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Effect of Cassava (*Manihot esculenta*) Foliage Supplementation to Calves on the Viability and Egg Count of *Haemonchus contortus* Nematodes

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Abstract: Thirty early-weaned 5±3 month-old calves with an average body weight of 96±23 kg were used; 24 were artificially infested with 90 larvae (L_3), of *H. contortus* per kilogram body weight. Calves grazed on African Star Grass (*Cynodon plectostachyus*) from 0600 to 1300 hours. Grazing was supplemented with lucerne hay (*Medicago sativa*) or cassava foliage (*Manihot esculenta*), and 500 g^{-1} $head^{-1}$ day^{-1} of a commercial feed containing 16% crude protein and trace-mineralized salt, offered during the time spent in confinement. Five treatments were compared: (1) infested, supplemented with lucerne; (2) as treatment (1), drenched; (3) infested, supplemented with cassava; (4) as treatment (3), drenched; (5) non-infested, supplemented with cassava. Each treatment had three replicates of two calves. Those that were drenched orally received 7 mg kg^{-1} albendazole on day 21 after being infested. The experiment lasted until 35 days after infestation. The response criteria were number of nematodes in the abomasum and egg count per gram of feces, both taken at weekly intervals. Numerical data were analysed as a completely randomized design, including the effect of sampling week for egg counts and feed intake. The average number of nematodes found in the abomasums did not differ between treatments ($P<0.05$), the observed values being from 200 to 496 *H. contortus*. Regarding the egg count in feces, the highest figure (822 g^{-1}) was observed in the undrenched control treatment and the lowest (115 g^{-1}) in the cassava-albendazole treatment. In general, there was a 50-85% reduction in egg counts when cassava foliage was used; the response seemingly being improved when the nematicide was administered. Dry matter intake was lower ($P<0.05$) for the cassava treatments. In conclusion, cassava foliage could be used for controlling and reducing the infestation of cattle by *H. contortus*.

Key words: calves, *Manihot esculenta* foliage, *Haemonchus contortus* eggs

INTRODUCTION

Beef cattle operations, especially those based on grazing systems, are constantly faced with diseases caused by different etiological agents (viruses, bacteria, parasites) that not only affect the animals' health, but also their productivity. Parasites, in general, can cause their hosts to suffer from anemia, edema, undernutrition, weight loss, slow growth, gastritis, and in acute cases, even death. Within parasitic diseases, those caused by helminths are the most relevant, and the one with the highest incidence is by *Haemonchus* spp., which affects sheep, cattle and other ruminants and has been reported to be the most common nematode in the tropical regions, because of the favourable climatic conditions that facilitate its development and survival [1].

Although nematode control strategies such as the use of anthelmintic drugs (benzimidazoles, imidazothiazoles and macrocyclic lactones) are in use, the problem has not been satisfactorily solved and, in some regions, it has even worsened due to the mismanagement of the drugs. To date there is no method which is efficient, practical and sustainable,

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that is, which does not present toxicity risks for both animals and humans, and for the environment [2].

An alternative would be the use of plants with nematicidal properties such as those which contain condensed tannins (CT). These are secondary metabolites that in the tropics can be found in fodder crops such as *Leucaena leucocephala*, *Gliricidia sepium*, *Manihot esculenta* [3]. The metabolic role of CT in plants is not well known, but it has been observed that they provide them with protection against microbes, insects and even large herbivores. Controlled-condition studies on their anthelmintic effect on larvae and adult parasites of both sheep and cattle suggest that they could be considered as alternatives against gastro-enteric parasites, although their presence in the diet could affect its digestibility and therefore its nutritive value. Concentrations of 60 g kg^{-1} in feed improve the utilization of protein by ruminants, although higher levels may depress voluntary intake [4, 5].

The objective of this experiment was to evaluate the anthelmintic effect of cassava foliage (*M. esculenta*) in calves infested with *H contortus*, raised in a restricted grazing system.

MATERIALS AND METHODS

The experiment was conducted in the Huimanguillo Experimental Station of the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, located in the State of Tabasco (17°50′ N; 20 m above sea level). The predominant climate of the State is classified as warm–humid (AmW), the temperature varies from 24 to 34°C, and the annual rainfall is 2,123 mm, 70% of which falls between May and November [6].

Thirty predominantly Brown Swiss X Zebu crossbred calves, weaned at 5±2 months and with an initial weight of 96±23 kg were used. All animals were maintained on an African Star Grass (*Cynodon plectostachyus*) subdivided pasture, with 5-day grazing periods, and 25 days of rest. Calves grazed from 0600 to 1300 hours and the rest of the time they were confined and supplemented with lucerne (*Medicago sativa*) hay or chopped cassava (*Manihot esculenta*) foliage. The amount of forage offered was adjusted daily, allowing for 5% refusal. The lucerne was commercially purchased. The cassava used was of the "Sabanera" cultivar (MPan 51), planted as a fodder crop with 25 cm spacing between plants and 30 cm between rows, and the foliage was harvested daily. In addition to the forage, each animal was offered 500 g of a 16% crude protein concentrate and 25 g of a trace-mineralized formula daily.

All feedstuffs were analysed for dry matter, crude protein and iron, according to the methods suggested by Tejada [7]. Free and reactive condensed tannins (CT) in forages were determined as described before [3]. *M. esculenta* had 55.5±4.0 g CT kg^{-1} of dry matter. Neither *M. sativa* nor *C. plectostachyus* had detectable levels of CT.

To remove all internal parasites, calves were subcutaneously medicated with Ivermectine (Iverfull, Aranda) at doses of 0.2 mg kg^{-1} live weight. After 35 days, they were orally given 10 mg kg^{-1} live weight of Fenbendazol (Actuol, Senosiain). Six days thereafter, a McMaster analysis of faeces [8], indicated that all animals were parasite-free. Although the oral anthelmintics are supposedly eliminated within 72 h after their application, a further period of 72 h was allowed before starting the experiment proper. During this time, animals were adapted to the consumption of the supplemental forages and concentrate.

A sample of faeces from a steer, orally infested with *H contortus* and free from other nematodes, was used. Starting at 2 weeks post-inoculation, the animal was sampled to detect for the elimination of eggs.

Larvae were extracted with the Baermann method, which is based on their ability to migrate within a semi-liquid substrate, through positive hydrotropism. Feces were covered with cheesecloth and placed in a funnel with distilled water. After a 12–24 h period, the larvae migrated, along with some escaped feces. The larvae-containing sediment was then centrifuged 3 min at 3,000 rpm, washed with a glucose solution, and the supernatant was siphoned and discarded. Two to three drops of the sediment were observed through a microscope to check the developmental stage and viability of the larvae; 1 mL of the sediment was then placed in refrigeration. The collected larvae were identified, quantified and the dosages calculated according to each animal's weight [9]. Twenty-four calves were infested with approximately 90 larvae (L_3) of *H. contortus* kg^{-1} live weight.

Five experimental treatments were tested: (1) infested with *H contortus*, supplemented with *Lucerne;* (2) as treatment (1), drenched with albendazole; (3) infested, supplemented with cassava; (4) as treatment (3), drenched with albendazole; (5) non-infested, supplemented with cassava. Each treatment had three replicates of two animals. The experiment lasted 35 days. On day 21 after infestation, treatments (2) and (4) received 7 mg kg^{-1} live weight of a commercial nematicide (Albendazole [methyl 5-6 propyl thio-1H-benzimidazole-2yl carbamate]; Lavet Laboratories).

Weekly feces collections from all animals were analysed as described above, to measure the effect of the treatments on *H. contortus* egg yield. Blood was collected in vaccutainer tubes and the serum separated [10]. Its iron content was determined by indirect atomic absorption (Nova 10 Analyzer). Hemoglobin was measured in EDTA-added blood.
Thirty-five days following medication, animals were slaughtered according to Mexican laws [11]. The extracted abomasums were placed in plastic bowls and cut open, collecting their contents in a separate container; the mucosa was washed with tap water. The collected volume was brought to 1–3 L, mixed, and a 10% aliquot saved. Live parasites were placed in a saline solution, and refrigerated for later identification and quantification.

The response criteria were: abomasal parasite yield and sex; egg numbers at weekly post-infestation periods; blood hemoglobin and serum iron at the start of the experiment, 21 days post-inoculation, and at slaughter; weekly supplemented forage intake.

The numbers of larvae were analysed as completely randomized designs, with data transformed to their square roots; the other parameters were analysed as measurements repeated over time. A SAS [12] statistical package was used.

RESULTS AND DISCUSSION

Anti-nutritional factors: Parasite numbers present inside the abomasums of calves infested with *H. contortus* and supplemented with *lucerne* or *cassava*, unmedicated or drenched with albendazole, are shown in Table 1. The total number of larvae in the abomasums did not differ significantly between treatments (P>0.05) and varied from 200 to 496, with no differences between the sexes. When we previously [5] fed confined Pelibuey sheep ad libitum amounts of tanniferous forages, the parasite yields were reduced 70–94%; however as our calves were on pasture, they could have been naturally re-infested.

Table 1: *Haemonchus contortus* numbers in the abomasums of grazing calves supplemented with lucerne or cassava, untreated or drenched with albendazole (mean ± SEM)

Treatment	Total	♂	♀
Lucerne, untreated	255±229	147±123	108±108
Lucerne, drenched	496±205	264±110	232±097
Cassava, untreated	466±229	245±123	221±108
Cassava, drenched	200±229	88±123	112±108
Cassava, non-infested	407±265	223±142	184±125

The drenched treatments were not 100% effective. However the literature also contains conflicting information, with some early authors reporting that the use of benzimidazoles result in the total elimination of parasites, while in more recent papers, others do not agree [13], possibly reflecting an increasing resistance of the parasites to the drugs.
Table 2 shows the weekly numbers of *H. contortus* eggs per gram of faeces from grazing calves supplemented with Lucerne or cassava, untreated or drenched with albendazole. From the first to the fourth week of the experiment, there was a gradual increment in the number of parasite eggs present in the calves' faeces, with no significant difference between treatments (P>0.05). Eight days after drenching (week 5), animals from the medicated treatment groups had significantly fewer eggs (P<0.05). By week 6, all treatments except the control showed a significant reduction in egg counts (P<0.05). The results are similar to those reported in cattle [14] and in goats [15], when fed tanniferous forages.

It has been reported that the mode of action of CT is to either bind specific nutrients, and thus inhibit their availability for the larvae, or to interfere with their metabolism through a disruption in the oxidative phosphorylation process, causing a reduction in egg yield or death [16]. It has been reported [14] that CT have a negative effect on the fecundity of female nematodes. Moreover, it has also been informed that CT and synthetic anthelmintic drugs have similar effects on egg yield [17].

Likewise, CT could have modified the rumen microbial digestion processes [18, 19] as it is known that the amount and quality of the diet, as well as its fermentation pattern, can have adverse effects on the development and establishment of the parasites, intensifying the effect of the anthelmintic drugs [20].
Iron concentrations in blood serum from grazing calves supplemented with Lucerne or cassava, untreated or drenched with albendazole, are shown in Table 3. There were no significant differences in serum iron values between treatments (P<0.05), and the observed values were much lower than those considered normal (100–170 μg 100 ml^{-1}) [21]. It has been demonstrated that hematophagous parasites, such as *Haemonchus*, consume daily up to 0.05 mL of blood, which is enough as to induce anemia in the host animals [22]. The observed blood serum iron values showed that the calves were anemic throughout the experiment; therefore they were not able to overcome the damage suffered before the initial medication. Furthermore, the effect was prevalent regardless of the adequate levels of dietary iron provided: cassava (200 mg kg^{-1}), lucerne (249 mg kg^{-1}), protein concentrate (185 mg kg^{-1}), trace-mineralized formula (513 mg kg^{-1}); their daily requirement being 50 mg kg^{-1} [23].

Table 2: Number of *Haemonchus contortus* eggs per gram of faeces from grazing calves supplemented with lucerne or cassava, untreated or drenched with albendazole, measured weekly (mean ± SEM).

Treatment/ Week	1	2	3	4	5	6
Lucerne, untreated	nd	112^{a}±48	304^{a}±89	773^{a}±176	921^{a}±210	822^{a}±117
Lucerne, drenched	nd	80^{a}±43	225^{a}±79	471^{a}±158	237^{b}±188	220^{b}±104
Cassava, untreated	nd	50^{a}±48	288^{a}±89	484^{a}±176	431^{a}±210	215^{b}±117
Cassava, drenched	nd	50^{a}±48	192^{a}±89	356^{a}±176	147^{b}±210	115^{b}±117
Cassava, non-infested	nd	50^{a}±55	306^{a}±102	504^{a}±204	337^{a}±243	383^{b}±135

Values with different superscripts are statistically significant.
nd = not detected.

Table 3. Iron (Fe) and haemoglobin (Hb) concentrations in blood serum from grazing calves supplemented with lucerne or cassava, untreated or drenched with albendazole (mean ± SEM).

Sample	Initial		When eggs appear		Final	
Treatment	Fe	Hb	Fe	Hb	Fe	Hb
Lucerne, untreated	83.7±13.03	9.05±0.70	82.5±7.9	9.05±0.44	78.7±9.5	8.83±0.54
Lucerne, drenched	80.0±11.65	8.88±0.63	76.2±7.1	8.70±0.39	95.2±8.5	9.65±0.48
Cassava , untreated	81.3±15.04	9.20±0.70	86.3±9.2	8.62±0.44	86.0±10.9	9.05±0.54
Cassava , drenched	86.5±13.03	8.81±0.70	74.7±7.9	8.74±0.44	82.2±9.5	8.59±0.54
Cassava , non-infested	81.3±15.04	8.85±0.81	86.3±9.2	9.22±0.50	86.0±10.9	9.27±0.63

Table 4. Weekly supplement intake of grazing calves supplemented with lucerne or cassava, untreated or drenched with albendazole, kg (mean ± SEM).

	Week					
Treatment	1	2	3	4	5	6
Lucerne, untreated	6.4±1.2	7.9±1.3	9.0±1.2	11.4±1.8	11.4^{a}±1.2	9.9^{a}±1.0
Lucerne, drenched	9.1±1.1	9.1±1.1	10.8±1.1	13.4±1.7	13.4^{a}±1.1	11.8^{a}±0.9
Cassava, untreated	5.5±1.2	5.6±1.3	6.9±1.2	8.9±1.8	6.4^{b}±1.2	6.0^{b}±1.0
Cassava, drenched	5.9±1.2	6.7±1.3	6.9±1.2	7.3±1.8	6.6^{b}±1.2	5.9^{b}±1.0
Cassava, non-infested	7.3±1.4	8.1±1.5	7.3±1.4	7.2±2.1	6.8^{b}±1.4	6.2^{b}±1.2

Values with different superscripts are statistically significant.

Table 3 also shows the hemoglobin concentrations in blood from grazing calves supplemented with lucerne or cassava, untreated or drenched with albendazole. There were no significant differences between treatments at any sampling time ($P>0.05$). Nevertheless the values fell within the lower limits of what would be considered as normal (8–15 g dl^{-1}) [24] and were also inferior to those previously observed in sheep [25]. Whereas it appears that infestation with nematodes decreases blood haemoglobin levels [26], the quality of the diet could exert a synergistic effect [20].

The weekly supplement intake of grazing calves, with lucerne or cassava, untreated or drenched with albendazole, is summarized in Table 4. There were no significant differences between treatments in supplement consumption during the first 4 weeks of the experiment ($P>0.05$); however, the intake in the lucerne, but not the cassava treatments, had gradually increased and therefore by the fifth week, the difference between forages had become significant ($P<0.05$). Although the supplemented cassava contained 56 g of CT kg^{-1} DM, and levels of 50–80 g of CT kg^{-1} of dietary DM do not affect the intake or nutritive value of the diet in sheep [27, 15], it has been suggested that about 60 g of CT kg^{-1} of DM, could decrease consumption, due to its astringency having an adverse effect on palatability [4, 28] and digestibility. In a previous experiment with confined sheep, we did not observe any differences in voluntary intake [5], but calves could have a lower threshold value [28, 29].

CONCLUSIONS

1. Supplementation of pasture-raised sheep with *M. esculenta* decreases faecal *H. contortus* egg counts.
2. Blood serum iron levels were lower than normal, probably due to the parasites.
3. Condensed tannins had an adverse effect on voluntary feed intake.

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