REVISTA CONTRIBUCIONES A LAS CIENCIAS SOCIALES

Mangifera indica L. (Anacardiaceae) leaf favor the control of *Haemonchus contortus*

Folhas de *Mangifera indica* L. (Anacardiaceae) favorece o controle de *Haemonchus contortus*

Las hojas de *Mangifera indica* L. (Anacardiaceae) favorecen el control de *Haemonchus contortus*

DOI: 10.55905/revconv.17n.6-350

Originals received: 05/21/2024
Acceptance for publication: 06/11/2024

Kaike Mágno de Macêdo
Master in Animal Production
Institution: Universidade Federal de Minas Gerais
Address: Montes Claros - Minas Gerais, Brasil
E-mail: kaikemacedo@hotmail.com
Orcid: https://orcid.org/ 0009-0004-0122-6877

Franciellen Morais-Costa
PhD in Sciences
Institution: Universidade Estadual de Montes Claros
Address: Montes Claros - Minas Gerais, Brasil
E-mail: franciellen.costa@unimontes.br
Orcid: https://orcid.org/0000-0002-6690-987X

Adriano Vinícius de Paiva Ferreira
Master in Ruminant Production and Nutrition
Institution: Universidade Federal de Lavras
Address: Laras - Minas Gerais, Brasil
E-mail: adriano_paivinh@yahoo.com.br
Orcid: https://orcid.org/0000-0002-0814-7141

Ana Cláudia Maia Soares
PhD in Zootechnics
Institution: Universidade Estadual do Sudoeste da Bahia
Address: Itapetinga - Bahia, Brasil
E-mail: aninha_maiamoc@yahoo.com.br
Orcid: https://orcid.org/0000-0003-0078-9518

Valdo Soares Martins Júnior
Master in Animal Production
Institution: Universidade Federal de Minas Gerais
Address: Montes Claros - Minas Gerais, Brasil
E-mail: valdo-soares1@hotmail.com
Orcid: https://orcid.org/0000-0001-5447-0970

Dheison Emanuel Augusto Pereira
Graduate in Zootechnics
Institution: Universidade Federal de Minas Gerais
Address: Montes Claros - Minas Gerais, Brasil
E-mail: dheison.emanuel@hotmail.com
Orcid: https://orcid.org/0000-0002-2882-3661

Viviane de Oliveira Vasconcelos
PhD in Parasitology
Institution: Universidade Estadual de Montes Claros
Address: Montes Claros - Minas Gerais, Brasil
E-mail: viviane.vasconcelos@unimontes.br
Orcid: https://orcid.org/0000-0001-5126-3124

Neide Judith Faria de Oliveira
PhD in Animal Science
Institution: Universidade Federal de Minas Gerais
Address: Montes Claros - Minas Gerais, Brasil
E-mail: neidejudith@hotmail.com
Orcid: https://orcid.org/0000-0003-0854-892X

Eduardo Robson Duarte
PhD in Biological Sciences, Microbiology
Institution: Universidade Federal de Minas Gerais
Address: Montes Claros - Minas Gerais, Brasil
E-mail: duartevet@hotmail.com
Orcid: https://orcid.org/0000-0002-2205-9412

Otávio Cardoso Filho
PhD in Health Sciences
Institution: Universidade Estadual de Montes Claros
Address: Montes Claros - Minas Gerais, Brasil
E-mail: otavio.filho@unimontes.br
Orcid: https://orcid.org/0000-0001-7694-0590

ABSTRACT
Bioactive compounds from plants are promising alternatives for controlling resistant nematode populations. However, these metabolites must be obtained from plants that are widely available. In this study, we evaluated the efficacy of *Mangifera indica* L. leaves for the control of *Haemonchus contortus*. Larval development inhibition (LDI) in quantitative fecal culture (FC)

as well as egg hatching inhibition (EHI) were evaluated. Two *in vivo* tests were performed to reduce fecal egg counts (FEC) in infected lambs. The concentration of condensed tannin (proanthocyanidin) was 3.79% ± 0.01 for aqueous extract (AE). Further, reverse phase high-performance liquid chromatography revealed the presence of tannins, flavonoids, and flavones in this extract. The leaf powder at 30 $mg \cdot g^{-1}$ displayed 100% efficacy in the LDI test and 20.7% FEC reduction after 14 days of oral treatment with 4.978 g (dm)/kg (bw). In addition, 30 $mg \cdot mL^{-1}$ of the AE displayed 81.65% efficacy in the EHI test and had an LC90 of 29.88 $mg \cdot g^{-1}$ in the LDI test. The FEC reduction test revealed an efficacy of 42.8% after 21 days of treatment with AE at 0.601 $g \cdot kg^{-1}$/bw. Lambs treated with AE had better ocular mucosa score and higher erythrocyte, hemoglobin, hematocrit, and β-globulin concentrations than untreated lambs. Overall, the AE of *M. indica* leaves promotes high and moderate anthelmintic efficacy in *in vitro* and in *vivo* tests, respectively, and increased erythrocyte count. Such findings suggest that the AE of *M. indica* leaves is an alternative or complementary treatment for hemonchosis.

Keywords: sheep, anthelmintic plant, mango, blood parameters, biochemical parameters.

RESUMO

Compostos bioativos de plantas são alternativas promissoras para o controle de populações de nematódeos resistentes. No entanto, esses metabólitos devem ser obtidos de plantas amplamente disponíveis. Neste estudo, foi avaliado a eficácia das folhas de *Mangifera indica* L. no controle de *Haemonchus contortus*. A inibição do desenvolvimento larval (IDL) em coprocultura, bem como, a inibição da eclosão larval (IEL) foram avaliadas. Dois testes *in vivo* foram realizados para a contagem da redução de ovos em cordeiros infectados. A concentração de tanino condensado (proantocianidina) foi de 3,79% ± 0,01 para o extrato aquoso (EA). Além disso, a cromatografia líquida de alta performance de fase reversa revelou a presença de taninos, flavonóides e flavonas neste extrato. O pó da folha a 30 mg g^{-1} exibiu 100% de eficácia no teste IDL e 20,7% de redução de CQF após 14 dias de tratamento oral com 4,978 g (dm) / kg (pc). Além disso, 30 mg mL^{-1} do EA exibiu 81,65% de eficácia no teste IEL e teve um CL90 de 29,88 mg g^{-1} no teste IDL. O teste de redução de ovos por grama de fezes revelou uma eficácia de 42,8% após 21 dias de tratamento com AE a 0,601 g kg^{-1}/pc. Cordeiros tratados com EA tiveram melhor escore de mucosa ocular e maiores concentrações de eritrócitos, hemoglobina, hematócrito e β-globulina do que cordeiros não tratados. De maneira geral, o EA das folhas de *M. indica* promove alta e moderada eficácia anti-helmíntica em testes *in vitro* e *in vivo*, respectivamente, e aumento na contagem de eritrócitos. Esses achados sugerem que o EA de folhas de *M. indica* é uma alternativa ou um tratamento complementar para a hemoncose.

Palavras-chave: ovinos, planta anti-helmíntica, manga, parâmetros sanguíneos, parâmetros bioquímicos.

RESUMEN

Los compuestos bioactivos de las plantas son alternativas prometedoras para controlar las poblaciones de nematodos resistentes. Sin embargo, estos metabolitos deben obtenerse de plantas que estén ampliamente disponibles. En este estudio evaluamos la eficacia de las hojas de *Mangifera indica* L. para el control de *Haemonchus contortus*. Se evaluó la inhibición del desarrollo larval (IDL) en cultivo fecal cuantitativo (CF), así como la inhibición de la eclosión de huevos (IEH). Se realizaron dos pruebas in vivo para reducir el recuento de huevos en heces (RHE) en corderos infectados. La concentración de tanino condensado (proantocianidina) fue de

3,79% ± 0,01 para el extracto acuoso (EA). Además, la cromatografía líquida de alta resolución de fase inversa reveló la presencia de taninos, flavonoides y flavonas en este extracto. El polvo de hoja a 30 mg·g-1 mostró una eficacia del 100% en la prueba IDL y una reducción de FEC del 20,7% después de 14 días de tratamiento oral con 4,978 g (dms)/kg (bw). Además, 30 mg•mL-1 del AE mostraron una eficacia del 81,65% en la prueba IEH y tuvieron una CL90 de 29,88 mg•g-1 en la prueba IDL. La prueba de reducción de RHE reveló una eficacia del 42,8% después de 21 días de tratamiento con AE a 0,601 g·kg^{-1}/pc. Los corderos tratados con AE tuvieron una mejor puntuación de la mucosa ocular y concentraciones más altas de eritrocitos, hemoglobina, hematocrito y β-globulina que los corderos no tratados. En general, el EA de las hojas de M. indica promueve una eficacia antihelmíntica alta y moderada en pruebas in vitro e in vivo, respectivamente, y un aumento del recuento de eritrocitos. Tales hallazgos sugieren que la EA de las hojas de *M. indica* es un tratamiento alternativo o complementario para la hemoncosis.

Palabras clave: ovino, planta antihelmíntica, mango, parámetros sanguíneos, parámetros bioquímicos.

1 INTRODUCTION

Ruminants produced on pastures represent an important sustainable part of the global agricultural economy. However, gastrointestinal nematodes (GIN) are limiting for the development of these animals (Sczesny-Moraes *et al*., 2010; Leathwick and Besier, 2014). *Haemonchus contortus* is the most common nematode in sheep reared in tropical areas. In fact, *H. contortus* promotes high mortality in young lambs and ewes during the peripartum period (Bastos *et al*., 2017). Hemonchosis causes reduced weight gain and feed conversion efficiency and decreases meat, wool, and milk production (Taylor *et al*., 2009).

Conventional anthelmintic treatment has served as a rapid method of control for *H. contortus*; however, *H. contortus* has shown resistance on several continents (Bastos *et al*., 2017, Lamb *et al*., 2017). The immune response and the associated resistance can be modified by the type of antigen that is recognized and by factors, such as age, nutrition, and the number of infections (Alba-Hurtado and Muñoz-Guzmán, 2013).

The geographical distribution of significant *H. contortus* infection and the existing resistance of *Haemonchus* strains to various anthelmintics of all classes are concerning, and should be considered in the formulation of health plans for controlling this infection (Arsenopoulos *et al*., 2021). The use of plant extracts to reduce populations of multiresistant

nematodes may be a key strategy for integrated control programs against GIN (Leathwick and Besier 2014; Morais-Costa *et al*., 2014; Tariq *et al*., 2017).

Anacardiaceae are commonly used for medicinal purposes, and contain phenols and tannins that have antimicrobial properties (Kabongo-Kayoka *et al*., 2016). *Mangifera indica* L. var. "Ubá" is a member of this family, is native to Asia, and is common on most continents, with a world production of approximately 40 million tons per year (Mitra, 2016). This fructiferous fruit is cultivated in many tropical and subtropical regions and produces one of the most popular fruits, which contributes to its economic and social importance (Saúco, 2004). This plant has been reported to have several therapeutic properties, including antioxidant, analgesic, anti-inflammatory, and immunomodulatory activities in the control of chronic bronchitis, dysentery, and intestinal bleeding in humans, and diuretic activity (González *et al*., 2007).

The use of plants with anthelmintic potential, regardless of the preparation process, has revealed the action of active substances with varying levels of efficacy in small ruminants (Mottin *et al*., 2019). In a previous parasitological study, the ethanolic extract from the seeds of this plant promoted 95.6% egg hatching inhibition (EHI) for *H. contortus* (Costa *et al*., 2002). Based on an assessment of the potential of immature mango juice as an anthelmintic, Nery *et al*. (2012) reported that the oral administration of 74 $g \cdot kg^{-1}$ of this fruit as a juice resulted in 53% reduction in fecal egg counts (FEC) in lambs. Although the *M. indica* materials are not produced throughout the year, its leaves are abundant during all seasons. However, little is known about the anthelmintic effects and potential benefits or toxicity of *M. indica* leaves when employed as a treatment for animals with hemonchosis.

In this study, we evaluated the anthelmintic activity of *M. indica* leaves against *H. contortus in vitro* and *in vivo*. To evaluate its beneficial or toxicological effects, we analyzed the clinical, hematological, and serum biochemical parameters of lambs treated with the aqueous extract (AE) of these leaves.

2 MATERIAL AND METHODS

2.1 PLANT COLLECTION AND EXTRACT PREPARATION

The leaves of *M. indica* were collected between May and July in Montes Claros city, north of Minas Gerais, Brazil (43 ° 51 '23.3″ "S and 16 ° 44' 02.08"″). According to Köppen, the area is part of the type, AW, with a tropical savanna climate, including dry winters and rainy summers (Alvares, *et al*., 2014). Precipitation in this area is approximately 700–1,200 mm.

Healthy leaves were selected and dried to constant weight in a forced air circulating dryer (TE 394/4, Tecnal Equipamentos CientíficosTecnal, Piracicaba, SP, Brazil) at 40 °C for 72 h. Dried leaves were ground in a Wiley mill (CE- 430/Macro, Cienlab, SP, Brazil) and the powder was stored in paper bags in the dark.

The AE was produced by placing the ground dried leaves in a distilled water bath (100 g/L) at 40 °C for 60 min. Subsequently, the extract was filtered through a gauze funnel and dehydrated at 40 °C for 48 h to obtain a residue with constant weight, which was stored in paper bags in the dark (Morais-Costa *et al*., 2016). Dry matter (dm) was verified at 105 °C according to the Association of Official Analytical Chemists (AOAC, 1990) on five subsamples of the AE and dry powder to calculate the concentrations to be tested.

2.2 EXTRACT CHARACTERIZATION AND TOTAL PROANTHOCYANIDINS

A Waters Alliance 2695HPLC system composed of a quaternary pump, auto-sampler, photodiode array detector (DAD) 2996, and Waters Empower Pro data handling system (Waters Corporation, Milford, Connecticut, USA) was used to characterize the extract. A LiChrospher 100 RP-18 column (5 µm particle size, L × internal diameter (ID) 250 × 4 mm) was used in combination with a LiChrospher 100 RP-18 guard column (5 µm particle size, L × ID × 40 × 4 mm) (Merck, Darmstadt, Germany) at 40 °C.

Water (A) and acetonitrile (B), both containing 0.1% (v/v) H3PO4, were used as eluents at a flow rate of 1.0 mL·min−1 as follows: 0 min, 95% A and 5% B; 60 min, 5% A, 95% B, followed by 10 min of isocratic elution. The solvents used were of HPLC grade (Merck,

Germany) and were degassed by sonication before use. The chromatograms were obtained at 210 nm, and the UV spectra were recorded online from 190 to 400 nm.

The dried extracts were dissolved in methanol (HPLC-grade), ultrapure water, or hydroethanolic solutions at a concentration of 10 mg/mL according to their solubility. After centrifugation at 8400 g for 10 min, 10 µL of the sample solution was automatically injected into the apparatus.

The total condensed tannin (proanthocyanidin) content of AE was determined according to the method described by Hiermann et al. (1986) by measuring the absorbance of cyanidin chloride resulting from acid-catalyzed solvolysis with n-BuOH/HCl 12 M (95:5) at 540 nm. The AE was analyzed in triplicate, and the results are expressed as the mean ± standard deviation. The total condensed tannin content, expressed as cyanidin chloride, was calculated using the following formula:

Condensed tannins% = absorbance sample − absorbance blank × 4.155/weight sample (g)

2.3 EGG HATCHING INHIBITION (EHI)

All procedures were performed in accordance with the principles of animal experimentation approved in the 42/2008 protocol of the Ethics Committee on Animal Experimentation (CETEA) of the Federal University of Minas Gerais, Brazil.

Two Santa Inês X Doper lambs were infected with 2.000 L3 of H. contortus resistant to albendazole. This strain was obtained from H. contortus females collected from the abomasum of a lamb in North Minas Gerais, Brazil (Duarte et al., 2012). After 22 days, lambs had a mean fecal egg count (FEC) >1000/g based on the modified McMaster technique, which has a minimum sensitivity of 25 eggs/g of feces (Gordon and Whitlock, 1939). Sedimentation in water, filtration, and flotation in saturated saline was conducted to obtain nematode eggs from lamb feces (Coles et al., 1992).

The AE was standardized to 30 mg·mL-1. Immediately after dilution in sterile purified water, this extract was subjected to the EHI test (Coles et al., 1992). Seven experimental groups were evaluated, each with five replicates. Positive controls were exposed to levamisole phosphate

(0.3 mg/mL) and the negative control consisted of sterile distilled water. Experimental treatments using AE were performed at concentrations of 30.0, 15.0, 7.5, and 3.75 mg·mL-1.

The mixtures comprised 100 µL of egg suspension containing an average of 150 fresh eggs and 100 µL of the extracts in 96-well microplates. Samples were homogenized and incubated at 28 °C for 48 h in a refrigerated incubator (TE 371, Tecnial Equipamentos Científicos, SP, Brazil). Subsequently, 15 µL of Lugol's solution was added to each tube, which was then stored at 4 °C, to count the unembryonated eggs, embryonated eggs, and L1 larvae.

The number of L1 eggs relative to the initial number of eggs (remaining eggs plus L1) was determined for each repetition and subjected to variance analysis. Means were compared using Duncan's test ($P < 0.05$). Probit regression was used in the estimation of concentrations sufficient to inhibit 90% (LC90) of egg hatching with SAEG 9.1 software. The formula of Coles et al. (1992) was used to determine the % EHI:

%EHI = 100 × (1-L1/initial number of eggs).

2.4 LARVAL DEVELOPMENT INHIBITION (LDI)

Pools of feces were collected from the two lambs described above and homogenized for five min. The LDI test was performed using the quantitative coproculture method (Borges, 2003; Nery *et al*., 2010; Morais-Costa *et al*., 2015). This LDI test may be considered more rigorous than the EHI test as the extract must exert anthelmintic effects on feces, which represent the natural development of nematode larvae (Borges 2003, Morais-Costa *et al*. 2016).

In two experiments, the powder and AE of *M. indica* leaves were evaluated at final concentrations of 166.7, 133.3, 100.0, 66.7, and 33.3 mg/g and 29.1, 15.55; 7.27; 1.81 mg g^{-1} fecal culture, respectively. The final concentration of the leaf powder was achieved by replacing vermiculite (substrate). Two mL of ivermectin solution (containing 40% glycerol formal, and propylene glycol, q.s.) at 3.3 mg/g fecal culture and 2 mL of 0.1 mg/mL levamisole phosphate were used as positive controls and 2 mL of sterile distilled water was employed as the negative control. Each tested solution was added to 2 g of feces with five replicates. The flasks with the fecal cultures were covered with a plastic film containing small holes for aeration and stored in plastic trays lined with a paper towel, which was moistened daily. The materials were incubated

at 28 °C for seven days. Water was added when necessary to maintain moisture (Nogueira *et al*., 2012). L3 was recovered as described by Morais-Costa *et al*. (2016), and larval suspensions were pipetted and stored at 4 °C for subsequent counting (Nogueira *et al*. 2012). One ml of 10% formaldehyde was also added for better preservation.

The number of L3 was divided by two to obtain the number of L3 g/feces (LPGF). For nematode identification, the slides were prepared with Lugol's iodine (Keith, 1953). The number of LPGFs was counted in a Sedgewick chamber with an optical microscope at 100x. The percent LDI was calculated according to the formula adapted from Borges (2003):

% LDI = 100 x (1 – LPGF of the treated group/LPGF of the untreated group).

The values of LDPG were transformed into log10 (x + 10) and subjected to ANOVA. Means were compared using Duncan's test, and the LC90 values were determined by probit analysis. The analyses were conducted using the Saeg 9.1 package with a significance of 5%.

2.5 *IN VIVO* ANTHELMINTIC ACTIVITIES

Two fecal egg reduction tests were performed to evaluate the powder and AE of the leaves. In the first trial, 24 Santa Inês male lambs (age, 6–8 months old; mean BW, 35.0 ± 1.1 kg) were used to evaluate the *in vivo* efficacy of leaf powder. Fourteen days prior to the beginning of the trial, all lambs were administered albendazole (LA Ranger, Vallée S.a, MG, Brazil) (10 $mg \cdot kg^{-1}$ bw) and levamisole phosphate (Protall, Vallée S.a, MG, Brazil) (0.6 $mg \cdot kg^{-1}$ bw) to ensure zero FEC. During the 14-day adaptation period, the animals were individually confined and fed a balanced diet containing sorghum silage, concentrate, mineral premix, and water *ad libitum*.

The lambs showing zero FEC on two counts were infected with 800 H. contortus L3 (strain previously described) per 10 kg BW. Twenty-eight days post-infection, lambs were assigned to one of three homogeneous groups based on FEC and weight.

A group of untreated sheep served as the negative control; the second group, which served as the positive control, received a single dose of albendazole at 10 mg·kg−1 (bw); and the third group was administered a single dose of 4.978 g (dm)/kg (bw) in the same volume of concentrate. The dose was based on the LC90 estimated by the LDI test considering the volumes of pre-

stomachs and abomasums of lambs (Nogueira et al., 2012). The treatment was conducted in the morning after 12 h of fasting. The animals were monitored for clinical signs and weighed in the morning before feeding on the day of treatment and on days 7 and 14 after treatment. The mean weight gain of the groups was compared by Duncan's test at a significance level of 5%.

The FEC was evaluated over two time periods at weekly intervals. Each period covered an average of three days, with two counts obtained each day (Nogueira et al., 2012). Fecal egg counts were recorded two days prior to treatment and on the day of treatment (initial period, before treatment), with the mean used to standardize the levels for each lamb group. Subsequently, the mean FEC was calculated on days 7, 8, and 9 (second period) and days 14, 15, and 16 (third period).

In the second trial, 20 Santa Inês lambs (age, 5-6 months old; gender, 10 males and 10 females; mean bw, 30.0 ± 2.1 kg) were evaluated in the anthelmintic analysis of AE. A group of untreated sheep served as the negative control and the second group was administered 0.601 mg (dm)/kg bw of AE via esophageal gavage. The procedures were similar to those described above, with analysis performed on days 21, 22, and 23 (fourth period) and days 26, 27, and 28 (fifth period). The lambs were weighed weekly, and the mucosae were examined using the Famacha method (Molento, 2004). All sheep were clinically inspected according to possible behavioral changes.

The modified McMaster technique was performed with saturated NaCl with a minimum sensitivity of 25 eggs/g of feces (Gordon and Whitlock, 1939). Morphological identification, according to Keith (1953), for L3 from fecal cultures showed 100% larvae of the genus, Haemonchus. The efficacies of treatments were calculated using a formula adapted from Coles et al. (1992):

%FEC reduction = 100 × [1-(FEC mean of treated group/ FEC mean of untreated group)]

The FEC data were transformed to log10 (x + 10) and subjected to analysis of variance in a split plot design with respect to the four evaluated periods. Means were compared using the Scott–Knott and Mann-Whitney tests ($P < 0.05$).

2.6 BLOOD AND BIOCHEMICAL PARAMETERS OF LAMBS

Blood samples were collected from the jugular vein of the males in the second *in vivo* trial on days 0, 7, 14, 21, and 28 into tubes containing ethylenediaminetetraacetic acid (EDTA) and stored at 4 °C. Blood cell numbers and hematocrit were evaluated using an electronic automatic analyzer (2.800 BCE Vet®, Mindray Medical International Ltd., Shenzhen, China). Creatinine and urea were determined using commercial kits (Bioclin, Quibasa Basic Chemistry Ltd., Belo Horizonte, MG, Brazil).

Total protein concentrations were determined by the biuret method using a UV spectrophotometer (NOVA® 1600/1800UV). Albumin (ALB) concentrations were measured using commercial kits (Bioclin®, Quibasa Química Básica Ltda., Belo Horizonte, MG, Brazil), and the concentration of globulin (GLO) was calculated as the difference between total protein and ALB.

The serum proteinograms were obtained by horizontal electrophoresis on a 12% agarose gel (CELMGEL ®) with TRIS buffer for 30 min. The gels were stained for 5 min in 200 mL of 0.1% starch black and were cleared after staining with acetic acid (7%) and ethanol until the bottom of the gel until the bottom of the gel is clear. The reading was performed using CELM SE-250® software. Relative protein concentrations within each fraction were determined as the optical absorbance percentage, and the absolute concentrations (g/dL) were calculated using the total protein concentration. The major protein fractions were divided according to the manufacturer's recommendations from cathode to anode as albumin, alpha 1, alpha 2, beta, and gamma globulins, respectively. Data were subjected to analysis of variance with split plots, and means were compared using the Scott–Knott test ($P < 0.05$) according to SAEG (2007).

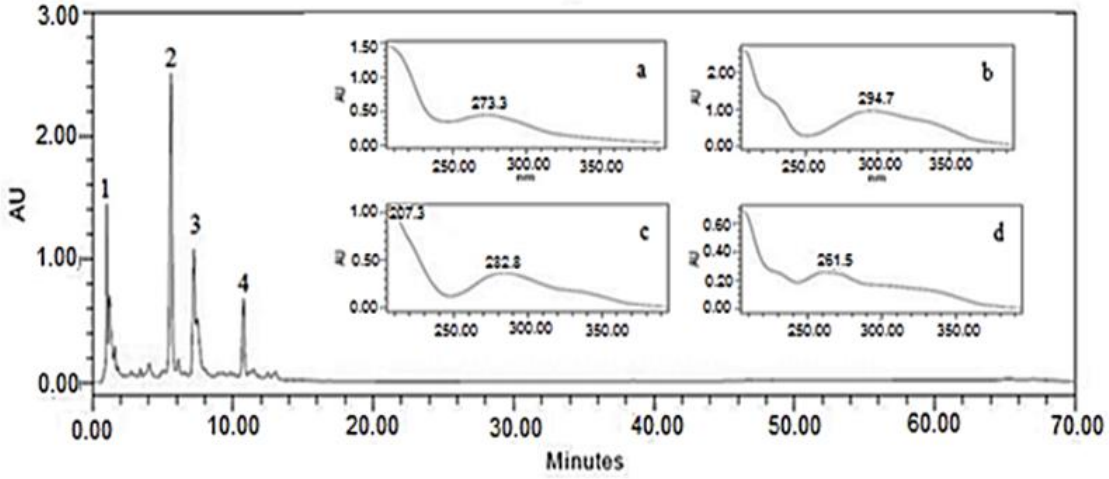
3 RESULTS

3.1 EXTRACT CHARACTERIZATION

The chemical composition of the AE based on its chromatographic profile recorded using HPLC-DAD revealed a predominance of peaks corresponding to polar compounds, with UV spectra compatible with polyphenols. The major peaks showed retention times of 1.02, 5.59,

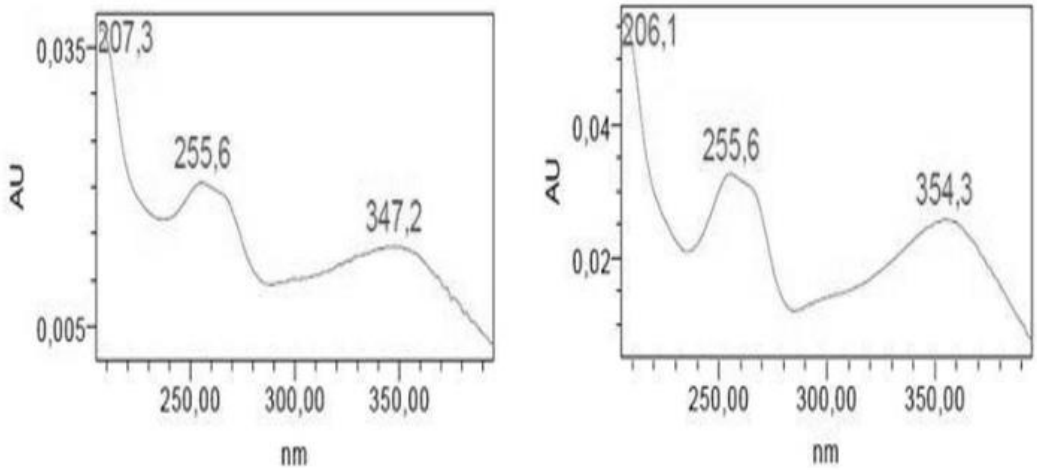
7.22, and 10.75 min, which are compatible with tannins (Figure 1). However, flavones and flavonoids were also detected, with peaks at retention times of 11.23 and 11.48 min, respectively (Figure 2). The condensed tannin level of AE was 3.79 ± 0.01%.

Figure 1. Chromatographic profile, obtained by HPLC-RF, for the aqueous extract of the plant species *Mangifera indica*. The UV letters are related to peaks 1, 2, 3 and 4, indicating tannins.



Source: From the authors

Figure 2. UV spectra: 347.2 and 354.3 indicating flavone and UV: 255.6 indicating a flavonoid.



Source: From the authors

3.2 EHI AND LDI

EHI increased with AE concentration, and at 30 mg/mL, EHI was 81.6% (Table 1). All tested concentrations of AE and treatment with levamisole phosphate promoted a higher number of unembryonated eggs than embryonated eggs and L1 ($P<0.05$).

Table 1. Efficacies of the aqueous extract of the leaves of *Mangifera indica* and levamisole phosphate (15mg ml^{-1}) on *Haemonchus contortus* hatchability inhibition.

Treatments	Unembryonated eggs mean	Embryonated eggs mean	L1 mean	Efficacy* (%)
Aqueous extract (mg/mL)				
30.00	87.00	31.75	26.75b	81.65
15.00	86.00	15.00	97.75cd	51.25
7.5	73.75	27.25	101.75d	49.87
3.75	71.00	37.75	79.5c	58.08
Levamisole phosphate (0.3 mg mL^{-1})	214.75	0.00	0.0a	100.00
Sterile distilled water	4.75	0.00	131.25e	
Variation coefficient (%)	8.60	33.08	12.60	

Different letters in columns indicates significant differences by Duncan's test ($P< 0.05$).
*% efficacy = 100 x (1 - L1/initial number of eggs).
Source: From the authors

In the LDI test, 7.8 $mg \cdot g^{-1}$ AE resulted in a significantly lower mean number of infective larvae than that found for the water control ($P<0.05$). Further, the anthelmintic efficacy increased with increasing concentration of this extract to an LC90 of 29.88 $mg \cdot g^{-1}$ (Table 2). The powder of leaves at 33.37 $mg \cdot g^{-1}$ promoted a lower mean number of infective larvae than the control with water ($P<0.01$). All concentration resulted in anthelmintic efficacies ≥ 90% (Table 2).

Table 2. Efficacy of aqueous extract and powder of *Mangifera indica* leaves at different concentrations to inhibit larval development of *Haemonchus contortus.*

Leaf powder			Aqueous extract		
mg/ g of fecal culture	LDGP mean	Efficacy (%)*	mg/ g of fecal culture	LDGP mean	Efficacy (%)*
166.67	0.0 a	100.0	29.10	50.8a	88.7
133.37	98.0a	89.0	14.55	210.0b	70.5
100.00	13.0 a	99.0	7.28	290.0b	59.3
66.67	1.0 a	100.0	1.82	410.0c	42.5
33.37	1.0 a	100.0			
Water	874.5 b	-	Water	450.0c	--
Ivermectin	0.0 a	100.0	Ivermetin	0.0 a	100.0
Levamisole	0.0 a	100.00	Ievamisole	0.0 a	100.0
CV	26.4%	-		3.3%	

Note: Means followed by the same are not statistically different by Skott-Knott test (5%). CV = Coefficient of variation

* Efficacy (%) = 100 - [(LDPG the treated group / LDPG negative control group) x 100].

Source: From the authors

3.3 *IN VIVO* ANTHELMINTIC ACTIVITY

In the first trial, the lambs did not reject the dried leaves supplied, and this treatment did not promote clinically visible changes during the 14 days. The natural ingestion of *M. indica* leaf powder had an efficacy of 22.7% in a single dose (Table 3).

Table 3. Average of eggs per gram of feces of sheep inoculated with *Haemonchus contortus* and treated with leaves of *Mangifera indica*, albendazole and untreated animals.

Treatment	Before treatment	First week		Second week		CV %
	FEC	FEC	Eff*	FEC	Eff*	
M. indica	837.5Aa	685.4 Aa	9.61%	843.5 Aa	22.7%	16.2
Untreated	845.8Ab	758.3 Ab	-	1091.7 Aa	-	11.3

Note: Means followed by the same letters, uppercase in columns, and lowercase in lines, do not indicate statistically different by Ducan test (5%). CV=Coefficient of variation; Efficacy (Eff)* (%) = 100 x (1 – FEC mean of treated group / FEC mean of control group).

Source: From the authors

However, the mean FEC of these lambs did not differ from that of the untreated sheep. The treated animals did not exhibit any behavioral changes. In the second *in vivo* experiment, all lambs initially showed a high FEC. However, after the third and fourth weeks of treatment, the AE led to a significant reduction in the FEC means compared to the control (Table 4).

For both lamb groups, the FEC was reduced in the second week after treatment ($P<0.05$). After 28 days of treatment, lambs treated with AE gained a daily average of 90.0 ± 0.07 g, which did not differ ($P>0.05$) from that of untreated lambs (107.0 ± 0.03 g). The animals treated with

AE did not exhibit behavioral changes, submandibular edema, weakness, or lack of appetite during the experiment.

In the clinical evaluation of the ocular mucosa of the animals using the Famacha method, no significant difference was observed between the evaluated periods. However, animals treated with the extract had a better score (1.93±0.70) than untreated animals (2.36±0.86) based on Mann-Whitney's test (P<0.05).

Table 4. Mean values of eggs per gram of feces for sheep treated with aqueous extract of *Mangifera indica* leaves (0.601 g/kg/PC) or untreated.

Treatments	Before treatment	First week		Second week		Third week		Forth week	
	FEC/g	FEC/g	Ef(%)	FEC/g	Ef (%)	FEC/ g	Ef (%)	FEC/ g	Ef (%)
Control	2888.4Aa	2268.7Aa	-	1924.6Ba	-	2008.3Ba	-	2033.3Ba	-
Aqueous extract	2128.7Aa	2530.6Aa	0.0	1741.5Ba	9.5	1147.5Bb	42.8*	1182.0Bb	41.8*

Different means followed by the same letters, uppercase in lines, and lowercase in columns lines, indicate significant differences by Scott–Knott's test (*P*< 0.05). (*P<0.01 by *Mann-Whitney*'s test)
Efficacy (Ef) = 100 x (1 – mean FEC of treated group/mean FEC of control group).
Coefficient of variation= 9.52%.
Source: From the authors

3.4 BLOOD PARAMETERS

The values of mean corpuscular hemoglobin, mean corpuscular volume, lymphocytes, eosinophils, rod neutrophils, segmented neutrophils, albumin, globulin, urea, albumin/globulin ratio, and β-globulins varied between the evaluated periods (Tables 5, 6, 7, 8). Lambs treated with AE showed significantly higher concentrations of erythrocytes, hemoglobin, hematocrit (Table 5), and β-globulin (Table 8) than untreated lambs. The concentrations of corpuscular hemoglobin, erythrocyte distribution range, platelets (Table 5), leukocytes, monocytes (Table 6), α1-globulin, α2-globulin, and γ-globulin (Table 8) levels were not influenced by the treatments and evaluated periods.

Table 5. Mean values for erythrocytes, hemoglobin, hematocrit, mean corpuscular hemoglobin, corpuscular hemoglobin concentration, mean corpuscular volume, range of distribution of erythrocytes and platelets of sheep with hemoncosis treated or not with the aqueous extract of *Mangifera indica* leaves.

Lamb groups	0 day	7 day	14 day	21 day	28 day	Average	Ref. [a]	CV (%)
				Erythrocytes (×10^6/µL)				
Untreated	11.33	11.28	10.78	11.56	11.11	11.21B	9-15	1.12
M. indica	12.71	12.75	12.20	12.34	12.96	12.59A		
				Hemoglobin (g/dL)				
Untreated	10.58	11.03	10.35	11.55	11.18	10.93B	9-15	17.56
M. indica	12.36	12.9	11.96	12.68	13.23	12.62A		

				Hematocrit (%)				
Untreated	32.01	33.53	32.18	35.91	33.35	33.39B	27-45	17.36
M. indica	36.96	39.50	37.71	39.55	41.03	38.95A		
				Corpuscular hemoglobin concentration (pg)				
Untreated	9.36	9.74	9.63	9.99	10.08			
M. indica	9.74	9.82	9.74	10.35	10.31		8-12	5.84
Average	9.55b	9.78b	9.68b	10.17a	10.19a			
				Mean corpuscular volume(fl)				
Untreated	28.39	29.74	30.08	30.99	30.02			
M. indica	29.08	29.84	30.93	32.39	32.12		28-40	7.33
Average	28.73b	29.79b	30.50a	31.69a	31.07a			
				Platelet (10^3)/µL				
Untreated	452.16	622.83	438.33	650.33	488.33		250-750	6.54
M. indica	517.50	609.33	473.00	747.83	635.33			

Lowercase letters in rows and capital letters columns in indicate significant differences (P <0,05 in Scott-Knott test). [a]Reference range for healthy sheep (PUGH, 2004).
Source: From the authors

Table 6. Mean values for leukocytes, lymphocytes, monocytes, eosinophils, rods and segments of sheep with hemoncosis treated or not with the aqueous extract of *Mangifera indica* leaves.

Lamb groups	0 day	7 day	14 day	21 day	28 day	Ref. [a]	CV (%)
				Leukocytes / (mm^3)			
Untreated	8150	8000	7283	7766	8800	4000-	3.09
M. indica	8000	8350	9333	8583	9033	12000	
				Lymphocytes / (mm^3)			
Untreated	3968	2848	3513	4266	4975	2.000 –	3.42
M. indica	3639	2927	4579	4618	5095	9.000	
Average	3803a	2887b	4046a	4442a	5035a		
				Monocytes / (mm^3)			
Untreated	362	355	290	277	325	0 –	7.02
M. indica	367	394	312	213	356	750	
				Eosinophils / (mm^3)			
Untreated	182	141	74	14	203	0 –	31.94
M. indica	150	71	112	49	109	1000	
Average	166a	106ab	93ab	31b	156a		
				Rods neutrophils / (mm^3)			
Untreated	0	96	48	141	238		
M. indica	0	79	228	231	211	*	24.53
Average	0c	87b	138ab	186a	224a		
				Segmented neutrophils / (mm^3)			
Untreated	3636	4559	3357	3067	3057		
M. indica	3843	4876	4100	3472	3260	400 - 6.000	3.60
Average	3739b	4717a	3729b	3269b	3158b		

Different letters indicate significant differences (P , 0,05) in Scott-Knott test. [a]Reference range for healthy sheep (FELDMAN *et al*.. 2000). *Reference value did not reported in the literature
Source: From the authors

Table 7. Mean serum concentrations of total protein, albumin, globulin, albumin/globulin ratio, urea and creatinine in sheep with hemoncosis treated or not with the aqueous extract of *Mangifera indica* leaves.

Lamb groups	0 day	7 day	14 day	21 day	28 day	Ref [a]	CV (%)
				Total protein (g/dL)			
Untreated	6.00Aa	5.90Aa	6.06Ba	6.10Ba	6.02Aa	6 - 7.9	5.87
M. indica	6.08Ab	6.03Ac	6.33Ab	6.76Aa	6.06Ab		
				Albumin (g/dL)			

Untreated	3.45	3.21	3.34	3.59	4.08		
M. indica	3.93	3.39	3.37	3.93	3.63	2.4- 3.5	12.15
Average	3.69a	3.3b	3.35b	3.76a	3.85a		
			Globulin (g/dL)				
Untreated	2.54	2.72	2.72	2.5	2.15		
M. indica	2.86	2.90	2.96	2.83	2.43	3.50 - 5.70	13.71
Average	2.70 a	2.81a	2.84a	2.66a	2.29b		
			Ureia (g/dL)				
Untreated	29.29	29.10	33.00	48.63	48.25		
M. indica	31.16	39.20	43.47	46.63	37.83	17.0 - 42.8	29.49
Average	30.22b	34.15b	38.23b	47.63a	43.04a		
			Creatinine (g/dL)				
Untreated	1.46Aa	1.25Ab	1.09Ac	0.91Bc	1.04Ac	1.2 - 1.9	12.66
M. indica	1.02Ba	0.99Ba	0.95Ba	0.93Ab	1.01Bb		

Lowercase letters in rows and capital letters columns in indicate significant differences (P <0.05 in Student Scott-Knott. test). [a]Reference range for healthy sheep (PUGH, 2004).
Source: From the authors

Table 8. Mean concentrations of α1-globulins, α2-globulins, β-globulins and γ-globulins in sheep with hemoncosis treated or not with the aqueous extract of *Mangifera indica* leaves.

Lamb groups	0 day	7 day	14 day	21 day	28 day	Averages	CV (%)
			α_1-globulin (g/dL)				
Untreated	0.46	0.52	0.47	0.48	0.44		21.92
M. indica	0.44	0.57	0.49	0.52	0.39		
			α_2-globulin (g/dL)				
Untreated	0.77	0.87	0.77	0.89	0.70		16.98
M. indica	0.73	0.79	0.71	0.70	0.78		
			β-globulin (g/dL)				
Untreated	0.28	0.27	0.40	0.22	0.32	0.29B	
M. indica	0.88	0.63	0.78	0.44	0.44	0.63A	50.41
Average	0.58a	0.45b	0.59a	0.33b	0.58b		
			γ-globulin (g/dL)				
Untreated	1.03	1.09	1.08	1.06	0.68		26.29
M. indica	0.79	0.90	0.87	1.15	0.97		

Lowercase letters in rows and capital letters columns in indicate significant differences (P <0,05 in Scott-Knott test)
Source: From the authors

We observed a significant interaction between the groups of lambs and the periods evaluated for total protein and creatinine concentrations. The total protein content in the extract-treated group was higher on days 14 and 21 post-treatment than in the untreated group. The creatinine levels of untreated lambs were higher than those observed for treated lambs, with the exception of day 21 after treatment (P <0.01) (Table 7).

4 DISCUSSION

The productivity of sheep infected with *H. contortus* is significantly compromised, and the concomitant presence of resistant nematode populations impede its control, thereby increasing production costs (Miller *et al*., 2012). In this study, the leaves of *M. indica*, which are widely available in tropical farms throughout the year, were found to promote high EHI and LDI, and moderate and significant FEC reduction in lambs infected with this nematode.

The efficacy of EHI was concentration-dependent for the AE, which promoted the inhibition of embryo development as depicted by the higher number of unembryonated cells than embryonated embryos. The metabolites of this extract could pass through the egg wrapper and possibly reduce the initial mitoses of nematode blastomeres.

The AE and powder of leaves induced an efficient reduction (≥90%) of L3 production in fecal cultures, exhibiting an anthelmintic effect under natural and biologic conditions, which are more similar to the biological cycle of GIN (Nery *et al*., 2010; Morais-Costa *et al*., 2014). We suggest that these mango materials could be used in mixtures with sheep feces in the composting process, contributing to the reduction of the environmental phase of the nematode cycle.

The anthelminthic potential of *M. indica* was analyzed by Costa *et al*. (2002), who reported an efficacy of 95.7% in EHI for the ethanolic fraction of the hexane extract of mango seeds at 50 $mg \cdot ml^{-1}$. The metabolites detected in this extract were proanthocyanidins, hydrolyzable tannins, triterpenes, and saponins. However, in this study, the AE of leaves showed lower LC90 (30 $mg \cdot mL^{-1}$), indicating a higher anthelminthic efficacy.

In another study, the juice of immature fruits of *M. indica* was found to contain tannins and flavonoids. The 100 $mg \cdot ml^{-1}$ AE induced 100% LDI in quantitative fecal cultures and the estimated LC90 was 35.9 $mg \cdot ml^{-1}$. In this study, a lower LC90 was observed for leaf AE (29.88 $mg \cdot ml^{-1}$); however, similar metabolites were detected. “Mango” fruit is an important source of polyphenols (catechins, quercetin, kaempferol, rhamnetin, anthocyanins, tannic acid, and mangiferin; carotenoids, organic acids, and volatile compounds), which are useful for medicinal applications and as indicators of fruit quality (Maldonado-Celis *et al*., 2019).

Other fructiferous trees have also demonstrated EHI properties for *H. contortus*. The AE of *Cariocar brasiliense* (epicarp and mesocarp) at 15 $mg \cdot mL^{-1}$ induced an EHI of 91.8%. Phytochemical tests indicated the presence of catechins, steroids, flavonoids, saponins,

xanthones, and tannins (Nogueira *et al*., 2012). The AE of *Anacardium humile* leaves at 150 $mg \cdot mL^{-1}$ resulted in an efficacy of 97.3% in LDI (Nery *et al*., 2010) using a similar methodology to that of this present study.

The oral administration of the AE of *M. indica* leaves to infected lambs promoted significant and moderate anthelminthic efficacy (>40%) at the third and fourth weeks post-treatment. This FEC reduction was concomitant with the concentration of erythrocytes and hematocrit for treated lambs after 21 and 28 days of treatment. These results suggest an indirect effect via the sheep immune system, increasing resistance to *H. contortus* infection. Corroborating these results, we detected a better color of the ocular mucosa using the Famacha method for treated lambs. Future studies should evaluate these effects to develop a better formulation and administration protocol for this extract or its metabolites to produce more effective *in vivo* control of *H. contortus.*

The animals did not reject the dried leaves supplied with the concentrate and did not show clinically visible changes during the trial period. The natural ingestion of leaf dry powder of *M. indica* leaves induced an efficacy of 22.7% to a single dose of 4.978 g/kg (bw) (Table 3), but did not differ from the negative control in any of the evaluated periods, indicating low efficacy in FEC. Additionally, during the 14 days after treatment, the FEC was constant for treated lambs, while lower FEC elevation was observed in the untreated group during this period. These results are promising because low doses and a single dose were employed. Future studies with a larger volume of ingested *M. indica* leaves at greater frequency may indicate better anthelminthic efficacy *in vivo*, and nutritional analysis could initiate their use with feed for sheep.

Another *in vivo* research reported a similar result regarding the efficacy of *M. indica* for FEC reduction in sheep. The juice of the immature fruit of *M. indica* was administered at 0.740 g (dm) kg^{-1} (bw), and was found to reduce 53% of the FEC of GINs for 14 days after treatment. The authors attributed this reduction to the tannins in the juice (Nery *et al*., 2012).

Compared with other FEC analysis using leaves of fructiferous, in the first and second week after treatment, 33.0 and 32.5% anthelminthic efficacy was observed for the AE of leaf of *Musa* sp. Cv. "Prata Anã" at 0.303 $g \cdot kg^{-1} \cdot bw$. This extract contained flavonoids, saponins, catechins, and condensed and gallic tannins (Nogueira *et al*., 2012). According to Olivo *et al*. (2007), the anthelminthic activity of banana leaves could be due to the tannins found in its many cultivars.

In this study, tannins, flavonoids, and flavones were detected in the AE of *M. indica* leaves, which showed high condensed tannin (CT) levels. In another study, the leaves were extracted with 70% ethanol-water and a total of 22 phenolics were isolated, including four new benzophenone glycosides. The major constituents in HPLC analysis were iriflophene, quercetin-3-O-β-d-glucoside, quercetin-3-O-β-d-galactoside, isoswertisin, and mangiferin. However, mangiferin was the main constituent, accounting for 7.43% of the extract. The three compounds exhibited pronounced anti-inflammatory activity on LPS-induced NO production, with the flavonoids identified as the most active components (Pan *et al*. 2018).

The fruits of *M. indica* have different classes of phenolic compounds, such as acids, esters, and flavonoids (Abdallaa *et al*., 2007). In other studies with AE and the ethanolic fraction of the hexane extract of the seed, proanthocyanidins, hydrolyzable tannins, and saponins were detected (Costa *et al*., 2002; El-Sherbin and Osman, 2013).

Secondary plant compounds, whose main function is to protect against herbivory, may be considered responsible for the anthelmintic activity (Chagas *et al*., 2004). This anthelminthic action has been primarily attributed to CTs (Githiori *et al*., 2006; Minho *et al*., 2008), which may interact with the proteins in the nematode cuticle (Hoste *et al*. 2006). Alteration of the hypodermis, the presence of numerous vesicles in the cytoplasm, and degeneration and/or death of muscle and intestinal cells of L3 of GINs were observed by transmission electron microscopy after direct contact with the CT-rich extract (Brunet *et al*. 2011).

In this study, the activity of EHI, LDI, and FEC reduction may be related to the phenolic compounds present in the AE, specifically CTs. However, the complex composition of these leaves suggests an association between different compounds and *H. contortus*. Flavonoids with anthelminthic efficacy should not be ignored, and the synergistic action of these components must be considered (Klongsiriwet *et al*. 2015; Morais-Costa *et al*. 2016). Thus, the anthelminthic activity of *M. indica* leaves could be related to the action of tannins or their interaction with other compounds, such as flavonoids and flavones. These possible integrations should be evaluated in future studies.

Based on the analysis of blood and serum biochemical parameters of lambs, the values obtained in the present study were within the normal range for sheep. However, when the two lamb groups were compared, the AE was found to increase the concentrations of erythrocytes, hemoglobin, hematocrit, and β-globulin, suggesting the recovery of lambs with hemonchosis,

which could be attributed to the anti-inflammatory action of flavonoids, which should be explained via future analysis.

For the assessment of infection by hematophagous nematodes, erythrocyte count and hematocrit are good indicators of anemia and represent important parameters (Sotomaior *et al*., 2007). Anemia is characterized by a progressive decrease in hematocrit and erythrocyte count in ruminants with hemonchosis (Taylor *et al*., 2009). Hematocrit values below 15% are concomitant with weakness and indicate poor prognosis (Bowman, 2010). However, in this study, the lambs were not anemic, and the diet could have favored clinical resistance to blood spoliation. In particular, for treated lambs, the flavonoids and other metabolites of AE promoted a better color of the ocular mucosa, which could be associated with a higher rate of erythropoiesis. In anemic rats, the combination of flavonoids has been reported as an erythropoiesis-stimulating agent, improving the levels of red blood cells, white blood cells, hemoglobin, and hematocrit (Zhang *et al*., 2017).

The hematophagy of *H. contortus* and abomasitis reduced protein digestion in the acute phase, which explains the low concentrations of serum proteins in ruminants that are highly infected (Borjesson *et al*., 2000). However, in this study, AE increased the total serum protein at days 14 and 21 post treatment in lambs with hemonchosis, suggesting a faster recovery.

In this research, the animals in both groups had high concentrations of albumin, with values above the reference. However, we detected hypoglobunemia due to abomasitis, which reduces protein digestion and consequently the absorption of amino acids important for the synthesis of these immunoproteins (Bricarello *et al*., 2004). Both albumin and globulin are useful and sensitive indicators for assessing an animal's protein status (Contreras *et al*., 2000).

In this study, the serum concentrations of urea and creatinine in lambs with hemonchosis were close to the reference limit for healthy sheep. However, higher urea values were observed on days 21 and 28, suggesting the recovery of both lamb groups. The concentration of serum urea is a direct and immediate reflection of the amount of protein nitrogen ingested or decreased absorption associated with lesions in the gastrointestinal mucosa (Ribeiro *et al*., 2004). An elevated concentration may also be associated with renal alteration (Bricarello *et al*., 2004).

Proteinogram analysis by electrophoresis is a useful tool for diagnosing the nutritional and immunological status of animals (Alberghina *et al*., 2011). However, these parameters in young sheep need to be better established, as well as the influence of hemonchosis. In this study,

the α1-globulin, α2-globulin, and γ-globulin fractions did not show alterations, a result that highlights the normal conditions of the animals. However, lambs treated with AE showed higher concentrations of β-globulin, suggesting a recovery of their immunological status relative to untreated lambs.

The globulin fractions are important indicators of inflammatory processes and protein metabolism in animals (González *et al*., 2007). Globulin reflects the animal's immunological status; therefore, high concentrations of this metabolite are generally associated with recent disease or vaccination management (Payne *et al*., 1987). In the study on selenium and copper supplementation by Fausto *et al*. (2014), the values of these globulin fractions were found to be similar to those observed in this study. However, this supplementation increased the total protein, as verified for AE in this study, and gamma globulin concentrations in treated lambs compared to untreated lambs with hemonchosis (Fausto *et al.*, 2014).

5 CONCLUSION

The aqueous extracts of mango plant leaves showed high efficacy for EHI or LDI. *In vivo* tests indicated that the leaf extract treatment had moderate anthelminthic activity and contributed to the elevation of erythrocytes, hematocrit, and hemoglobin, plasma total proteins, and beta globulin levels. These results suggest that *M. indica* is an alternative or complementary treatment for hemonchosis.

REFERENCES

ABDALLAA, E.M., DARWISH, S.M., AYADA, E.H.E., EL-HAMAHMY, R.M. **Egyptian mango by-product. Compositional quality of mango seed kernel.** Food Chem 2007; 103(4): 1134-1140. Disponível em: Disponível em: https://doi.org/10.1016/j.foodchem.2006.10.017. Acesso em: 17 jun. 2024.

ALBA-HURTADO, F., MUÑOZ-GUZMÁN, M.A. **Immune Responses Associated with Resistance to Haemonchosis in Sheep.** BioMed Res Int 2013; (1): 16215. Disponível em: Disponível em: https://doi.org/10.1155/2013/162158. Acesso em: 17 jun. 2024.

ALBERGHINA, D., GIANNETTO, C., VAZZANA, I., FERRANTELLI, V., PICCIONE, G. **Reference intervals for total protein concentration, serum protein fractions, and albumin/globulin ratios in clinically healthy dairy cows.** J Vet Diagn Invest 2011; 23(11); 111- 114. Disponível em: https://doi.org/10.1177/104063871102300119. Acesso em: 17 jun. 2024.

ALVARES, C.A., STAPE, J.L., SENTELHAS, P.C., GONÇALVE, J.L.M., SPAROVEK, G. **Köppen's climate classification map for Brazil.** Meteorol Z 2014; 22(6): 711–728.

ARSENOPOULOS, K.V., FTHENAKIS, G.C., KATSAROU, E.I., PAPADOPOULOS, E. **Haemonchosis: A Challenging Parasitic Infection of Sheep and Goats.** Animals 2021; 11(2): 363. Disponível em: https://doi.org/10.3390/ani11020363.

BASTOS, G.A., FONSECA, L.D., FERREIRA, A.V.P., COSTA, M.A.M.S., SILVA, M.L.F., VASCONCELOS, V.O., *et al*. **Helminthiasis characterization and anthelmintic efficacy for ewes and lambs raised in tropical semiarid region.** Trop Ani Healt Produc 2017; 49(5): 937–943. Disponível em: https://doi.org/10.1007/s11250-017-1276-x. Acesso em: 17 jun. 2024.

BORGES, C.C.L. **Anthelmintic´s activities in vitro about infected worms from caprine gastrointestinal nematodes using quantitative faeces culture technic (Ueno, 1995).** Parasitol Latinoam 2003; 58(3-4): 142-147.

BORJESSON, D.L., CHRISTOPHER, M.M., BOYCE, W.M. **Biochemical and hematologic reference intervals for free-ranging desert bighorn sheep.** J Wildl Dis 2000; 36(2): 294-300. Disponível em: https://doi.org/10.7589/0090-3558-36.2.294. Acesso em: 17 jun. 2024.

BOWMAN, D.D. (Ed.). **Georgis Parasitologia Veterinária**. Elsevier; 2010.

BRICARELLO, P.A, GENNARI, S.M., OLIVEIRA-SEQUEIRA, T.C.G., VAZ, C.M.S.L., GONÇALVES DE GONÇALVES, I,, *et al*. **Worm burden and immunological responses in Corriedale and CrioulaLanada sheep following natural infection with *Haemonchus contortus.*** Small Ruminant Res 2004; 51(1): 75-83. Disponível em: https://doi.org/10.1016 / S0921-4488 (03) 00188-3. Acesso em: 17 jun. 2024.

BRUNET, S., FOURQUAUX, I., HOSTE, H. **Ultrastructural changes in the third stage, infective larvae of ruminant nematodes treated with sainfoin (Onobrychis viciifolia)**

extract. Parasitol Internat 2011; 60(4): 419–424. Disponível em: https://doi.org/10.1016/j.parint.2010.09.011. Acesso em: 17 jun. 2024.

CHAGAS, A.C.S. **Controle de parasitas utilizando extratos vegetais.** Rev Bras Parasitol Vet 2004; 13, 156-160.

COLES, G.C., BAUER, C., BORGSTEEDE, F.H., GEERTS, S., KLEI, T.R., TAYLOR, M.A., *et al*. **World Association for the Advancement of VETERINARY PARASITOLOGY (WAAVP) – methods for detection of anthelmintic resistance in nematodes of veterinary importance.** Vet Parasitol 1992; 44(1-2): 35-44. Disponível em: Disponível em: http://doi.org/10.1016/0304-4017(92)90141-U. Acesso em: 17 jun. 2024.

COSTA, C.T.C., MORAIS, S.M., BEVILAQUA, C.M.L., SOUZA, M.M.C., LEITE, F.K.A. **Ovicidal effect of *Mangifera indica* L. seeds extracts on *Haemonchus contortus.*** Rev Bras Parasitol Vet 2002; 11, 57-60.

DUARTE, E.R., SILVA, R.B., VASCONCELOS, V.O., NOGUEIRA, F.A., OLIVEIRA, N.J.F. **Diagnóstico do controle e perfil de sensibilidade de nematoides de ovinos ao albendazol e ao levamisol no norte de Minas Gerais.** Pes Vet Bras 2012; 32(1): 147-152.

EL-SHERBINI, G.T., OSMAN, S.M. **Anthelmintic activity of unripe *Mangifera indica* L. (Mango) against *Strongyloides stercoralis*.** Int J Curr Microbiol Appl Sci 2013; 2(5): 401-409.

FAUSTO, G.C., PIVOTO, F.L., COSTA, M.M., DOS ANJOS LOPES, S.T., FRANÇA, R.T., *et al*. **Protein profile of lambs experimentally infected with *Haemonchus contortus* and supplemented with selenium and copper.** Parasitol Vectors 2014; 7(1): 355. Disponível em: http://doi.org/10.1186/1756-3305-7-355. Acesso em: 17 jun. 2024.

GITHIORI, J.G., ATHANASIADOU, S., THAMSBORG, S.M. **Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants**. Vet Parasitol 2006; 139(4): 308-320. Disponível em: Disponível em: http://doi.org/10.1016/j.vetpar.2006.04.021. Acesso em: 17 jun. 2024.

GONZÁLEZ, J.E., RODRÍGUEZ, M.D., RODEIRO, I., MORFFI, J., GUERRA, E., LEAL, F., *et al*. **Lack of *in vivo* embryo toxic and genotoxic activities of orally administered stem bark aqueous extract of *Mangifera indica* L. (Vimang®).** Food Chem Toxicol 2007; 45(12): 2526-2532. Disponível em: http://doi.org/10.1016 / j.fct.2007.05.029. Acesso em: 17 jun. 2024.

GORDON, H.M., WHITLOCK, H.V. **A new technique for courting nematode eggs in sheep faeces.** J Counc Sci Ind Res 1939; 12(1): 50-52. Acesso em: 17 jun. 2024.

HOSTE, H., JACKSON, F., ATHANASIADOU, S., THAMSBORG, S.M., HOSKIN, S.O. **The effects of tannin-rich plants on parasitic nematodes in ruminants.** Trends Parasitol 2006; 22(6): 253-261. Disponível em: http://doi.org/10.1016/j.pt.2006.04.004. Acesso em: 17 jun. 2024.

LAMB, J., ELLIOTT, T., CHAMBERS, M., CHICK, B. **Broad spectrum anthelmintic resistance of *Haemonchus contortus* in Northern NSW of Australia.** Vet Parasitol 2017; 241, 48-51. Disponível em: http://doi.org/10.1016/j.vetpar.2017.05.008. Acesso em: 17 jun. 2024.

KABONGO-KAYOKA, P.N., ELOFF, J.N., OBI, C.L., MCGA,W.L.J. **Antimycobacterial Activity and Low Cytotoxicity of Leaf Extracts of Some African Anacardiaceae Tree Species.** Phytother Res 2016; 30(12): 2001-2011. Disponível em: http://doi.org/10.1002 / ptr.5706. Acesso em: 17 jun. 2024.

KEITH, R.K. **The differentiation of infective larvae of some common nematode parasites of cattle.** Aust J Zool 1953; 1, 223–235.

KLONGSIRIWET, C., QUIJADA, J., WILLIAMS, A.R., MUELLER-HARVEY, I., WILLIAMSON, E.M., HOSTE, H. **Synergistic inhibition of *Haemonchus contortus* exsheathment by flavonoid monomers and condensed tannins.** Int J Parasitol Drugs Drug Resist 2015; 5(3): 127–134. Disponível em: https://doi.org/10.1016/j.ijpddr.2015.06.001. Acesso em: 17 jun. 2024.

LEATHWICK, D.M., BESIER, R.B. **The management of anthelmintic resistance in grazing ruminants in Australasia--strategies and experiences.** Vet Parasitol 2014; 204, 44-54. Disponível em: http://dx.doi.org/10.1016/j.vetpar.2013.12.022. Acesso em: 17 jun. 2024.

MALDONADO-CELIS, M.E., YAHIA, E.M., BEDOYA, R., LANDÁZURI, P., LOANGO, N., AGUILLÓN, J., *et al*. **Chemical Composition of Mango (Mangifera indica L.) Fruit: Nutritional and Phytochemical Compounds.** Front Plant Sci 2019; 10:1073. Disponível em: Disponível em: http://dx.doi.org/10.3389/fpls.2019.01073. Acesso em: 17 jun. 2024.

MILLER, C.M., WAGHORN, T.S., LEATHWICK, D.M., CANDY, P.M., OLIVER, A.M.B., WATSON, T.G. **The production cost of anthelmintic resistance in lambs.** Vet Parasitol 2012; 186, (3-4): 376–381. Disponível em: http://dx.doi.org/10.1016 / j.vetpar.2011.11.063. Acesso em: 17 jun. 2024.

MINHO, A.P., BUENO, I.C.S., GENNARI, S.M., JACKSON, F., ABDALLA, AL. ***In vitro* effect of condensed tannin extract from acacia (*Acacia mearnsii*) on gastrointestinal nematodes of sheep.** *Rev Bras Parasitol Vet* 2008; 17(1): 144-148. Acesso em: 17 jun. 2024.

MITRA, S.K. **Mango production in the world – present situation and future prospect.** Acta Hortic 2016; 1111, 287-296. Disponível em: https://doi.org/10.17660/ActaHortic.2016.1111.41. Acesso em: 17 jun. 2024.

MOLENTO, M.B., TASCA, C., GALLO, A., FERREIRA, M., BONONI, R., STECCA, E. **Método FAMACHA como parâmetro clínico individual de infecção por *Haemonchus contortus* em pequenos ruminantes.** Ciênc Rural 2004; 34(4): 1139-1145. Acesso em: 17 jun. 2024.

MORAIS-COSTA, F., BASTOS, G.A., SOARES, A.C.M., COSTA, E.G.L., VASCONCELOS, V.O., OLIVEIRA, N.J.F., *et al*. ***In vitro* and *in vivo* action of *Piptadenia viridiflora* (Kunth) Benth against *Haemonchus contortus* in sheep.** Vet Parasitol 2016; 223, 43-49. Disponível em: https://doi.org/10.1016/j.vetpar.2016.04.002. Acesso em: 17 jun. 2024.

MORAIS-COSTA, F., SOARES, A.C.M., BASTOS, G.A., NUNES, Y.R.F., GERASEEV, L.C., BRAGA, F.C., *et al*. **Plants of the Cerrado naturally selected bygrazing sheep may have potential for inhibiting development of *Haemonchus contortus* larva.** Trop Anim Health Prod 2015; 47(7), 1321–1328. Disponível em: https://doi.org/10.1007 / s11250-015-0866-8. Acesso em: 17 jun. 2024.

MORAIS-COSTA, F., VASCONCELOS, V.O., DUARTE, E.R., LIMA, W.S. **Anthelmintics: Clinical pharmacology, uses in veterinary medicine and efficay In: Plants from Cerrado for the control of gastrointestinal nematodes of ruminats.** NSP: 2014. p 89-107.

NERY, P.S., NOGUEIRA, F.A., MARTINS, E.R., DUARTE, E.R. **Effect of Anacardium humile on the larval development of gastrintestinal nematodes of sheep.** Vet Parasitol 2010; 171(3-4): 361–364. Disponível em: https://doi.org/10.1016/j.vetpar.2010.03.043. Acesso em: 17 jun. 2024.

NERY, P.S., NOGUEIRA, F.A., OLIVEIRA, N.J.F., MARTINS, E.R., DUARTE, E.R. 2012. **Efficacy of extracts of immature mango on ovine gastrointestinal nematodes.** J Parasitol Res 2012; 111(6): 2467–2471. Disponível em: https://doi.org/10.1007 / s00436-012-3017-4.

NOGUEIRA, F.A., FONSECA, L.D., SILVA, R.B., FERREIRA, A.V.P., NERY, P.S., GERASEEV LC, *et al*. ***In vitro* and *in vivo* efficacy of aqueous extract of *Caryocar brasiliense* Camb. to control gastrointestinal nematodes in sheep.** Parasitol Res 2012; 111(1): 325-330. Disponível em: https://doi.org/10.1007/s00436-012-2843-8. Acesso em: 17 jun. 2024.

NOGUEIRA, F.A., OLIVEIRA, L.N., SILVA, R.B., NERY, P.S., VIRGÍNIO JUNIOR, G.F., GERASEEV, L.C., *et al*. **Anthelminthic eficacy of banana crop residues on gastrointestinal nematodes of sheep: *in vitro* and *in vivo* tests.** J Parasitol Res 2012; 111(1): 317-323. Disponível em: https://doi.org/10.1007 / s00436-012-2842-9. Acesso em: 17 jun. 2024.

MOTTIN, V.D., CRUZ, J.F., TEIXEIRA NETO, M.R., MARISCO, G., FIGUEREDO, J.S., SOUSA, L.S. **Eficácia, toxicidade e letalidade de plantas com potencial atividade anti-helmíntica em pequenos ruminantes no Brasil.** Rev Bras Saúde Prod Anim 2019; 20, 01-23, e0232019. Disponível em: http://dx.doi.org/10.1590/S1519-9940200232019. Acesso em: 17 jun. 2024.

OLIVO, C.J., PEREIRA, L.E.T., CARVALHO, N.M., VOGEL, F.F., HEINZMANN, B.M., NEVES, A.P. **Use of the banana plant (Musa spp.) for the control of parasites in domestic animals: from empiricism to science.** Livest Res Rural Dev 2007; 19, 11.

PAN, J., YI, X., ZHANG, S., CHENG, J., WANG, Y., LIU, C., *et al*. **Bioactive phenolics from mango leaves (*Mangifera indica* L.).** Ind Crops Prod 2018; 111, 400-406. Disponível em: https://doi.org/10.1016/j.indcrop.2017.10.057. Acesso em: 17 jun. 2024.

PAYNE, J.M., PAYNE, S. **The metabolic profile test.** Oxford, Oxford University Press, 1987.

RIBEIRO, L.A.O., MATTOS, R.C., GONZALEZ, F.H.D., WALD, V.B., SILVA, M.A., LA ROSA, L.V. **Perfil metabólico de ovelhas Border Leicester x Texel durante a gestação e lactação.** *Revista* Port Cienc Vet 2004; 99 (551): 155-159.

SAEG. **Sistema para Análises Estatísticas, Versão 9.1.** Viçosa: Fundação Arthur Bernardes – UFV. 2007.

SAÚCO, V.G. **Mango production and world market: Current situation and future prospects.** Acta Hortic 2007; 645, 107-116. Disponível em: https://doi.org/10.17660/ActaHortic.2004.645.7. Acesso em: 17 jun. 2024.

SCZESNY-MORAES, E.A., BIANCHIN, I., SILVA, K.F., CATTO, J.B., HONER, M.R., PAIVA F. **Anthelmintic resistance of gastrointestinal nematodes in sheep, Mato Grosso do Sul, Brazil.** Pesq Vet Bras 2010; 30(3): 229–236. Disponível em: https://doi.org/10.1590/S0100-736X2010000300007. Acesso em: 17 jun. 2024.

SOTOMAIOR, C.S., CARLI, L.M., TANGLEICA, L., KAIBER, B.K., SOUZA, F.P. **Identificação de ovinos e caprinos resistentes e susceptíveis a helmintos gastrintestinais.** Ciênc Anim Bras 2007; 5(4): 397-412.

TARIQ, K.A. **Anthelmintics and emergence of anthelmintic resistant nematodes in sheep: need of an integrated nematode management.** J Anim Sci 2017; 2, 13-19.

TAYLOR, M.A., LEARMOUNT, J., LUNN, E., MORGAN, C., CRAIG, B.H. **Multiple resistance to anthelmintics in sheep nematodes and comparison of methods used for their detection**. Small Rumin Res 2009; 86, 67–70.

ZHANG, L., GONG, A.G.W., RIAZ, K., DENG, J.Y., HO, C.M., LIN, H.Q., *et al*. **A novel combination of four flavonoids derived from Astragali Radix relieves the symptoms of cyclophosphamide-induced anemic rats.** FEBS Open Bio 2017; 7(3): 318–323. Disponível em: http://doi.org/10.1002/2211-5463.12146. Acesso em: 17 jun. 2024.