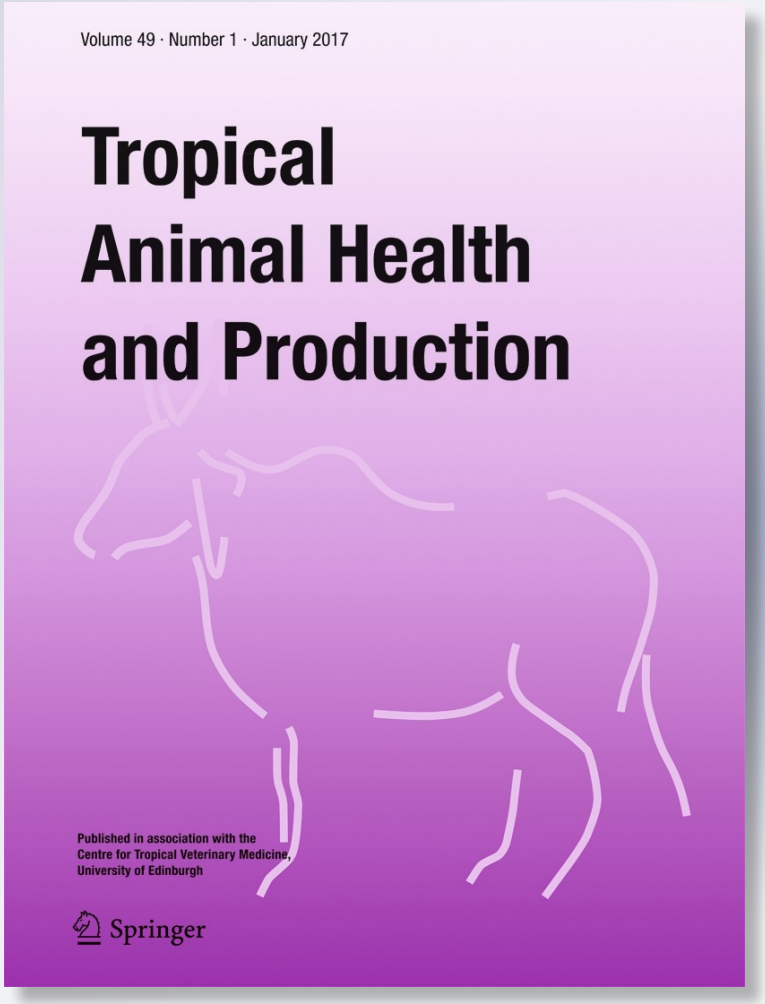

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REGULAR ARTICLES

Nutraceutic effect of free condensed tannins of *Lysiloma acapulcensis* (Kunth) benth on parasite infection and performance of Pelibuey sheep

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Abstract Forty-five Pelibuey sheep were experimentally infested with nematodes to evaluate the effect of three free condensed tannin (FCT) levels of *Lysiloma acapulcensis* on fecal egg counts (FECs), packed cell volumes (PCV), ocular mucosa colors (OMC), average daily gain (ADG), and adult nematode count. Five treatments were used: 12.5, 25.0, and 37.5 mg of FCT kg^{-1} of body weight (BW); sterile water (control); and ivermectine (0.22 mg kg^{-1} of BW) as chemical group. The data were processed through repeated measurement analysis. Even though the three FCT doses decreased ($P < 0.05$) the FEC, the highest reduction was obtained with 37.5 mg kg^{-1} of BW. No differences were observed in PCV and OMC. Higher ADG ($P < 0.05$) was observed with 37.5 mg kg^{-1} of BW of FCT. The count of adult nematodes (females and males) in the higher dose of FCT was similar to chemical treatment. Dose of 37.5 mg kg^{-1} of BW decreased the parasite infection and improved the lamb performance. Therefore, this dose could be used as a nutraceutic product in sheep production.

Keywords Pelibuey · Sheep · Tannins · Nematodes · *Lysiloma acapulcensis* · Ivermectine

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Introduction

Actually, the problem of resistance to pharmaceuticals by gastrointestinal nematodes (GIN) has become aggravated, representing one of the principal challenges for parasite control in small ruminants (Torres-Acosta et al. 2012; Arece et al. 2014; Cedillo et al. 2015). As an alternative to this problem, new parasite control methods have been evaluated, highlighting the use of secondary plant components (Olmedo et al. 2014; Kommuru et al. 2015; Saric et al. 2015). Thus, in vivo studies have demonstrated the effectiveness of plants rich in tannins for the reduction of the parasite load (Martínez-Ortiz de Montellano et al. 2009; Debela et al. 2012; Ahmed et al. 2014) and egg excretion by direct affectation of fertility in females (Mupeyo et al. 2011). *Lysiloma acapulcensis* is a perennial tree, which is abundant in the south of Mexico state. Studies have been made to evaluate its nutritive potential (Camacho et al. 2010; Olmedo et al. 2015) and in vitro studies have been made to validate its possible anti-parasitic effects in ruminants (Olmedo et al. 2014). This represents a promising alternative to chemotherapy, especially when it is used as nutraceutic alternative, which combines the effects of a reduction of the parasite infection and a better nutritional status of the animals. Therefore, the objective of the present study was

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to evaluate the effect of different levels of free condensed tannins (FCTs) of *L. acapulcensis* in the diet on parasite infection of experimentally infested sheep and on some bioproductive indicators.

Materials and methods

Location

The study was developed in the Estación Experimental de Pastos y Forrajes "Indio Hatuey" Cuba. Plant material of *L. acapulcensis* was collected in the southwestern of the State of Mexico, Mexico, with 1740 m asl, and subhumid climate with rains in summer (Aw) (INEGI 2014).

Collection of plant material

For the collection of the plant material, seven sites were delimited in which trees of *L. acapulcensis* were found as part of the natural vegetation. Later, during the morning, the foliage (mature and young leaves) from seven trees was collected on each site; it was taken in a thermal container with artificially cooled temperature and was carried out to the University Center UAEM Temascaltepec (CUT) for chemical analyses. A total of 49 individual samples were collected, which corresponded to seven true replicates.

Processing of the plant samples

At the laboratory, samples were dried in a forced air oven at 48 ° C during 72 h until constant weight was reached. Afterward, a pool was made with all the samples to attain homogeneity; then, they were ground in a Willey mill to reach a particle size of 2-mm diameter and were high vacuum packed to be transported to Cuba.

Proximal chemical analysis and fractioning of the tannins in the foliage of *L. acapulcensis*

Dry matter (DM) was determined in the samples (DM, method 934.01) along with ash (CEN, method 942.05), crude protein (CP, method 954.01) (AOAC 1997), neutral detergent fiber (NDF), and acid detergent fiber (ACF) (Van Soest et al. 1991). Condensed total tannins (CTT) were determined using the method of butanol-HCl (Terrill et al. 1992), with the modifications of López et al. (2004). The analysis of free condensed tannins (FCTs) was determined using the method reported by Porter et al. (1986) (Table 1).

Animals and feed

Forty-five Pelibuey male lambs (21.9 ± 1.5-kg body weight (BW), 6 months of age) were individually housed in pens equipped with shade, feed through an automatic waterer. The lambs were dewormed (Levamisol 10 %® at 7.5 mg/kg of BW, LABIOFAM, Cuba) 12 days before the start of the experiment and were maintained in total enclosure. During the experimental period, all animals received a mixture of fresh fodder ad libitum composed of 50 % of the sugar cane plant (*Saccharum officinarum*, 6 months of age) and 50 % king grass (*Pennisetum purpureum*, clone OM-22, 60 days of regrowth); the fodder was supplied chopped to a particle size of 3 cm. Additionally, all animals were supplemented with 400 g/animal/day of concentrate with 22.3 % of CP (NRC 2007), fractioned in two doses (09:00 and 14:00 h). Every 15 days, adjustments were made to the dietary balance as a function of the live weight of the animals. The Animal Protection Commission and the Scientific Council of the Estación Experimental de Pastos y Forrajes Indio Hatuey approved all the procedures to be carried out with the animals.

Infective larvae (L_3)

An infested animal (donor) was used with a high parasite load of a mixture of gastrointestinal strongilids (95 % *Haemonchus contortus*, 2 % *Trichostrongylus colubriformis*, and 3 % of *Oesophagostomum columbianum*) and was maintained in a metabolism cage for the collection of feces, which were used to obtain the fecal cultures (Roberts and O'Sullivan 1952) for the collection of infesting larvae. The larvae were identified, quantified, and conserved in refrigeration (8 °C) until the experimental infection.

Treatments, experimental procedure, and measurements

The animals were randomly distributed (Table 2) in five experimental groups: two control groups (positive and negative) and three doses (12.5, 25.0, and 37.5 mg/kg of live weight) of FCT added to the diet every 3 days after the confirmation of an established infection. The doses were calculated based on the concentration of FCTs, for which the animals were offered daily the following amount of fodder of *L. acapulcensis*: 2.14, 3.22, and 6.5 g of DM. After verifying the efficacy of Levamisol 10 %® administered prior to the start of the experiment (12 days), the animals were infested (day zero) with 3000 larvae L_3 of a mixture of GIN previously mentioned.

Fecal egg count

Every 3 days, the feces of each animal were extracted directly from the rectum for fecal egg count (FEC) determination, which

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Table 1 Chemical composition and condensed tannin profile (g/kg DM) in leaves of *Lysiloma acapulcensis*

Specie	OM	CP	NDF	ADF	FCT	CT-CP	CT-F	TCT
L. acapulcensis	945.9	177.0	607.3	500.8	116.3	67.8	3.7	187.8

OM organic matter, *CP* crude protein, *NDF* neutral detergent fiber, *ADF* acid detergent fiber, *FCTs* free condensed tannins, *CT-CP* condensed tannin-bound crude protein, *CT-F* condensed tannin-bound fiber, *TCT* total condensed tannins

was expressed in eggs per gram (EPG) of feces according to the McMaster technique (Arece et al. 2002).

Packed cell volumes and ocular mucosa colors

Blood was drawn by puncture of the jugular vein and deposited in tubes with EDTA as anti-coagulant for packed cell volumes (PCV) determination by microcentrifugation. Briefly, a capillary was filled and sealed at one end and, after that, was centrifuged at 12,000 rpm during 5 min, and the relative value occupied by the PCV was determined. OMC was determined with the color chart for the detection of anemia FAMACHA©, which has five categories, where one corresponds to an animal with bright red coloration and five to one with pale membranes (Van Wyk and Bath 2002).

Measurement of live weight gain

The animals were weighed every third day using an OHAUS-3370 (100 ± 0.050 kg) scale to determine daily live weight gain, and health status was monitored daily.

Count of adult nematodes

With the approval of the Animal Protection Commission and the Scientific Council of the Estación Experimental de Pastos y Forrajes Indio Hatuey, five animals per group were necropsied to count all adult parasites (male and female) in abomasum.

Statistical analysis

The analysis of the information was performed using SAS® software version 9.1.3 (SAS, 2014) through the Proc Mixed, in which three covariance structures were evaluated, and the unstructured (UN) covariance resulted in the best fit. Prior to this analysis, normal distribution of the data and homogeneity of the variance were verified, so it was necessary to transform the percent variable (PCV) through the Arcsin$\sqrt{X}$ and the variable FEC using Log 10 ($x + 1$). The differences among means were determined with the Tukey test using an error of 0.05 (Steel et al. 1997).

Table 2 Description of experimental treatments

Treatment	Description	*L. acapulcensis* forage
Control	Without treatment	
Chemical	Ivermectine 0.22 mg kg^{-1} of BW	
12.5 FCT	12.5 mg kg^{-1} of BW of FCT	0.107 g kg^{-1} of BW
25.0 FCT	25.0 mg kg^{-1} of BW of FCT	0.214 g kg^{-1} of BW
37.5 FCT	37.5 mg kg^{-1} of BW of FCT	0.322 g kg^{-1} of BW

FCTs free condensed tannins, *BW* body weight

Results

Fecal egg count

The egg count reduction dynamic of GIN when supplemented with dehydrated foliage of *L. acapulcensis* is presented in Fig. 1. The experimental infection was carried out on day "0," and a prepatent period started on day 18 post-infection for all groups. From this time, different trends of egg count reduction were observed according to the treatment group. The control group showed the highest FEC ($p < 0.05$) with respect to the other treatment groups, which demonstrates the effectiveness in reduction of the parasite infection by this plant.

Packed cell volumes and ocular mucosa colors

The principal health indicators monitored during the experiment are presented in Fig. 2. No differences ($p > 0.05$) were observed in any of these indicators during the entire experimental period. The OMC showed little variation during the experimental period and is the result of having PCV values higher than 20 %.

Live weight gain

Live weight gain of the animals throughout the experimental period is shown in Fig. 3. Lambs of the control group presented the worst productive behavior ($p \leq 0.05$), while the best weight gain was found in lambs that received the highest dose of FCT ($p \leq 0.05$) with averages higher than 120 g/animal/day. The low and medium groups of FCT showed similar behavior to that of the chemical group (treated with ivermectine).

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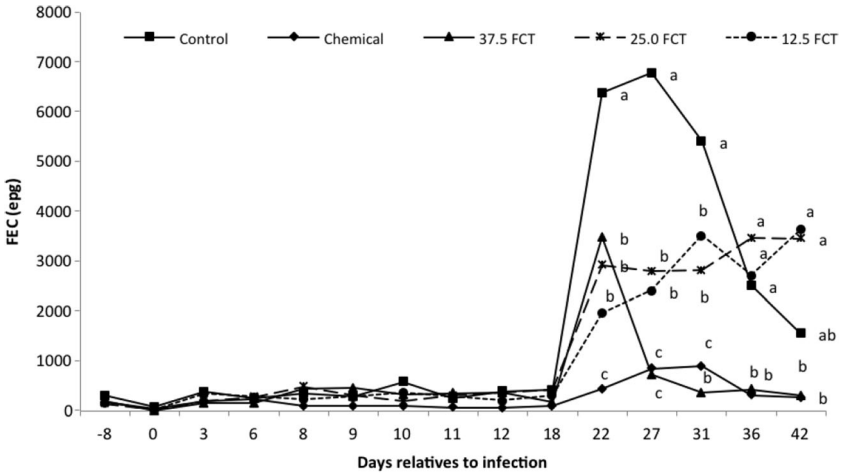

Fig. 1 Fecal egg count dynamic in the five experimental groups

Adult nematodes

The adult nematode count in the animals is observed in Fig. 4, where the groups with the lowest doses of FCT had similar amount of parasites as the control, both in male and female parasites. The treatment of 37.5 mg/kg of BW eliminated the adult parasites at the same level as the chemical product.

Discussion

Fecal egg count

This study showed that the highest dose of FCT (37.5 mg/kg^{-1} of BW) had higher efficacy in the reduction of FEC, with respect to the control group. In addition, a similar trend was observed in the chemical group starting on day 27 post-infection. The intermediate and low doses also reduced the amount of eggs eliminated in feces with lower values. It is probable that higher doses of FCT had better effects on the parasites already established in the abomasum; however, it is also possible that it would have interfered with the absorption of nitrogen (Min et al. 2003). On the other hand, Olmedo et al. (2015) found a quadratic effect when 2.0, 5.0, and 7.5 g of FCT day^{-1} were used in sheep during 60 days. In addition, there are studies (Min et al. 2005) that mention that ruminal microorganisms exposed to low doses of condensed tannins have the capacity to adapt to these secondary compounds.

Based on these results, we inferred that for the doses used in the present study, mainly in the group with the highest concentration (37.5 mg of FCT kg^{-1} of BW), the metabolism of the nutrients does not represent a problem, given that it did not exceed 1 % of the FCT consumption in lambs.

Several studies have demonstrated the effects of the inclusion of tanniferous plants in diets of animals on the establishment and maintenance of parasite infection (Brunet et al. 2008, Manolaraki et al. 2010, Martínez et al. 2013). At present time, this is an important alternative given that anthelmintic resistance represents a threat for an adequate development of sheep production (Torres-Acosta et al. 2012).

In in vitro studies, the efficacy of *L. acapulcensis* was demonstrated in the reduction of the hatching capacity of eggs, inhibiting the development of larvae from stages L_1/L_2 to L_3, and also interfered in the migration of L_3 (Olmedo et al. 2014);

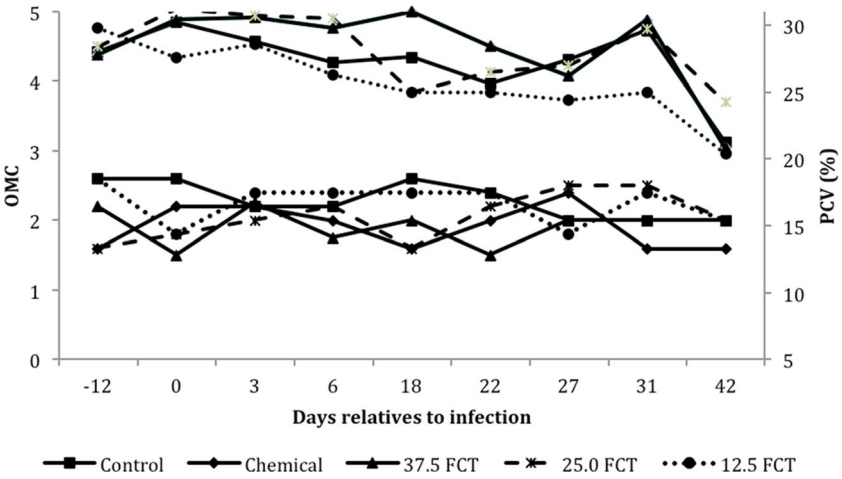

Fig. 2 Means of ocular mucosa colors (OMC) and packed cell volumes (PCV) in the five experimental groups

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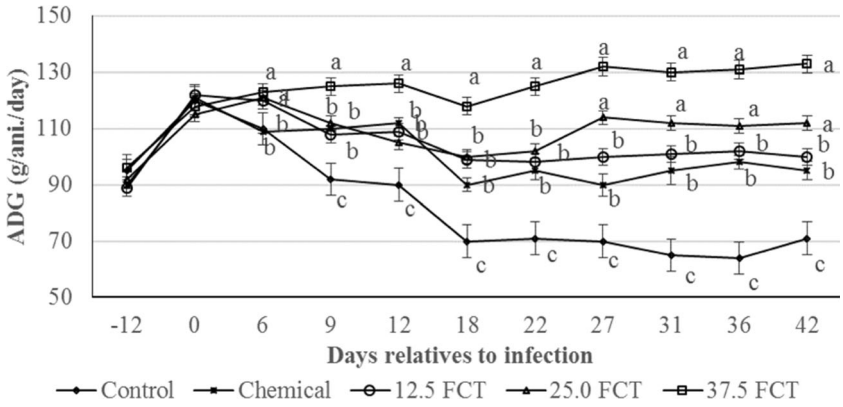

Fig. 3 Average live weight gain in the five experimental groups

these results make possible to deduce the importance of this plant as a potential species for the control of GIN in extensive productive systems of small ruminants.

Brunet et al. (2008) found that supplementation of fresh foliage of *Lysiloma latisiliquum* at doses of 1.4 mg/kg of BW of TCT reduced the establishment of *H. contortus* and *T. colubriformis*, while Martínez et al. (2013) when administered foliage of the same plant found that in doses of 16.12 g/day, there was a dramatic reduction in FEC and also in fertility of females of *H. contortus*. These results, along with those obtained in the present study, demonstrate the effectiveness of supplying plants rich in CT for short periods.

The direct effects are related with structural damage in the adult nematodes that prevented their appropriate feeding, movement, and mating, as was demonstrated by Martínez et al. (2013) in studies using an electronic microscope.

Relative to the efficacy of each group calculated as a function of relative reduction to the control group, it was observed that ivermectine showed an efficacy lower than 95 %, which indicates the presence of low resistance or tolerance to this medication (Coles et al. 1992). On the other hand, the group of animals that received the highest dose of FCT showed an efficacy on reduction of FEC higher than 80 % after day 22 post-infection, while in the groups of minimum dose (12.5 mg of FCT kg^{-1} of BW) and median dose (25.0 mg of FCT kg^{-1} of BW), the efficacy was erratic given that, first, the count was reduced in more than 35 % and reaching values higher than 68 %. However, after 36 days, average fecal counts in these groups were higher than those found in the control group. This behavior is probably the result of a response of the parasites to non-lethal doses that interfered in their feeding and egg-laying capacity but did not eliminate them completely. It is likely that during the period of study, the parasites created adaptation mechanisms which started at day 31, allowing the elimination of eggs through the feces.

In concordance with the present results, a study developed in Brazil reported no effect of the administration of pure marketable extract of *Acacia mearnsii* on the number of adult parasites. However, the viability of the eggs *T. colubriformis* (Minho et al. 2010) was compromised, a variable that was not evaluated in the present study. It is probable that, in addition to the dose used, there are other factors such as the nature of the tannins and its chemical structure (Min et al. 2003) that could interfere with their general biological activity (Hoste et al. 2006, Quijada et al. 2015).

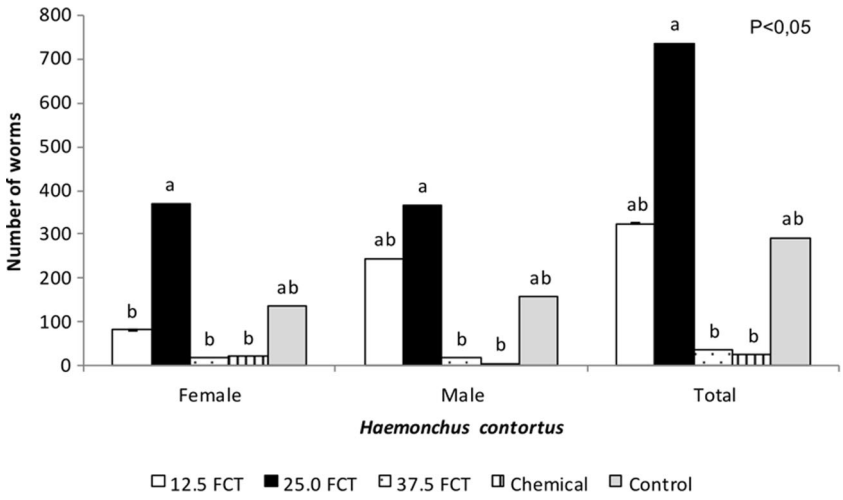

Fig. 4 Adult nematode (*Haemonchus contortus*) count in the five experimental groups

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Packed cell volumes and ocular mucosa colors

The values of PCV and OMC observed did not indicate anemia in the animals; thus, the inoculated dose of larvae did not have significant effect on health and the animals maintained hemostasia; furthermore, the physiopathological changes induced in them are directly related to the nutritional plan to which they were subjected. In this sense, Arece et al. (2013) found that under similar conditions, no important variations in health were detected in the animals.

Live weight gain

The weight differences observed are probably related to a better use of the protein in the diet as a result of a greater supply of tannins. In rumen, at pH between 6 and 7, the complexes that form between the FCT and the proteins are stable (Butter et al. 2000) and contribute to protect the proteins from ruminal degradation. The formation of these complexes between the tannins and the dietary proteins or microbial enzymes globally reduces the ruminal proteolysis (Min et al. 2003), favoring the flow toward the abomasum where they are degraded by the gastric juices (HCl) and digestive proteases. This in turn increases the flow of amino acids to the duodenum, which, together with the microbial protein, increases the pool of metabolizable nitrogen compounds, promoting the anabolism of muscular protein.

Probably, if higher doses of FCT were used in the present study, there would be more interference in the use of protein, NDF, and organic matter as a result of the union of the FCTs to the enzymes produced by the ruminal microorganisms, which would also reduce the nitrogen content and consequently would lead to a reduction in the digestion of the potentially degradable fractions (Romero et al. 2000).

The *L. acapulcensis* had 61.9 % of the CT in free form (FCT), similar to those reported by other trees such as *Guazuma ulmifolia* (72.2 %), *Leucaena leucocephala* (73.8 %), and *Gliricidia sepium* (71 %) (López et al. 2004). This caused the possible effects in the formation of complexes with the diet and endogenous protein to be greater (Scull and Savón 2003), which reflected an improved productive behavior of the animals.

Adult nematode count

The present study demonstrates the direct effect of tannins on adult parasites by decreasing the egg-laying capacity on one hand and eliminating adult parasites on the other, as a function of the dose used. In the dose of 37.5 mg of FCT kg^{-1} of BW, more than 90 % of adult nematodes were eliminated. As previously mentioned, it is probable that this dose caused physical damage to the parasites, preventing their permanence in the digestive system of the animals and caused them to be eliminated in feces. In the lowest dose, reduction registered in egg count was related to variations in the fertility of the parasites as an adaptation mechanism, as other studies have been shown (Mupeyo et al. 2011).

Conclusion

The dose of 37.5 mg/kg of FCT of the leaves of *L. acapulcensis* eliminated the already established parasites and consequently decreased the elimination rate of eggs. In addition, it improved the live weight gain of the male lambs, which demonstrates a nutraceutic effect.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicting interests.

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