IEEE Access
Multidisciplinary | Rapid Review | Open Access Journal

Received 9 July 2024, accepted 25 August 2024, date of publication 28 August 2024, date of current version 10 September 2024.

Digital Object Identifier 10.1109/ACCESS.2024.3451363

RESEARCH ARTICLE

Analyzing *Morinda citrifolia's* Potential for *Haemonchus contortus* Control in Lambs Using an Artificial Immune/Neural Approach

GABRIELE L. S. SILVA[1], LAURA L. S. OLIVEIRA[2], FREDSON V. SILVA[2], MURILO O. CAMARGOS[3], MATHEUS P. LIBÓRIO[4], MARCOS F. S. V. D'ANGELO[5], ALISSON S. P. CALDEIRA[6], LUIS MIRALLES-PECHUÁN[7], AND HAMID RABIEI[8]

[1]Graduate Program in Animal Science, State University of Montes Claros (UNIMONTES), Montes Claros 39401-089, Brazil
[2]Department of Agricultural Sciences, State University of Montes Claros (UNIMONTES), Montes Claros 39401-089, Brazil
[3]Graduate Program in Computer Modeling and Systems, State University of Montes Claros (UNIMONTES), Montes Claros 39401-089, Brazil
[4]Institute of Continuing Education, Pontifical Catholic University of Minas Gerais, Belo Horizonte 30535-901, Brazil
[5]Department of Computer Science, State University of Montes Claros (UNIMONTES), Montes Claros 39401-089, Brazil
[6]Fiocruz Minas, Belo Horizonte 30190-002, Brazil
[7]School of Computer Science, Technological University Dublin, Dublin 7, D07 H6K8 Ireland
[8]School of Architecture, Planning and Environmental Policy, University College Dublin (UCD), Dublin 4, D04 V1W8 Ireland

Corresponding author: Luis Miralles-Pechuán (luis.miralles@tudublin.ie)

This work was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), in part by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and in part by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

This work involved human subjects or animals in its research. Approval of all ethical and experimental procedures and protocols was granted by the Ethics Committee on Animal Use of the State University of Montes Claros (UNIMONTES) under Approval No. 209/2020.

ABSTRACT Sheep farming faces significant health challenges, particularly from gastrointestinal nematodes like *Haemonchus contortus*, which can severely impact animal health. In response, there is growing interest in herbal medicines derived from medicinal plants as alternative solutions for parasite control. This approach offers multiple benefits, including local availability, reduced risk of anthelmintic resistance, decreased dependency on synthetic chemical treatments, and improved shelf life. Among the promising alternatives, *Morinda citrifolia* has shown potential for effective nematode control. This study evaluates the anthelmintic efficacy of the hydroalcoholic crude extract (EHMC) of *Morinda citrifolia* using a clustering approach based on an Artificial Immune/Neural System to determine the optimal dosage. The findings reveal that the EHMC of *Morinda citrifolia* inhibits the hatching of *Haemonchus contortus* eggs, increases the mortality of adult nematodes, and demonstrates anthelmintic activity in lambs without causing adverse effects on animal health or negatively impacting meat quality. This suggests that *Morinda citrifolia* extract is a promising natural alternative for controlling parasitic infections in sheep.

INDEX TERMS Artificial immune/neural system, morinda citrifolia, haemonchus contortus control.

I. INTRODUCTION

For decades, anthelmintics have been the primary measure for controlling nematodes in sheep farming [1]. These drugs, while effective and fast-acting, have led to a significant problem: nematode resistance. This resistance has been documented in several states across Brazil, including Minas Gerais [2], Ceará [3], São Paulo [4], Rio Grande do Sul [5], Santa Catarina [6], Mato Grosso do Sul [7], and Maranhão [8]. The development of resistance has rendered many anthelmintics less effective, posing a serious threat to sheep farming sustainability.

In many developing regions, anthelmintics are not only expensive but also limited in availability, underscoring the unsustainability of relying solely on these drugs for nematode control [9], [10]. Furthermore, the overuse of these drugs

The associate editor coordinating the review of this manuscript and approving it for publication was Kah Phooi (Jasmine) Seng.

FIGURE 1. Leaves and fruits of *Morinda citrifolia*: https://pt.wikipedia.org/wiki/Noni.

has environmental and health implications, as chemical residues can accumulate in meat and the environment. Consequently, there is a pressing need for alternative methods to manage nematodes in sheep effectively. One promising alternative is the use of medicinal plants with anthelmintic properties. Numerous studies have highlighted the beneficial effects of plants and plant extracts, such as improved nutrient digestibility and absorption, modification of the intestinal microbiota, stimulation of the immune system, and antibacterial, coccidiostatic, antiviral, anti-inflammatory, and antioxidant properties. These benefits are particularly noted in plants containing secondary metabolites [11], [12], [13], [14].

The presence of drug-resistant helminths represents a significant limitation to the sustainability of current helminth control strategies, as sheep farmers heavily rely on the constant and repetitive use of commercial anthelmintic (AH) drugs [9]. The only realistic strategy for the sustainable control of nematode parasites is to develop new non-chemical approaches that reduce the need for treatment and to use the anthelmintics that remain effective in a more intelligent manner [10].

Given these challenges, there is increasing interest in developing sustainable production systems that utilize low-cost alternatives, yielding products free from chemical residues and less harmful to human health and the environment [15]. In this context, *Morinda citrifolia* (Figure 1), commonly known as Noni, is being investigated for its potential in nematode control.

Noni has been used traditionally for various medicinal purposes, and its potential as an anthelmintic agent offers a sustainable and environmentally friendly solution. Besides being sustainable and environmentally friendly, Noni could enhance farming profitability by reducing reliance on conventional anthelmintics and extending the efficacy of existing chemicals [16]. However, further research is needed to identify the most effective molecules against nematodes and their structural characteristics. Understanding these characteristics will be crucial for developing effective plant-based anthelmintics.

Notably, we have not found scientific studies that have investigated the effects of *Morinda citrifolia* on controlling *Haemonchus contortus*, one of the most pathogenic nematodes affecting sheep, or its impact on the quality of sheep carcasses and meat. Therefore, this study aims to fill this research gap by evaluating the anthelmintic activity of *Morinda citrifolia* extract and its effects on animal performance, carcass quality, and meat quality in lambs infected with *Haemonchus contortus*.

The main contribution of the study is to determine whether *Morinda citrifolia* can be a viable alternative to synthetic anthelmintics, thus contributing to more sustainable sheep farming practices. Additionally, we employ an Artificial Immune/Neural clustering algorithm to detect changes in the measured variables compared to a control group, thereby indicating whether the use of *Morinda citrifolia* had a significant impact. This innovative approach allows for a detailed analysis of the effects of the plant extract, providing insights that could help refine and optimize its use.

The Artificial Immune/Neural system is designed to mimic the adaptive and learning capabilities of the biological immune system, making it well-suited for detecting subtle changes and patterns in complex data sets. By applying this algorithm, we aim to provide a comprehensive evaluation of the potential benefits and limitations of *Morinda citrifolia* as an anthelmintic agent.

The remainder of this paper is structured as follows: Section II describes the Artificial Immune/Neural system-based clustering approach. Section III outlines the experimental design, including the preparation of *Morinda citrifolia* extracts, the infection protocol for lambs, and the methodology for assessing animal performance, carcass quality, and meat quality. Section IV presents the results, including statistical analyses and comparisons with the control group. Finally, Section V concludes the study, discussing our findings' implications and potential future research directions.

A. RELATED WORK

It is evident that farmers need to reduce their dependence on conventional anthelmintic drugs as the sole control method against gastrointestinal nematodes. To this end, the selective use of anthelmintic treatments, combined with one or more alternative approaches for gastrointestinal nematode control, should be adapted to the conditions of each farm [17].

Helminth infections are the most prevalent, pathogenic, and economically significant among all parasitic diseases in small ruminants. Among all helminths that can infect sheep, gastrointestinal nematodes are the most relevant in terms of health and economic impact, both in industrialized and developing countries [18].

In an attempt to control helminth infections, producers and technicians often use chemical anthelmintics indiscriminately. This disordered use results in a serious problem for

farms: anthelmintic resistance [2]. Gastrointestinal nematodes are becoming increasingly resistant to commercial products used to control them. The cost of routine applications of anthelmintics in herds and the problems related to residues in animal products and the environment have driven research on the anthelmintic activity of plant extracts [19].

Phytochemical analyses of plants and controlled experiments, along with recent knowledge about parasite control strategies, can offer new alternatives for the effective and economical control of parasitic diseases [20].

Various plants are popularly cited as having anthelmintic activity, among them *Morinda citrifolia*, known as noni. This is a small tree of the Rubiaceae family, originating from Southeast Asia, which spread through India and the Pacific Ocean to the islands of French Polynesia. The traditional use of noni by Polynesians is attributed to its antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, anti-inflammatory, hypotensive, and immunostimulant effects, and it has been used for over 2000 years [21].

Studies conducted with bird helminths have shown that in *vivo tests* using a 10% concentration, the aqueous and ethanolic fruit extract showed low efficacy against *Ascaridia galli*, not ruling out the possibility that extracts at higher concentrations may exhibit effective anthelmintic activity [22]. In in-vitro tests, higher concentrations of aqueous and ethanolic extracts of *M. citrifolia* showed high efficacy against *Heterakis gallinarum*, with a mortality rate above 90%. However, the low efficacy observed in the in vivo test indicates the need for studies with higher concentrations [23].

The use of clustering techniques to evaluate the efficacy of anthelmintics in the control of nematodes has gained increasing support in recent scientific literature. Studies demonstrate that the application of advanced data analysis methods, such as clustering algorithms, is crucial for identifying patterns and conducting detailed evaluations of the antiparasitic activity of natural compounds. Thus, the proposal to employ clustering techniques in this context is not only innovative but also grounded in robust evidence, enhancing the possibilities for sustainable and efficient biological control in parasite management.

In [24], non-hierarchical cluster analysis using the k-means algorithm was employed to determine resistance to parasitic infections in Santa Inâs sheep. The authors combined different sets of indicators of resistance to gastrointestinal parasites and formed three groups based on a reduced set of the animals' phenotypes, thus increasing the efficiency and efficacy of selecting resistant individuals.

In [25], cluster analysis was performed to explore additive-genetic patterns and identify sheep as resistant, resilient, or susceptible to gastrointestinal nematodes. Using R software, the authors classified 747 animals into three distinct groups based on predictive genetic values. This study highlights the importance of clustering analysis in the selection of the most suitable candidates to improve the herd genetically for parasite resistance and productivity.

The work of [26] applied fuzzy logic techniques and cluster analysis to evaluate the response of goats to worm infection, considering resistance, resilience, or sensitivity to parasitism. The study utilized phenotypic data and artificial intelligence techniques to categorize the animals' responses to parasitic infections. The authors showed that fuzzy logic was able to distribute individuals into infection response categories in a way that aligns better with livestock objectives compared to traditional statistical techniques.

II. IMMUNE NEURAL CLUSTERING

The clustering procedure used in this work is based on the immune-inspired algorithm ClonALG [27], aided by the Kohonen neural network [28] training algorithm to increase its performance, as presented in [29]. The ClonALG approach explicitly takes into account the antibodies' affinity, where only the fittest are selected to proliferate through a process called maturation (or mutation). The affinity criterion is

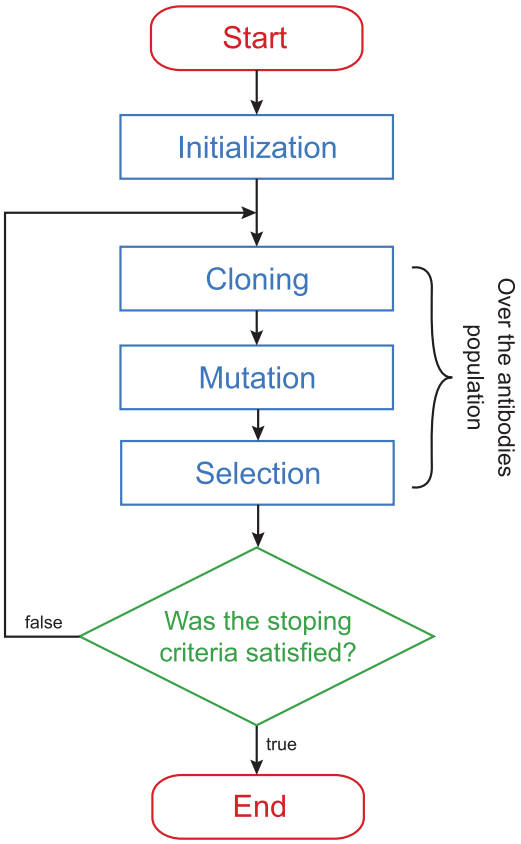


FIGURE 2. Algorithm flow chart, where a part of the mutation mechanism is carried out by the Kohonen neural network.

determined by the Euclidean distance. Every antigen used in the training step was tagged with its type so that the algorithm could train each antibody to recognize a specific type of antigen. The metric used to determine if an antibody recognized an antigen was the Euclidian distance, in which the antigen will be recognized by the nearest antibody. The training process begins with one antibody created in a central

position (related to the antigens' positions in space). Then, all antibodies are submitted to three steps that iterate until the stopping criteria are satisfied: cloning, mutation and selection (Figure 2). It is important to know that this approach is based on a non-fixed size population of antibodies.

As in almost all evolution-based algorithms, this approach uses the concept of populations and relations between individuals to proliferate the fittest. In this specific case, we have a population of antigens and a population of antibodies that will recognize the antigens. Both populations can be seen as matrices Eq. (1), with each line corresponding to an individual with dimension m

$$X = \left[x_1, \ldots, x_k\right]^\top \quad \text{and} \quad B = \left[b_1, \ldots, b_n\right]^\top \tag{1}$$

where $X \in \mathbb{R}^{k\times m}$ is the antigens' population and $B \in \mathbb{R}^{n\times m}$ the antibodies' population; $x_i \in \mathbb{R}^m$ and $b_i \in \mathbb{R}^m$. The general idea of an evolution-based algorithm is to repeat the mechanisms of cloning, mutation and selection until a stopping criterion is satisfied.

A. STOPPING CRITERIA

Two stopping criteria were employed in this study:

1) **Generation limit:** A predefined maximum number of generations, where each generation consists of cloning, mutation, and selection, serves as the first stopping criterion.
2) **Antibody balance:** The algorithm terminates when the number of deleted antibodies in a given generation meets or exceeds the number of newly created antibodies.

B. CLONING

Each antibody is cloned twice, and each one of these clones undergoes a random mutation using a uniform distribution between -1 and 1 on its space position, as shown in Eq. (2), guaranteeing diversity in the antibody population.

$$B = \{B + \lambda\,\mathcal{U}\} \;\cup\; \{B + \lambda\,\mathcal{U}\} \;\cup\; B \tag{2}$$

Here, $\mathcal{U}$ represents a matrix with $n \times m$ uniformly distributed random variables between -1 and 1, and λ is a constant that will be chosen with respect to the data; it could be max (B) or even mean(B); the designer will choose whatever fits best.

C. MUTATION

The mutation begins with the cloning mechanism and is improved by a Kohonen neural network where each antigen linearly reduces the distance between itself (x_j) and the closest antibody $\hat{b}_{x_j}$ given by Eq. (3). This algorithm changes the antibody's position as if it were the weights of a neuron, as shown in Eq. (4).

$$\hat{b}_{x_j} = \underset{b_i}{\operatorname{argmin}} \left\{ \left\| x_j - b_i \right\| : b_i \in B \right\} \tag{3}$$

$$\hat{b}_{x_j} = \hat{b}_{x_j} + \alpha_k (x_j - \hat{b}_{x_j}) \tag{4}$$

$$\alpha_k = \alpha_{k-1} \left(1 - \frac{k}{K_m}\right) \tag{5}$$

Note that Eq. (3)–Eq. (5) iterate over k with $0 < k \leq K_m$ for all $x_j \in X$. To make this linear reduction work, we must define α_0 and K_m; in this paper, we defined $\alpha_0 = 0.8$ and $K_m = 2$.

D. SELECTION

The selection uses two pieces of information to prune antibodies. The first one is the type of antigens mostly recognized by an antibody, given by Eq. (6); the second one uses the proximity between two antibodies, where the threshold for considering an antibody close to another is defined in Eq. (7).

$$r(b_i) = \text{Mo}(\{t(x_j) : x_j \in X \text{ and } \hat{b}_{x_j} = b_i\}) \tag{6}$$

where $t(x_j)$ represents the class of antigen x_j, and Mo$(\cdot)$ is the mode of the set.

$$c = 0.25 \left(\frac{2}{3n(3n-1)} \sum_{i=0}^{3n-1} \sum_{j=i+1}^{3n} \left\| b_i - b_j \right\| \right) \tag{7}$$

In other words, the threshold is 25% of the average distance between every two antibodies.

We start by removing antibodies who did not recognize any class of antigen Eq. (8).

$$B = \{\hat{b}_{x_j} : x_j \in X\} \cap B \tag{8}$$

Then, all combinations of two elements out of the antibodies set are analyzed; when the set of antigens recognized by two of them is of the same type and they are close to each other Eq. (9), a new antibody is created between them Eq. (10), and those two must be deleted Eq. (11).

$$B_1 = \left\{ (b_i, b_j) : (b_i, b_j) \in \binom{B}{2} \right. \\ \text{and} \quad r(b_i) = r(b_j) \\ \left. \text{and} \quad \left\| b_i - b_j \right\| < c \right\} \tag{9}$$

$$B_2 = \left\{ \frac{b_i + b_j}{2} : (b_i, b_j) \in B_1 \right\} \tag{10}$$

$$B = (B \setminus B_1) \cup B_2 \tag{11}$$

From Eq. (11), it is possible to see that this method does not guarantee the final population of antibodies to be of size n.

E. GENERIC EXAMPLE

To illustrate the proposed methodology, a generic example was conducted using five types of antigens randomly created. Figure 1 shows the algorithm initialization, where the first antibody is placed in the central position of the space.

Initially, the selection mechanism would take place, but when the number of antibodies is smaller than the number of types of antigens, no antibody will be deleted. Therefore, only the mechanisms of cloning and mutation were depicted. Figure 2 illustrates the first antibody being cloned twice, with each clone undergoing a random mutation using a uniform distribution, ensuring diversity in the antibody population.

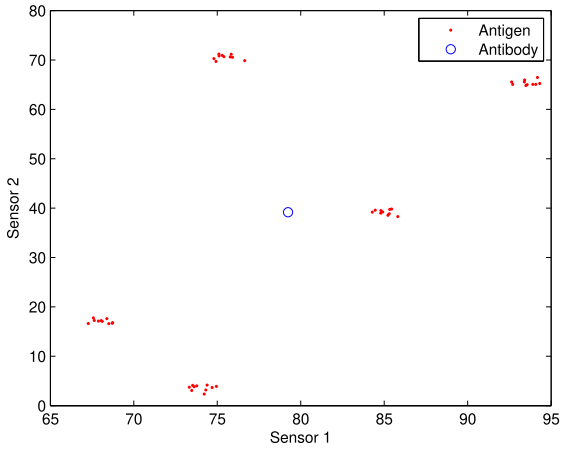


FIGURE 3. Initialization with one antibody.

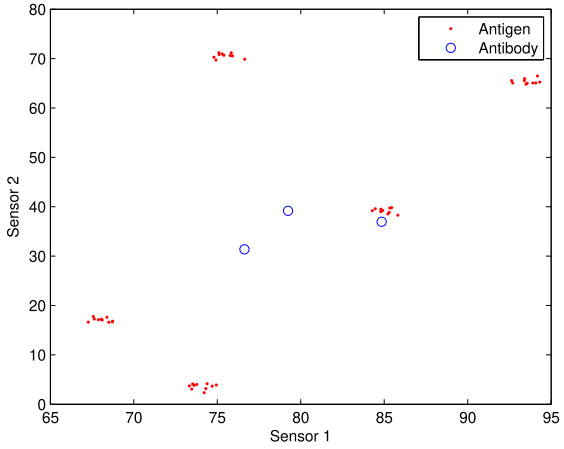


FIGURE 4. Cloning and first mutation of that antibody.

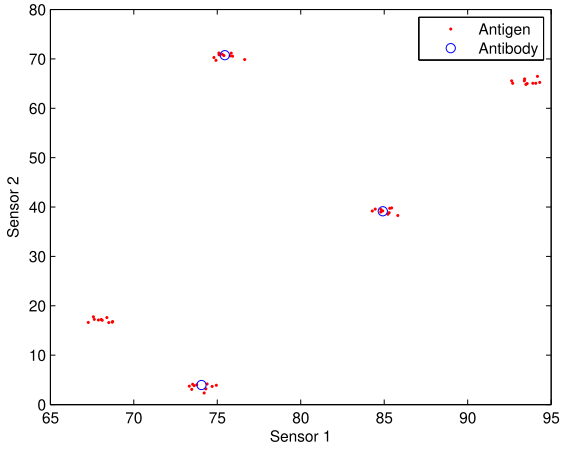


FIGURE 5. Kohonen neural network position adjustment.

The Kohonen neural network is responsible for the second mutation process, as shown in Figure 3, where the positions of the previously created antibodies are adjusted to a new set of antigens. The selection mechanism is not depicted at this stage due to the lower number of antibodies created so far.

Figure 4 demonstrates the cloning mechanism applied to three antibodies, followed by the application of the Kohonen network algorithm to increase the affinity of an antibody with a set of antigens (Figure 5).

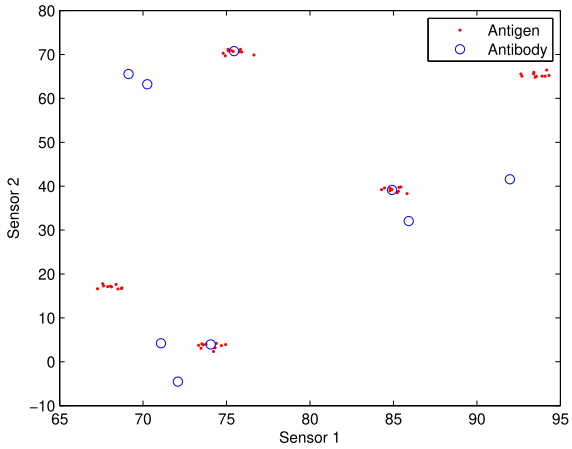


FIGURE 6. Cloning and mutating each antibody.

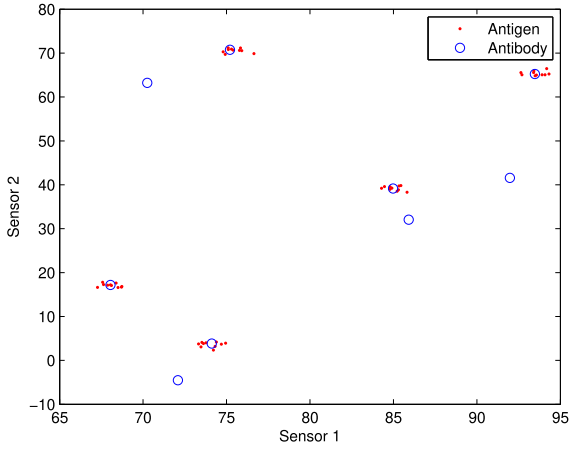


FIGURE 7. Kohonen neural network position adjustment.

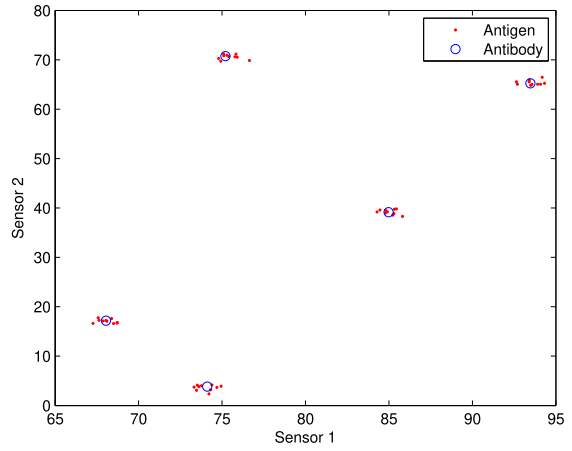


FIGURE 8. Removing antibodies who did not recognize any pattern.

Finally, every antibody that did not recognize at least one antigen must be deleted, as depicted in Figure 6, which shows the final situation of the training.

The figures accompanying the text provide a visual representation of the algorithm's initialization, cloning, mutation, and selection processes, helping the reader to understand how the Artificial Immune/Neural System for clustering operates.

III. EXPERIMENT

This section presents the design of the experiment carried out and how the data were obtained. The experiment was approved by the Ethics Committee on Animal Use of the State University of Montes Claros (approval number 209/2020).

A. EXPERIMENT LOCATION AND WEATHER CONDITIONS

The experiment was conducted at the Experimental Farm of the State University of Montes Claros - UNIMONTES, Campus Janaúba, Brazil, located in the irrigated perimeter of Gorutuba, in the Municipality of Janaúba, MG, at 15° 52'38'' S, 43° 20' 05 W. The average annual precipitation is 800 mm with an average annual temperature of 28 °C, relative humidity around 65%, and, according to Köppen's climate classification, the predominant type of climate in the region is Aw.

B. PLANT COLLECTION, EXTRACTION, AND PREPARATION OF CRUDE EXTRACT (CE)

To obtain the extract, the fruits of *Morinda citrifolia* were collected in the municipality of Janaúba, North of Minas Gerais. The collected fruits were dried in an oven at 40 °C and then ground into a powder using a Wiley knife mill. The powdered material was stored in a freezer until further use. A 3 L flask was filled with the material, and 2 L of ethanol 95% P.A. was added. The flask was then attached to a ball condenser and heated to reflux using a heating mantle for 20 minutes. After the reflux period, the mixture was filtered through cotton wool, and the hot extraction process was repeated 15 times with additional ethanol. The liquid extract was combined and concentrated using a rotary evaporator until dry. The resulting crude extract was then freeze-dried for 96 hours and kept frozen until diluted for supplying to the animals.

C. DESCRIPTION OF ANIMALS, EXPERIMENTAL INFECTION BY HAEMONCHUS CONTORTUS, AND EXPERIMENTAL GROUPS

A total of 32 lambs, non-castrated males with no defined breed pattern, were used. The lambs had an average age of 75 days and an initial weight of 14.87 ± 0.49 kg. The animals were housed in stalls with environmental enrichment, each stall housing two animals. The stalls were equipped with feeders, drinking fountains with clean water, and wood shavings bedding. Fecal samples were collected from each animal and refrigerated until examination. The fecal samples were analyzed for the number of eggs per gram of feces (EPG) using the Gordon technique modified by Whitlock [30]. All animals received albendazole anthelmintic treatment before the experimental phase. EPG counts were conducted until they resulted in zero for the experimental infection. The animals were then orally infected with approximately 1,000 infective *Haemonchus contortus* larvae before receiving the extract. After 24 days of experimental infection, the animals were divided into four groups of eight animals each. The group assignment was conducted randomly using a draw. The groups were as follows:

- **Group 1**: Control group, animals that received a hydroalcoholic solution for 7 consecutive days.
- **Group 2**: Animals that received a hydroalcoholic extract of *Morinda citrifolia* at a dose of 2 mg/kg of body weight for 7 consecutive days.
- **Group 3**: Animals that received a hydroalcoholic extract of *Morinda citrifolia* at a dose of 6 mg/kg of body weight for 7 consecutive days.
- **Group 4**: Animals that received a hydroalcoholic extract of *Morinda citrifolia* at a dose of 12 mg/kg of body weight for 7 consecutive days.

D. PARASITOLOGICAL AND PERFORMANCE EVALUATIONS

A weekly clinical examination was conducted on the animals to assess their general health status. The total experimental period lasted 68 days, and a total of 32 male sheep were involved in the study (Figure 9). The animals were housed in pairs and provided with ad libitum water and feed in a ratio of 52:48 concentrate to volume. The diet consisted of Vaquero grass hay (*Cynodon dactylon*) and corn grain concentrate, soybean meal, and a mineral mixture. The diet was formulated to meet the crude protein and metabolizable energy requirements for a daily weight gain of 200 g during the feedlot, following the guidelines of the National Research Council [31] (Table 1).

The daily feed intake of the sheep was measured by weighing the provided feed and the leftovers in the trough. Samples of the diets and leftovers were collected and stored for later analysis. Using the data on dry matter intake and individual animal weights, the average daily weight gain and feed efficiency were calculated.

Samples of the diet ingredients were ground and analyzed in the laboratory for their dry matter content, crude protein, ether extract, mineral matter, and neutral detergent fiber [32].

The experimental design utilized in this study was a completely randomized design, with the treatments consisting of various doses of the crude extract of *Morinda citrifolia*.

Over the next seven days, the animals were treated with different doses of *Morinda citrifolia* extract (FEHMC). Throughout the experiment, a total of eleven fecal collections were performed to examine and track EPG counts [30].

The animals were weighed (BW) every 14 days, following a 16-hour fasting period at the end of the feedlot. The spruce was conducted 42 days after the initiation of FHEMC. Using this information, along with data on dry matter intake, average daily weight gain, and feed efficiency, we further evaluated the results.

E. SLAUGHTER, CHARACTERISTICS OF CARCASSES, AND MEAT QUALITY

The animals underwent a humane slaughter process in accordance with current technical guidelines [33], [34]. The abomasum of the animals was identified, and the enumeration

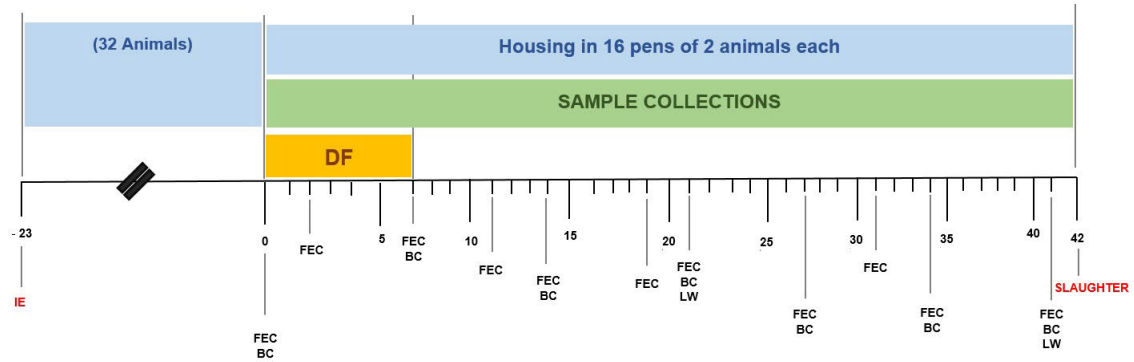


FIGURE 9. Overview of experimental design, recorded parameters and collected samples. **FHEMC: Supply of *Morinda citrifolia* extract. IE: Experimental infection; PC: Weighing of lambs; EPG: Carrying out the EPG; CS: Blood collection.**

TABLE 1. Proportion of ingredients and chemical composition of the experimental diet.

Item	Experimental diet (g/kg of dry matter)
Vaquero grass hay (*Cynodon dactylon*)	490,00
Ground corn	336,83
Soybean meal	143,25
Mineral mix[1]	21,12
Calcitic limestone	8,80
Item	**Chemical composition (g/kg)**
Dry matter	904,40
Crude protein[2]	141,94
Ether extract[2]	25,92
Ash[2]	89,97
Total carbohidrates[2]	646,57
Non-fibre carbohydrates[2]	261,77
Neutral detergent fibre[2]	384,80

[1]Mineral Mix, content per kilogram of product: calcium, 135 g; phosphorus, 65 g; sodium, 107 g; sulfur, 12 g; magnesium, 6000 mg; cobalt, 175 mg; copper, 100 mg; iodine, 175 mg; manganese, 1440 mg; selenium, 27 mg; zinc, 6000 mg; iron, 1000 mg; fluoride, 650 mg.
[2]on a dry matter basis.

of H. contortus specimens was conducted, distinguishing between females and males. Subsequently, the carcasses were weighed and placed in a cold chamber. Following this cooling period, the carcasses underwent another weighing.

The Longissimus lumborum muscles were extracted from the carcasses to measure pH, color, water-holding capacity, and shear force. The pH was gauged using a digital pH meter inserted into the Longissimus lumborum muscle. For meat color assessment, a 2.54 cm-thick sample of Longissimus lumborum was exposed to atmospheric air at 4°C for 30 minutes before measurements. Meat color determination employed a Hunter spectrophotometer, Miniscan EZ model, in the CIE system (L*, a*, and b*). Sub-samples were derived from the same sample to assess water-holding capacity using the pressure method with filter paper [35].

Two 2.54-cm samples were utilized for shear force measurement; these samples were weighed, cooked on a grill until reaching 71°C [36], and then cooled in a climate-controlled room at 16°C for 15 minutes before post-cooking weighing. Subsequently, six cylindrical sub-samples, measuring 1.27 cm, were longitudinally taken along the muscle fibers for shear force measurement [37].

F. STATISTICAL ANALYSES

All statistical analyses were performed using R Statistical Software (R Core Team, 2021). The pen was the experimental unit ($n = 16$) to determine the effect of the crude hydroalcoholic extract of *Morinda citrifolia* (CHEMC) on nutrient intake and feed efficiency. The effect of CHEMC (fixed effect) on nutrient intake and feed efficiency was adjusted with generalized linear models (GLM); ''glm'' function. The initial body weight of the animals was included in the GLMs as a covariate.

The lamb was the experimental unit ($n = 32$) to determine the effects of CHEMC on average daily weight gain, final body weight, and carcass and meat characteristics. The effect of CHEMC on blood variables, final body weight, and carcass and meat characteristics was adjusted with generalized linear mixed models (GLMM). Model assumptions were tested by observing model residuals for deviations from normality or homogeneity of variance. The ''glmer.nb'' function (negative binomial model) from the ''lme4'' package [38] was used for estimating *H. contortus* counts. For the shear force variable, the ''glmer'' and ''inverse.gaussian'' functions (link $= 1/\mu^2$) were used, respectively. For other variables analyzed with GLMM, the ''lmer'' function from the same package was used.

The model for *H. contortus* included CHEMC as a fixed effect, and the animals nested within pens were included as a random effect. For estimating average daily weight gain, final body weight, and carcass and meat characteristics, CHEMC was included as a fixed effect, and pen was included as a random effect in the GLMMs. Initial body weight was

TABLE 2. Experiment and your variables.

Experiment	Variables
Nutrients Intake and Feed Efficiency	Dry matter intake Crude protein intake Ether extract intake Neutral detergent fiber intake Feed efficiency Initial live weigh
Eggs per Gram of Feces	Eggs per gram of feces Initial live weight
Average Daily Weight Gain and Final Live Weight	Initial live weight Average daily weight gain Final live weight
Carcass Characteristics	Initial live weight Hot carcass Cold carcass
Haemonchus Contortus Count	*Haemonchus contortus*(males) *Haemonchus contortus*(females)
Meat Characteristics	PH Shear force Lightness (L*) Red intensity (a*) Yellow intensity (b*) Water holding capacity

included in the models as a covariate. When initial body weight was not significant ($p \geq 0.05$), the covariate was excluded.

Linear and quadratic regressions were evaluated. When both regressions were significant ($p < 0.05$), an ANOVA was performed between the models to determine if there was a better fit with the quadratic regression; as there was not, the more parsimonious model was chosen. Graphs, p values, model estimates, and standard errors were generated/calculated using the ''lme4'', ''lmerTest'', and ''sjPlot'' packages [38], [39], [40].

IV. RESULT

In order to categorize the data, four classes were employed based on the groups outlined in subsection III-C. Following the timeline depicted in Figure 9, a total of six experiments were conducted, and the respective variables analyzed in these experiments are detailed in Table 2.

The confusion matrix results suggest that when the Artificial Immune/Neural system struggles to classify correctly, it hints at changes in behavior compared to the control group. On the other hand, accurate classification implies potential behavior changes if the medoids deviate from the control group's medoid. Furthermore, the results indicate that *Morinda citrifolia* had no impact on this variable if the medoids stayed close to the control group's medoid.

A. NUTRIENTS INTAKE AND FEED EFFICIENCY EXPERIMENT

The experimental study demonstrated that animals administered *Morinda citrifolia* extract exhibited no adverse reactions or signs of intoxication. Furthermore, the administration of varying levels of hydroalcoholic crude extract of *Morinda citrifolia* (EHMC) did not significantly impact nutrient intake or feed efficiency. As shown in Table 3, the regression estimates, standard errors, and P-values, along with the confusion matrix in Table 4, indicate that most individuals were consistently placed in group 1, reflecting uniformity in their nutrient intake and feed efficiency behaviors.

TABLE 3. Regression estimates, standard errors, and P-values of nutrient intake and feed efficiency of lambs receiving doses of crude hydroalcoholic extract of *M. citrifolia*.

	Intercept	EHMC[1]	PVI[2]
Dry matter intake (kg)			
Estimate	1.05	0.003	0.08
SE[3]	0.38	0.01	0.02
P-value	0.016	0.777	0.005
Crude protein intake (g)			
Estimate	169.37	0.89	13.42
SE	59.92	1.89	3.83
P-value	0.014	0.646	0.005
Ether extract intake (g)			
Estimate	31.11	0.17	2.41
SE	11.03	0.35	0.71
P-value	0.015	0.636	0.005
Neutral detergent fiber intake (g)			
Estimate	433.18	1.71	31.26
SE	148.08	4.66	9.46
P-value	0.012	0.720	0.006
Feed efficiency (g/g)			
Estimate	0.214	-0.002	-
SE	0.008	0.001	-
P-value	<0.001	0.106	-

[1]EHMC = crude hydroalcoholic extract of *M. citrifolia* (doses = 0, 2, 6, or 12 mg/kg body weight); [2]PVI = initial body weight (kg) (covariate); [3]SE = standard error.

TABLE 4. Confusion matrix of the nutrients intake and feed efficiency experiment.

	Group 1	Group 2	Group 3	Group 4
Group 1	8	0	0	0
Group 2	4	4	0	0
Group 3	4	4	0	0
Group 4	4	0	0	4

B. EGGS PER GRAM OF FECES EXPERIMENT

Doses of *Morinda citrifolia* extract resulted in a significant reduction in the hatching of *Haemonchus contortus* eggs. The 12 mg/kg body weight dose of EHMC exhibited a more pronounced inhibitory effect on egg hatching compared to the 2 mg/kg and 6 mg/kg body weight doses. Initially, there were no significant differences among the groups, as seen in the first sample. However, subsequent samples showed an evolution in the results (Table 5). The control group exhibited a decrease in each sample, with group 2 showing a greater reduction than the control group, group 3 showing a greater reduction than group 2, and group 4 showing the greatest reduction.

The first confusion matrix in Table 5, which includes combinations such as (group 2, group 1), (group 3, group 1), and (group 4, group 1), reflects the results of the first samples. The combinations (group 1, group 2), (group 3, group 2), and (group 4, group 2) refer to samples subsequent to the initial one. In the confusion matrix, the combinations (group 1, group 3), (group 2, group 3), and (group 4, group 3) reflect

results from samples taken after the previous one. Finally, the confusion matrix combinations (group 1, group 4), (group 2, group 4), and (group 3, group 4) are from the last samples.

The classification of individuals in the confusion matrix indicates a noticeable anthelmintic effect between the second and third samples. This suggests a significant reduction in the presence of *Haemonchus contortus* eggs in the groups studied from that point onward.

TABLE 5. Confusion matrix of the eggs per gram of feces experiment.

	Group 1	Group 2	Group 3	Group 4
Group 1	47	10	9	6
Group 2	13	45	7	7
Group 3	12	6	39	14
Group 4	7	10	15	40

C. AVERAGE DAILY WEIGHT GAIN AND FINAL LIVE WEIGHT EXPERIMENT

The levels of the hydroalcoholic crude extract of *Morinda citrifolia* (EHMC) did not significantly impact the average daily weight gain and final live weight. This conclusion is supported by the regression estimates, standard errors, and P-values in Table 6, as well as the confusion matrix in Table 7, which shows that most individuals were classified into either group 1 or group 2. Notably, these two groups exhibit very similar behaviors in terms of average daily weight gain and final live weight, indicating a consistent pattern across the dataset.

TABLE 6. Regression estimates, standard errors, and P-values of average daily weight gain and final body weight of lambs receiving doses of crude hydroalcoholic extract of *M. citrifolia*.

	Intercept	EHMC[1]	PVI[2]
Average daily weight gain (g)			
Estimate	151.96	-2.05	6.19
SE[3]	47.71	1.95	3.00
P-value	0.004	0.312	0.049
Final body weight (kg)			
Estimate	13.25	-0.06	1.28
SE	2.77	0.11	0.17
P-value	<0.001	0.565	<0.001

[1]EHMC = crude hydroalcoholic extract of *M. citrifolia* (doses = 0, 2, 6, or 12 mg/kg body weight); [2]PVI = initial body weight (kg) (covariate); [3]SE = standard error.

TABLE 7. Confusion matrix of the average daily weight gain and final live weight experiment.

	Group 1	Group 2	Group 3	Group 4
Group 1	6	1	0	1
Group 2	0	8	0	0
Group 3	4	2	2	0
Group 4	2	3	0	3

D. CARCASS CHARACTERISTICS EXPERIMENT

EHMC levels did not significantly impact carcass characteristics or the weights of meat cuts in lambs, except for an increase in fat thickness with higher doses of EHMC. The confusion matrix in Table 8 indicates that the classes were correctly separated, although the medoids of groups 2, 3, and 4 remained close to the medoid of the control group. This is further confirmed by the regression estimates, standard errors, and P-values of carcass traits for lambs receiving doses of the crude hydroalcoholic extract of *M. citrifolia*, as presented in Table 9.

TABLE 8. Confusion matrix of the carcass characteristics experiment.

	Group 1	Group 2	Group 3	Group 4
Group 1	8	0	0	0
Group 2	0	8	0	0
Group 3	1	0	7	0
Group 4	0	0	0	8

TABLE 9. Regression estimates, standard errors, and P-values of carcass traits of lambs receiving doses of crude hydroalcoholic extract of *M. citrifolia*.

	Intercept	EHMC[1]	PVI[2]
Hot carcass weight (kg)			
Estimate	4.79	0.02	0.58
SE[3]	1.29	0.05	0.08
P-value	0.001	0.655	<0.001
Cold carcass weight (kg)			
Estimate	4.70	0.02	0.55
SE	1.17	0.05	0.07
P-value	<0.001	0.710	<0.001

[1]EHMC = crude hydroalcoholic extract of *M. citrifolia* (doses = 0, 2, 6, or 12 mg/kg body weight); [2]PVI = initial body weight (kg) (covariate); [3]SE = standard error.

TABLE 10. Regression estimates, standard errors, and P-values of the number of *Haemonchus contortus* in lambs receiving doses of crude hydroalcoholic extract of *M. citrifolia*.

	Intercept	EHMC[1]	SQEHMC[2]
H. contortus (females)[3]			
Estimate	5.80	-0.60	0.047
SE[4]	0.39	0.19	0.01
P-value	<0.001	0.001	0.001
H. contortus (males)[3]			
Estimate	5.24	-0.58	0.046
SE	0.41	0.20	0.02
P-value	<0.001	0.004	0.003

[1]EHMC = crude hydroalcoholic extract of *M. citrifolia* (doses = 0, 2, 6, or 12 mg/kg body weight); [2]SQEHMC = crude hydroalcoholic extract of *M. citrifolia* squared; [3]logarithmic scale; [4]SE = standard error.

E. HAEMONCHUS CONTORTUS COUNT EXPERIMENT

EHMC significantly increased mortality rates among adult *Haemonchus contortus* specimens compared to the control group. Treatment with EHMC demonstrated effective anthelmintic activity in controlling *Haemonchus contortus* in lambs. This treatment resulted in a noticeable reduction in both OPG counts and the number of adult specimens (both females and males) of *Haemonchus contortus*.

The regression estimates, standard errors, and P-values of the number of *Haemonchus contortus* in lambs receiving doses of the crude hydroalcoholic extract of *M. citrifolia* are presented in Table 10. The confusion matrix in Table 11 accurately distinguished between groups 2 and 3 but failed to properly classify group 4. Additionally, the medoids of

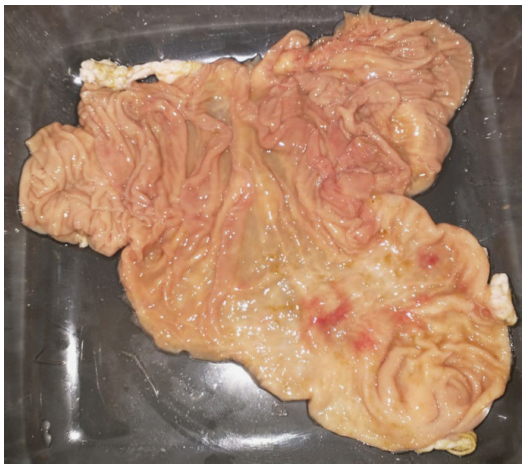


FIGURE 10. Visual aspects of abomasum from the control group, lamb infected with *Haemonchus contortus* that received hydroalcoholic solution, noting the intensely reddened mucosa.

TABLE 11. Confusion matrix of the haemonchus contortus count experiment.

	Group 1	Group 2	Group 3	Group 4
Group 1	8	0	0	0
Group 2	0	8	0	0
Group 3	1	0	7	0
Group 4	3	1	1	3

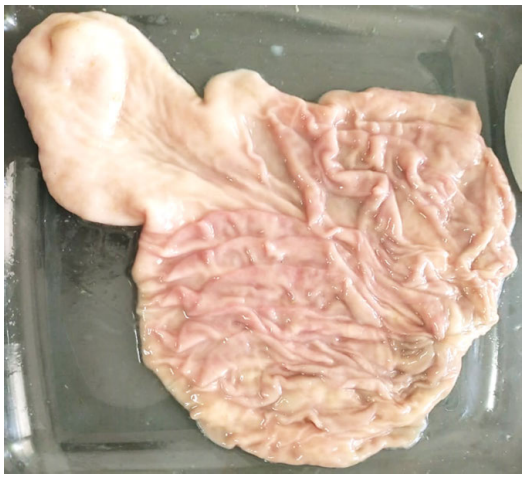


FIGURE 11. Visual aspects of abomasum from the experimental group, lamb infected with *Haemonchus contortus* that received crude hydroalcoholic extract of *Morinda citrifolia L.*

groups 2 and 3 showed a significant divergence from the medoids of the control group. Visual aspects of the abomasum of a lamb with haemonchosis are shown in Figures 10 and 11.

F. MEAT CHARACTERISTICS EXPERIMENT

No notable variations were found in variables such as pH, luminosity (''L*''), red intensity (''a*''), yellow intensity (''b*''), conductivity, water holding capacity, and cooking losses with increasing EHMC dosage. However, the shear force of the meat decreased as the EHMC dosage increased.

TABLE 12. Regression estimates, standard errors, and P-values of meat characteristics of lambs receiving doses of crude hydroalcoholic extract of *M. citrifolia*.

	Intercept	EHMC[1]
pH		
Estimate	5.61	0.01
SE[2]	0.04	0.01
P-value	<0.001	0.167
Shear force (N)[3]		
Estimate	0.237	0.0052
SE	0.01	0.002
P-value	<0.001	0.024
Lightness (L*)		
Estimate	39.20	-0.09
SE	0.79	0.12
P-value	<0.001	0.449
Redness (a*)		
Estimate	7.96	0.003
SE	0.42	0.06
P-value	<0.001	0.959
Yellowness (b*)		
Estimate	10.67	-0.04
SE	0.24	0.04
P-value	<0.001	0.254
Conductivity (mV)		
Estimate	96.18	-0.40
SE	1.77	0.26
P-value	<0.001	0.137
Water holding capacity (%)		
Estimate	14.42	0.02
SE	0.41	0.06
P-value	<0.001	0.77
Cooking losses (%)		
Estimate	27.05	-0.02
SE	1.40	0.21
P-value	<0.001	0.934

[1]EHMC = crude hydroalcoholic extract of *M. citrifolia* (doses = 0, 2, 6, or 12 mg/kg body weight); [2]SE = standard error; [3]$\hat{Y} = 1/x^2$.

TABLE 13. Confusion matrix of the meat characteristics experiment.

	Group 1	Group 2	Group 3	Group 4
Group 1	8	0	0	0
Group 2	0	8	0	0
Group 3	0	0	8	0
Group 4	0	0	0	8

The regression estimates, standard errors, and P-values of meat characteristics for lambs receiving doses of the crude hydroalcoholic extract of *M. citrifolia* are presented in Table 12. The confusion matrix displayed in Table 13 indicates that the classes were accurately distinguished, although the medoids of groups 2, 3, and 4 progressively diverged from the medoid of the control group with increasing EHMC dosage.

V. CONCLUSION

In conclusion, the experiments on the effects of *Morinda citrifolia* extract, specifically the hydroalcoholic crude extract (EHMC), as also suggested by the results presented by the Artificial Immune/Neural System, yielded various outcomes. The extract did not induce any adverse reactions or intoxication in the animals, and it did not significantly affect nutrient intake and feed efficiency. However, it did have a notable impact on inhibiting the hatching of *Haemonchus contortus* eggs, with the 12 mg/kg body weight dose exhibiting the

strongest effect. The extract also increased the mortality of adult *Haemonchus contortus* specimens and showed anthelmintic activity in controlling the parasite in lambs. It did not significantly affect average daily weight gain, final live weight, carcass characteristics, and meat characteristics, except for an increase in fat thickness and a decrease in shear force with higher doses of EHMC. Overall, these findings suggest the potential benefits of *Morinda citrifolia* extract in controlling parasites without negative effects on animal health and meat quality.

REFERENCES

[1] R. M. Kaplan, "Biology, epidemiology, diagnosis, and management of anthelmintic resistance in gastrointestinal nematodes of livestock," *Veterinary Clinics North America, Food Animal Pract.*, vol. 36, no. 1, pp. 17–30, Mar. 2020.

[2] L. F. Batista, L. L. D. S. Oliveira, F. V. E. Silva, W. D. S. Lima, C. A. D. J. Pereira, R. H. F. Rocha, I. S. Santos, J. A. D. Junior, and C. A. Alves, "Anthelmintic resistance in sheep in the semiarid region of Minas Gerais, Brazil," *Vet. Parasitology, Regional Stud. Rep.*, vol. 37, Jan. 2023, Art. no. 100821.

[3] A. C. F. L. Melo, I. F. Reis, C. M. L. Bevilaqua, L. D. S. Vieira, F. A. M. Echevarria, and L. M. Melo, "Nematodes resistant to anthelmintics in sheep and goat flock in the state of Ceara, Brazil," *Ciencia Rural*, vol. 33, no. 2, pp. 339–344, Apr. 2003.

[4] A. F. T. do Amarante, M. A. Barbosa, M. de Oliveira, and E. R. Siqueira, "Eliminacao de ovos de nematodeos gastrintestinais por ovelhas de quatro racas durante diferentes fases reprodutivas," *Pesquisa Agropecuaria Brasileira*, vol. 27, no. 1, pp. 47–51, 1992.

[5] F. Echevarria, M. F. S. Borba, A. C. Pinheiro, P. J. Waller, and J. W. Hansen, "The prevalence of anthelmintic resistance in nematode parasites of sheep in southern Latin America: Brazil," *Veterinary Parasitology*, vol. 62, nos. 3–4, pp. 199–206, Apr. 1996.

[6] F. Rosalinski-Moraes, L. H. Moretto, W. S. Bresolin, I. Gabrielli, L. Kafer, I. K. Zanchet, F. Sonaglio, and V. Thomaz-Soccol, "ResistÊncia anti-helmíntica em rebanhos ovinos da regiao da associacao dos municipios do alto irani (AMAI), oeste de Santa Catarina," *Ciencia Animal Brasileira Brazilian Animal Sci.*, vol. 8, no. 3, pp. 559–566, 2007.

[7] E. A. Sczesny-Moraes, I. Bianchin, K. F. d. Silva, J. B. Catto, M. R. Honer, and F. Paiva, "Anthelmintic resistance of gastrointestinal nematodes in sheep, Mato Grosso do Sul, Brazil," *Pesquisa Veterinaria Brasileira*, vol. 30, no. 3, pp. 229–236, Mar. 2010.

[8] S. C. P. Soares, A. C. Reis, R. L. P. Castro, P. C. S. P. Filho, C. S. M. Cabral, D. O. D. Junior, C. B. Costa, D. M. da Costa Pinheiro, Y. S. A. Pinheiro, N. H. S. Buna, L. M. C. Junior, and D. R. B. Brito, "Resistance of gastrointestinal nematoids of goats and sheep to the antihelmintics levamisol, ivermectin and albendazole," *Ciencia Animal Brasileira Brazilian Animal Sci.*, vol. 24, Jul. 2023, Art. no. e75316E.

[9] M. B. Molento, "Parasite control in the age of drug resistance and changing agricultural practices," *Veterinary Parasitology*, vol. 163, no. 3, pp. 229–234, Aug. 2009.

[10] R. M. Kaplan, "Drug resistance in nematodes of veterinary importance: A status report," *Trends Parasitology*, vol. 20, no. 10, pp. 477–481, Oct. 2004.

[11] R. K. Prichard, "Anthelmintic resistance in nematodes: Extent, recent understanding and future directions for control and research," *Int. J. Parasitology*, vol. 20, no. 4, pp. 515–523, Jul. 1990.

[12] M. T. Fox, "Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: Recent developments," *Veterinary Parasitology*, vol. 72, nos. 3–4, pp. 285–308, Nov. 1997.

[13] J. A. Hammond, D. Fielding, and S. C. Bishop, "Prospects for plant anthelmintics in tropical veterinary medicine," *Veterinary Res. Commun.*, vol. 21, no. 3, pp. 213–228, Apr. 1997.

[14] L. Costa, V. Almeida, B. Berenchtein, M. Tse, A. Andrade, and V. Miyada, "Phytobiotic additives and sodium butyrate as alternatives to antibiotics for weanling pigs," *Archivos de zootecnia*, vol. 60, no. 231, pp. 733–744, 2011.

[15] A. H. Atta, S. A. Atta, S. M. Nasr, and S. M. Mouneir, "Current perspective on veterinary drug and chemical residues in food of animal origin," *Environ. Sci. Pollut. Res.*, vol. 29, no. 11, pp. 15282–15302, Mar. 2022.

[16] S. Rochfort, A. J. Parker, and F. R. Dunshea, "Plant bioactives for ruminant health and productivity," *Phytochemistry*, vol. 69, no. 2, pp. 299–322, Jan. 2008.

[17] J. F. J. Torres-Acosta, P. Mendoza-de-Gives, A. J. Aguilar-Caballero, and J. A. Cuéllar-Ordaz, "Anthelmintic resistance in sheep farms: Update of the situation in the American continent," *Veterinary Parasitology*, vol. 189, no. 1, pp. 89–96, Sep. 2012.

[18] D. Traversa and G. von Samson-Himmelstjerna, "Anthelmintic resistance in sheep gastro-intestinal strongyles in Europe," *Small Ruminant Res.*, vol. 135, pp. 75–80, Feb. 2016.

[19] A. C. S. Chagas, L. S. Vieira, A. R. Freitas, M. R. A. Araujo, J. A. Araujo-Filho, W. R. Araguão, and A. M. C. Navarro, "Anthelmintic efficacy of neem (Azadirachta Indica a. Juss) and the homeopathic product fator Vermes in morada Nova sheep," *Veterinary Parasitology*, vol. 151, no. 1, pp. 68–73, Jan. 2008.

[20] M. S. Akhtar, Z. Iqbal, M. N. Khan, and M. Lateef, "Anthelmintic activity of medicinal plants with particular reference to their use in animals in the Indo–Pakistan subcontinent," *Small Ruminant Res.*, vol. 38, no. 2, pp. 99–107, Oct. 2000.

[21] M.-Y. Wang, B. J. West, C. J. Jensen, D. Nowicki, C. Su, A. K. Palu, and G. Anderson, "Morinda citrifolia (Noni): A literature review and recent advances in noni research," *Acta Pharmacologica Sinica*, vol. 23, no. 12, pp. 1127–1141, Dec. 2002.

[22] D. R. B. Brito, R. M. Fernandes, M. Z. D. L. C. M. Fernandes, M. D. D. S. Ferreira, F. R. L. Rolim, and M. L. D. S. Filho, "Atividade anti-helmíntica dos extratos aquoso e etanólico do fruto da morinda citrifolia sobre ascaridia galli," *Revista Brasileira De Parasitologia Veterinária*, vol. 18, no. 4, pp. 32–36, 2009.

[23] D. R. B. Brito and R. M. Fernandes, "Ação anti-helmíntica da morinda citrifolia (Noni) sobre heterakis gallinarum," *Semina, Ciencias Agrárias*, vol. 34, no. 4, pp. 1–29, Aug. 2013.

[24] J. I. M. Araujo, N. P. da Silva Santos, M. B. de Oliveira, L. S. Sena, D. Biagiotti, A. de Araujo Rego Neto, and J. L. R. Sarmento, "Non-hierarchical cluster analysis for determination of resistance to worm infection in meat sheep," *Tropical Animal Health Prod.*, vol. 53, no. 1, pp. 1–12, Nov. 2020.

[25] L. A. D. Freitas, R. P. Savegnago, L. S. Menegatto, R. D. D. Bem, N. B. Stafuzza, A. C. A. R. D. Paz, B. V. Pires, R. L. D. D. Costa, and C. C. P. D. Paz, "Cluster analysis to explore additive-genetic patterns for the identification of sheep resistant, resilient and susceptible to gastrointestinal nematodes," *Veterinary Parasitology*, vol. 301, Jan. 2022, Art. no. 109640.

[26] W. P. D. S. Oliveira, N. P. D. S. Santos, M. B. D. Oliveira, A. F. Evangelista, R. T. D. C. Filho, and A. M. D. Araujo, "Fuzzy logic discriminant function for evaluating goats exposed to verminosis occurrence regarding resistance, resilience, or sensitivity to parasitism," *Ciencia Animal Brasileira*, vol. 24, pp. 1–18, Aug. 2023.

[27] L. N. de Castro and F. J. Von Zuben, "Learning and optimization using the clonal selection principle," *IEEE Trans. Evol. Comput.*, vol. 6, no. 3, pp. 239–251, Jun. 2002.

[28] T. Kohonen, *Self-Organizing Maps*. Cham, Switzerland: Springer, 2001.

[29] M. F. S. V. D'Angelo, R. M. Palhares, M. C. O. C. Filho, R. D. Maia, J. B. Mendes, and P. Y. Ekel, "A new fault classification approach applied to Tennessee eastman benchmark process," *Appl. Soft Comput.*, vol. 49, pp. 676–686, Dec. 2016.

[30] H. Ueno and V. C. Gutierres, *Manual Para Diagnostico Das Helmintoses De Ruminantes*. Tokyo, Japan: Japan International Cooperation Agency, 1998.

[31] N. R. Council, *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*. New York, NY, USA: Academic, 2007.

[32] E. Detmann, L. F. C. E Silva, G. C. Rocha, M. N. N. Palma, and J. P. P. Rodrigues, *Metodos Para Analise De Alimentos*. Porto, Portugal: Livraria, 2021.

[33] *Technical Regulation for Pre-Slaughter and Humane Slaughter Handling, and the Authorized Methods of Stunning by the Ministerio da Agricultura*, Pecuaria e Abastecimento, Brazil, 2021. [Online]. Available: https://www.in.gov.br/en/web/dou/-/portaria-n-365-de-16-de-julho-de-2021-334038845

[34] F. V. E. Silva, I. Borges, S. K. Garcia, H. C. M. D. Sá, V. L. Silva, A. R. Araujo, J. A. de Lima, L. L. D. S. Oliveira, A. M. Matos, and F. L. B. Toral, "Effect of space allowance during transport of dorper X santa ines lambs on biochemical stress parameters and meat quality," *Small Ruminant Res.*, vol. 219, Feb. 2023, Art. no. 106910.

[35] R. Hamm, "Functional properties of the myofibrillar system and their measurements," in *Muscle as Food*. New York, NY, USA: Academic, 1986, pp. 135–199.
[36] E. Ramos and L. Gomide, *Meat Quality Assessment: Fundamentals and Methodologies*. Rio de Janeiro, Brazil: Editora, 2017.
[37] T. L. Wheeler, S. D. Shackelford, and M. Koohmaraie, "Shear force procedures for meat tenderness measurement," Animal Res., 2005, pp. 1–7. [Online]. Available: https://www.ars.usda.gov/ARSUserFiles/30400510/protocols/shearforceprocedures.pdf
[38] D. Bates, M. Mächler, B. Bolker, and S. Walker, "Fitting linear mixed-effects models using lme4," *J. Stat. Softw.*, vol. 67, no. 1, pp. 1–24, 2015.
[39] A. Kuznetsova, P. B. Brockhoff, and R. H. B. Christensen, "LmerTest package: Tests in linear mixed effects models," *J. Stat. Softw.*, vol. 82, no. 13, pp. 1–26, 2017.
[40] D. Lüdecke, "sjPlot: Data visualization for statistics in social science," CRAN, 2019, doi: 10.5281/zenodo.1308157.

GABRIELE L. S. SILVA received the B.S. and M.S. degrees in animal science from the State University of Montes Claros, in 2021 and 2023, respectively.

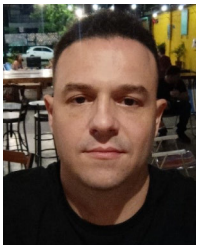
LAURA L. S. OLIVEIRA received the degree in veterinary medicine from the State University of Santa Cruz, the master's degree in animal science from the State University of Southwest Bahia, and the Ph.D. degree in sciences, specializing in parasitology, from the Federal University of Minas Gerais. She is currently a Lecturer with the Department of Agricultural Sciences, State University of Montes Claros (UNIMONTES), Brazil. Her research interest includes veterinary helminthology.

FREDSON V. SILVA received the degree in animal science from the Federal University of Viçosa, the master's degree in animal science from the State University of Southwest Bahia, and the Ph.D. degree in animal science from the Federal University of Minas Gerais. He is currently a Lecturer with the Department of Agricultural Sciences, State University of Montes Claros (UNIMONTES), Brazil. His field of expertise is animal production, with a focus on research involving small ruminants.

MURILO O. CAMARGOS received the bachelor's degree in systems engineering from the State University of Montes Claros (UNIMONTES), Brazil, and the Ph.D. degree in electrical engineering from the Federal University of Minas Gerais (UFMG), with a focus on detection, diagnosis, and prognosis of failures in dynamic systems using data-based methodologies, financed by Petróleo Brasileiro S.A. (Petrobras). He is currently a Lecturer with the Graduate Program in Computer Modeling and Systems, UNIMONTES. Before that, he was a Senior Research Associate with Lancaster University, U.K.

MATHEUS P. LIBÓRIO received the double bachelor's degree in economic sciences and business administration, the master's degree in spatial information processing (geography), specialization in geoprocessing, the M.B.A. degree in strategic planning and competitive intelligence, and the Ph.D. degree in administration. He was a Postdoctoral Researcher in business administration from the Pontifical Catholic University of Minas Gerais (PUC Minas) and in production and transport engineering. He has experience in the areas of operational research (consideration of the uncertainty factor, multi-criteria analysis, and decision-making methods), statistics (multivariate data analysis and time series), and applied geography (remote sensing and geostatistics). He is a journal reviewer for scientific journals from the most renowned publishing houses in the world, such as Elsevier, Springer Nature, and IEEE Xplore.

MARCOS F. S. V. D'ANGELO received the B.S. and M.S. degrees in electrical engineering from the Pontifical Catholic University of Minas Gerais, in 1998 and 2000, respectively, and the Ph.D. degree in electrical engineering from the Federal University of Minas Gerais, in 2010. In 2000, he joined the State University of Montes Claros (UNIMONTES), Brazil, as an Associate Professor in information science. In a broad sense, his main research interests include dynamic systems and optimization theory/applications, and soft computing. In particular, his work has focused on maintenance engineering.



ALISSON S. P. CALDEIRA received the degree in industrial pharmacy from the Federal University of Minas Gerais, the master's degree in research and development in the pharmaceutical industry from Farmanguinhos/Fiocruz, and the Ph.D. degree in pharmaceutical sciences, specializing in phytochemistry, from the Federal University of Minas Gerais. He is currently a Researcher at Fiocruz Minas, Brazil, precisely assigned to the Natural Products Chemistry Laboratory. His field of expertise is the chemistry of natural products, focusing on the analysis of plant and fungal extracts by liquid chromatography hyphenated to high-resolution mass spectrometry (LC-HRMS).

LUIS MIRALLES-PECHUÁN received the bachelor's and Ph.D. degrees in computer science from the University of Murcia, Spain. He was a full-time Researcher/Lecturer at Universidad Panamericana, Mexico, for three years. In 2012, he started his Ph.D. studies on creating new approaches within the online advertising world. During his Ph.D. studies, he got familiar with ML and many papers on how to apply ML to online advertising. He is currently a Lecturer with Technological University Dublin. After finishing the Ph.D. studies, he was in postdoctoral levels I and II in CeADAR, University College Dublin, and there, he won the prize for supervising the best student paper at the Digital Forensic Conference. His topic is applying reinforcement learning to fight the COVID-19 pandemic and plan the containing levels, considering public health and the economy. After that, he has expertise in human activity recognition and generalized zero-shot learning (GZSL) and applying machine learning to improve the accessibility of websites.

HAMID RABIEI received the Ph.D. degree in spatial planning and urban development from Politecnico di Mialno with a strong background and interest in spatial data, methods, and application. He is currently a Teaching Fellow and a Senior Researcher with the School of Architecture, Planning and Environmental Policy, University College Dublin (UCD). He is also a multidisciplinary and interdisciplinary researcher with a background in engineering, social science, and data science. His focus has been on sustainable development and global challenges. His research area is different and diverse, including smart, sustainable cities, environmental policy, remote sensing and GIS applications, housing, gentrification, spatial inequality, spatial data analytics, big data research applications, spatial demography, natural hazards, and social vulnerabilities. He serves as an editor, a member of the board, and a senior editor of academic journals in environmental studies, urban planning, social science, and computer science.