

ANTHELMINTIC EFFECTS OF DRIED GROUND BANANA PLANT LEAVES (*MUSA SPP.*) FED TO SHEEP ARTIFICIALLY INFECTED WITH *HAEMONCHUS CONTORTUS* AND *TRICHOSTRONGYLUS COLUBRIFORMIS*

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Abstract

Background: Helminths is a endoparasites that cause the major losses for profitable sheep production in Brazil. The increased development of resistant strains of endoparasites have enforced the search for sustainable alternatives. The aim of this paper was to provide information about endoparasites control with banana leaves in infected sheep as alternative control strategies and see its viability.

Materials and Methods: In this study, we performed two trials to investigate the anthelmintic properties of banana leaves on endoparasites in sheep. In Trial 1, twelve sheep were artificially infected with *Trichostrongylus colubriformis*; in Trial 2, eleven sheep were artificially infected with *Haemonchus contortus*. Clinical examinations, packed cell volume, total protein, faecal egg counts (FECs) and egg hatchability tests (EHTs) were performed. At the end of the trials, the sheep were humanely slaughtered, and total worm counts were performed.

Results: In Trial 1 and 2, no significant FEC decreases were note but significant diference in EHTs were observed. Total worm counts, clinical and haematological parameters did not reveal significant changes between the treatment and control groups. These results suggest that feeding dried ground banana plant leaves to sheep may reduce the viability of *Trichostrongylus colubriformis* eggs, and this anthelmintic activity is potentially exploitable as part of an integrated parasite management programme.

Conclusion: However, further investigation is needed to establish the optimal dosage, develop a convenient delivery form and confirm the economic feasibility of using banana plantation byproducts as feed for ruminant species.

Keywords: alternative parasite control, endoparasites, small ruminant.

Abbreviations: Coproculture test (CT), Faecal egg count (FEC), Egg hatchability test (EHT)

Introduction

Gastrointestinal helminthiases are a major sanitary concern in sheep herds. Classical clinical signs include loss of appetite, weight loss, melenas and anaemia in the case of *Haemonchus*, whereas severe *Trichostrongylus* infections may result in diarrhoea (West et al., 2009). The parasitological control of nematodes is based on repeated treatments with anthelmintic drugs (Amarante et al., 1997). The practice of repeated treatments favors the emergence of resistant helminth to existing medication (Torres-Acosta e Hoste, 2008). Currently, there is very resistant to endoparasites anthelmintics available. (Max et al., 2010) generating increasing concern about the presence of residues in meat and milk influencing the environment (Athanasidou et al., 2008). Anthelmintics derived bioactive plants can be an alternative for the treatment of parasitic infections (Akhtar et al. 2000). Several studies confirm that tanniniferous plants have been used for control of gastrointestinal nematodes in small ruminants, using extracts, leaves, fruits or seeds from different regions of the world and obtained with different techniques (Min et al., 2004, Martinez-Ortiz-De-Montellano et al., 2010, Novobilský et al, 2013) Sheep that grazed on French honeysuckle (*Hedysarum coronarium*), a source of condensed tannins, exhibited lower FECs and reduced nematode burdens at *post-mortem* examination compared with animals that grazed on lucerne (*Medicago sativa*) (Niezen et al., 1995).

Analyzing the effect of *Acacia molissima* extract lambs naturally infected with *H. contortus* and *T. colubriformis*, Minho et al. (2008) reported that there was a reduction in egg counts per gram of feces and parasite load of *H. contortus* in the abomasum, a fact not observed in the parasite load of *T. colubriformis* in intestine. Nery et al., 2012 studied the efficacy of extracts of immature mango on ovine gastrointestinal nematodes and proved that this fruit could be used to ovine nematodes control. Cala et al., 2014 used *Artemisia annua* L. extracts in naturally infected sheep

and discovered that this plant combined with commercial anthelmintics have been one potential synergism to control *Haemonchus contortus*.

Knowledge of the traditional use of the banana plant (*Musa* spp.) in medicine in Asia reached Europe as early as the XVI century (Touwaide and Appetiti, 2013). Currently, bananas are one of the most important fruit crops in the world, with a global production of approximately 102 million tons (FAO, 2012). Banana plants yield approximately 13 tons/ha/year of leaves and pseudostems, a biomass that could be exploited as fodder for ruminants (Foulkes et al., 1977). The crude protein content of banana leaves ranges from 14.6 g to 17.9 g (Heuzé and Tran, 2013), and its use as a feed source for cattle in the tropics has been explored with positive results (Llosa, 1950). The anthelmintic effect of the banana plant has been studied both *in vitro* and *in vivo*. Aqueous extracts from the leaves, false stems and flowering stalks of banana plants significantly reduced the *in vitro* recovery of *Haemonchus* larvae from pooled faeces from naturally infected sheep, with the average reduction in worm recovery ranging from 98.5 to 100% (Oliveira et al., 2010). Lambs that were artificially infected with *H. contortus* and fed with banana plant leaves for 21 consecutive days demonstrated significant FECs reduction compared with the control group, suggesting that the treatment reduced the fecundity of the worms (Marie-Magdalene et al., 2010). The anthelmintic properties of aqueous extract of leaves, stems and heart banana reported by Nogueira et al. (2012) state that *in vitro* test was inhibition of hatching eggs of *H. contortus*. In the *in vivo* test using naturally infected sheep, the authors report a reduction in the elimination of eggs. These studies have demonstrated that feeding banana plant to sheep may have some efficacy in controlling nematode infections, although it is unclear how it could be used in a feasible and effective manner at the farm level. Recommendations to use the banana plant as fodder date back to the 1950s (Vaitsman, 1954), but its use has not reached any significant level. In addition, gaps persist in the literature regarding its economic viability and effective dosage. The aerial parts of the banana plant are commonly used to cover and protect the soil (Borges, 2006), and the productive chain lacks reliable information to subsidize any efforts towards the use of this biomass in animal nutrition. This study describes two trials designed to assess the efficacy and safety of a daily dose of 150 g of dried ground banana plant leaves during ten consecutive days against artificial *Haemonchus* and *Trichostrongylus* infections in sheep by comparing physical, haematological and parasitological parameters.

Materials and Methods

The trials were conducted at the Veterinary Hospital of FMVZ - USP, Section in accordance with internationally accepted principles for laboratory animal use and care expressed in Brazilian Federal Law No. 11794/2008 and its regulations, as approved by the local Institutional Animal Care and Use Committee.

Fresh banana plant leaves were collected from plantations in Registro city, São Paulo state and the leaves were then stored in an open shed, chopped in the direction of the fibres and oven dried at 40 °C (as higher temperatures can damage condensed tannins). The dried material was then coarsely ground to be fed to the sheep later. Quantification of the condensed tannins in the dried leaves was performed at the Animal Nutrition Laboratory of the Centre for Nuclear Energy in Agriculture - CENA – Luís de Queiróz Superior School of Agriculture - ESALQ - USP. Twenty-three Santa Inês wethers ranging from 6 months to 1 year in age were used in the trials. The animals were obtained from free-range farms located in the state of São Paulo. The average weights of the twelve sheep obtained for Trial 1 and the eleven animals obtained for Trial 2 were 29.0 ± 4 kg and 16.0 ± 4 kg, respectively.

To ensure that the animals were parasite-free before the start of the trials, the sheep were treated with levamisole hydrochloride (Ripercol L, Fort Dodge) at 10 mg/kg b.w. and albendazole (Valbazen, Pfizer) at 10 mg/kg b.w. for three consecutive days. Next, the animals were treated with trichlorfon (Neguvon, Bayer Health Care, São Paulo-SP, Brazil) at 100 mg/kg b.w. for another three consecutive days. FECs were performed for all sheep seven days after the last anthelmintic treatment. FECs were conducted according to the McMaster technique (Whitlock, 1948). The same protocol was applied to all animals that still carried worms until gastrointestinal nematodes were completely eliminated.

Once the sheep were worm-free, they were artificially infected with one of two parasites. For Trial 1, twelve animals were infected with *T. colubriformis*, and eleven animals were infected with *H. contortus* for Trial 2. Infective larvae from both species were obtained from the Department of Parasitology, Institute of Biosciences, Paulista State University – UNESP, Botucatu Campus. Each animal received 3,000 larvae per week by gavage for two weeks. After 21 days, FECs revealed that the artificial infections failed in both groups. Therefore, the sheep were injected with dexamethasone (Dexin, Fagra) at 0.5 mg/kg b.w. twice a week to induce immunosuppression, and the larvae were re-administered until patent monospecific infections were achieved, as confirmed by FECs of 1,000 eggs or higher. Coproculture tests (CTs) were conducted to confirm the monospecific infections. CTs were conducted according to a modified O'Sullivan technique (Roberts and O'Sullivan, 1950).

In each trial, once a patent monospecific infection was achieved, the sheep were randomly assigned to the control or treatment groups. In Trial 1 (monospecific infection with *T. colubriformis*), the control group was composed of 6 animals, and the treatment group contained 6 animals. In Trial 2 (monospecific infection with *H. contortus*), there were 5 sheep in the control group and 6 animals in the treatment group.

Until the trials began, the sheep were fed 300 g of concentrated feed formulated to deliver 15 % raw protein and coast cross (*Cynodon dactylon*) hay *ad libitum*. Beginning on Day 0 for both trials, the treatment groups were fed

150 g of dried ground banana plant leaves mixed with 150 g of concentrated feed/animal/day for 10 days plus coast cross hay *ad libitum*. Sheep in the control groups were fed the initial diet throughout the experimental period. Throughout the trials, the animals were subjected to physical examination by a veterinarian as described by Dirksen et al. (1983). The packed cell volume and total plasma protein were determined at Days 0, +12 and +17 in Trial 1 and at Days 0, +9, +15 and +17 in Trial 2. To evaluate the anthelmintic effect of *Musa* spp. leaves, FECs and egg hatchability tests (EHTs) were performed at Days 0, +5, +9, +12 and +17 in Trial 1, whereas faecal exams were conducted at Days 0, +6, +9, +12, +15 and +17 in Trial 2. EHTs were conducted according to a technique adapted from Coles et al. (1992) using pooled faeces. At the end of the trial, the sheep were humanely slaughtered, and the gastrointestinal contents and mucosae were collected for total worm counts.

Statistical analyses were performed using SAS 9.3 (SAS Institute, USA). In both trials, the design of this experiment was completely randomised with two treatments (with or without banana leaves) and animals were the experimental unit. The data were tested for normality and variance homogeneity for the proc univariate normal procedure. The analysis of variance was performed using the proc mixed with repeated measurements procedure in SAS®. The FEC and worm count were transformed by log₁₀ (x + 10). Comparisons of means were carried out by pdiff. The probability level for acceptance or rejection of the hypothesis was 5 %.

Results

Analysis of the fresh and dried ground *Musa* spp. leaves used in Trials 1 and 2 are presented in Table 1.

Table 1: Analysis of the fresh and dried ground *Musa* spp. leaves used in the trials.

Sample	Dry	Total	Total	Condensed
	Matter	phenols**	tannins**	tannins***
	100°C*			
Fresh banana plant	810.21	60.73	48.51	26.30
Leaves				
Dried ground leaves used in Trial 1	-	11.99	8.11	6.88
Dried ground leaves used in Trial 2	-	13.93	8.61	9.90

Values expressed as g/kg of dry matter

** Values expressed as gram equivalents of tannic acid per kg dry matter

*** Values expressed as gram equivalents of leucocyanidin per kg dry matter

No significant difference was noted between the weight of the animals in the control and treatment groups in Trial 1 and 2 (monospecific infection with *T. colubriformis*). In addition, no significant difference was noted in the packed cell volume of the control and treatment groups. Although the sheep in the control group exhibited a higher total protein concentration at Week 3 than the sheep in the treatment group (P=0.001), all values were within the normal range for the species. In Trial 1, the EHT was significantly decreased in the group fed dried ground banana plant leaves (*Musa* spp.) (Table 2). Larval hatchability was not inhibited in the control or treatment groups infected with *H. contortus* (Trial 2) (Table 2).

Table 2: Egg hatchability tests (EHTs) for Trial 1 (artificial infection with

	Day	Control (%)	Banana (%)	P-value
Trial 1	0	86	77	0.099
	+5	84	1	0.001
	+9	82	0	0.001
	+12	77	48	0.001
	+17	70	86	0.005
Trial 2	0	25	76	0.001
	+6	71	73	0.753
	+9	94	85	0.036
	+12	100	75	0.001
	+15	98	91	0.028
	+17	80	90	0.046

Trial 1: Control group n=6, Treatment group n=6; Trial 2: Control group n=5, Treatment group n=6.

No significant difference was noted in FECs between the control and treatment groups infected with *T. colubriformis* (Trial 1) and *H. contortus* (Trial 2) (Table 3).

We made the differentiation of larvae between male and female, after treatment in animals mono-specifically infected with *T. colubriformis* (Trial 1) (Table 4).

No significant difference was observed in the weight of the animals in the control and treatment groups infected with *H. contortus* throughout the duration of the experiment.

There was no significant difference in the total number of males and females in control sheep and sheep treated with *H. contortus* monospecific infections (Trial 2) (Table 4).

Table 3: Faecal egg counts (number eggs per g faeces - original date) for Trial 1 (artificial infection with *Trichostrongylus colubriformis*) and Trial 2 (artificial infection with *Haemonchus contortus*) in sheep treated with or without banana leaves.

	Day	Control (fec)*	Banana (fec)*	P-value*
Trial 1	0	504.2 ± 115.6	913 ± 586	0.14
	+5	483.3 ± 204.1	333 ± 258	0.18
	+9	300 ± 108.4	363 ± 321	0.92
	+12	738 ± 283	542 ± 323	0.26
	+17	1192 ± 855	1588 ± 1115	0.53
Trial 2	0	5250 ± 3174	7179 ± 6201	0.73
	+6	5955 ± 3147	5067 ± 3847	0.49
	+9	5200 ± 2437	5079 ± 2039	0.94
	+12	6025 ± 4667	5188 ± 2333	0.92
	+15	8905 ± 5555	1272 ± 5456	0.23
	+17	17080 ± 11966	22063 ± 23286	0.74

*

fec = faecal egg count; mean± standard deviation

Table 4: Total worm counts (original date) for Trial 1 (artificial infection with *Trichostrongylus colubriformis*) and Trial 2 (artificial infection with *Haemonchus contortus*) in sheep treated with or without banana leaves.

Control			Banana	P-valuee
Trial 1	Total	325.4±162.6	509.4±240.1	0.14
	Male	126.9±70.74	197.7±85.04	0.11
	Female	198.5±92.85	311.7±163.61	0.18
Trial 2	Total	151±86.85	168.6±63.21	0.67
	Male	77.6±48.22	86±33.29	0.88
	Female	73.8±40.70	90.2±30.43	0.50

(TW)*=Total worms

Discussion

The banana extract evaluated in concentration was well accepted by the animals, since the offered material was ingested by them, demonstrating having good palatability. In the trial period, the animals showed no diarrhea and changes in clinical parameters. The results strongly suggest that the ingestion of dried ground banana does not cause any deleterious effects

The effectiveness of Acacia extract was tested *in vivo* by Cenci et al. (2007) and Max (2010) when they used naturally infected sheep and demonstrated a decrease in OPG count and reduction in parasite burden of gastrointestinal nematodes. Minho et al. (2008) analyzed, *in vitro*, the effect of condensed tannins from *Acacia molissima* on lambs naturally infected with *H. contortus* and *T. colubriformis* and reported that there was a reduction in OPG count and parasite load of *H. contortus* in the abomasum when they received 1.6 g / kg body weight extract. The anthelmintic effect of condensed tannins in gastrointestinal nematodes in sheep was confirmed by the authors. Manolaraki et al. (2010) tested the action anthelmintic of condensed tannins present in *Pistacia lentiscus*, *Quercus coccifera* and *Ceratonia soliqua* in sheep infected with *H. contortus* and *T. colubriformis*. There was a reduction in the elimination of eggs brought about mainly by the decrease in fecundity of females of both species. These results suggested that tannins could be an alternative method for controlling endoparasites in ruminants raised in a pasture management system, thereby reducing reliance on anthelmintics. We did not observe a similar reduction in FECs in either of the trials reported herein most likely because banana plant leaves have low tannin concentrations compared with other tanniferous plants. Ribas et al. (2009) evaluated the efficacy *in vivo* of banana leaf in the control of gastrointestinal worms in small ruminants, and observed no reduction of OPG in treated animals compared to the control group. According to the authors, this fact may be due to the short period of administration of banana leaves, or the restricted supply of 1 kg / animal / day. Assessing the anthelmintic efficacy of waste from the banana crop in gastrointestinal nematodes of sheep, Nogueira et al. (2012) reported that the *in vivo* test, the extract showed no efficacy, while the extract of leaves showed moderate efficacy. The authors suggest that low efficacy may be related to dose, extraction process or frequency of administration. The findings in these studies also report the absence of the anthelmintic action of banana extract *in vivo* tests, corroborating the results found in this study.

In this study, there was a significant reduction in hatching eggs *Trichostrongylus*, but was not observed for *Haemonchus* eggs. In *in vitro* tests, Nogueira et al. (2012) evaluated the effectiveness of the aqueous extract of banana at the outbreak of *Haemonchus* spp. eggs, at a concentration of 2.5 mg / mL and reported that there was a significant inhibition compared to control treatment. Batatinha et al. (2004) evaluated the effectiveness of the aqueous extract of banana leaves on of gastrointestinal nematodes larvae of goats culture and report that the concentration of 130.6 mg / mL reduction in the number of larvae *Trichostrongylus*, *Oesophagostomum* and *Haemonchus* were 71.52; 95.80 and 97.92% respectively. The results show a greater reduction in the number of *Haemonchus* larvae compared to *Trichostrongylus* genre. In *in vitro* tests, we observed anthelmintic action of the aqueous extract of banana, a fact that may be related to the development phase of the parasites, since according to Paolini et al. (2003) the divergence of effects depends on the parasite development stage exposed to the extract. In addition to the development phase of the parasite, Marie-Magdalene et al. (2010) suggested that other compounds, such as terpenoids and flavonoids,

potentially play a role in the anthelmintic effect exhibited by *Musa* spp. both *in vitro* and *in vivo*. The aqueous extraction method used by Oliveira et al. (2010) reinforces this hypothesis because it required heating the samples at 60 °C for one hour, which would denature most condensed tannins. Although we did not observe a decrease in FECs in either trial, a significant reduction in *in vitro* EHT was observed in the animals infected with *T. colubriformis*, demonstrating an inhibitory effect of the banana leaf on egg development. It is likely that the eggs counted at these time points were not viable, suggesting that dried ground banana plant leaves could be used to decrease pasture contamination, even at a dose of 150 g per sheep.

The use of natural products would further reduce the presence of chemical residues in foods of animal origin, especially in small ruminants that have short meat production cycles. Given the increasing pressure from consumer markets for foods that are free of chemical residues, this type of management correlates well with global efforts to reduce environmental pollution. The diversity of the Brazilian flora allows for the possibility of utilising various plant products to control parasitic diseases in livestock. A collective, systematic effort is necessary to incorporate functional or therapeutic foods into feed for small ruminants to control worm infections.

Our results suggest that *Musa* spp. has anthelmintic properties, as treatment completely inhibited *Trichostrongylus colubriformis* larval hatchability *in vitro* at two consecutive time points.

The presence of tannins *Musa* spp. can promote the health of the animal. However, side effects are concentration dependent manner and extraction of these metabolites. Thus, studies are needed to define how to use, methods of extraction, analysis of secondary metabolites and dose in order to facilitate the use of these compounds in nematode control properties and, consequently, increase in the productivity of sheep industry. Therefore, bio panning of bioactive compounds and the development of an anthelmintic product containing condensed tannin would have great commercial potential.

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Conflict of Interest

There are no conflicts of interest.

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