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Anthelmintic efficacy of neem (*Azadirachta indica* A. Juss) and the homeopathic product Fator Vermes® in Morada Nova sheep

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Abstract

Gastrointestinal nematodes are becoming increasingly resistant to the commercial products used to control them. The cost of routine vermifuge applications on herds and the problem of residues in animal products and the environment have prompted research on the anthelmintic activity of plant extracts. This work examines the anthelmintic action of neem and the homeopathic product Fator Vermes® in sheep kept in a pasture for 18 months. Forty sheep of the Morada Nova breed were divided into four treatments and the control, according to the EPG. During the experiment, each animal received 100 g/day of shredded corn and did not receive protein supplementation. In treatment 1 (control), the animals received only shredded corn. Treatment 2 received 1.6 g/(animal day) of the homeopathic product mixed with the shredded corn, and treatments 3, 4 and 5 received, respectively, 12.5, 25.0 and 37.5 g/(animal day) of dried *Azadirachta indica* leaves mixed with the shredded corn. The neem was administered for alternating 15-day periods and the homeopathic product daily for 18 months. There were 39 fortnightly fecal collections made to count the EPG, and fecal cultures were performed monthly. The following genera, in percentage, were identified: *Haemonchus*: 65.58 ± 3.27, *Trichostrongylus*: 15.92 ± 7.38 and *Oesophagostomum*: 18.50 ± 6.22. The treatments evaluated were not effective in controlling gastrointestinal nematodes ($P > 0.05$), whose mean $\log_{10}$ counts (EPG +1) and standard errors for treatments 1–5 were respectively 3.55 ± 0.28; 3.48 ± 0.31; 3.90 ± 0.29; 2.78 ± 0.29 and 3.48 ± 0.30. A significant effect ($P < 0.0001$) was observed of the periods of the year when the 39 collections occurred. Because of the diet deficient in raw protein, the sheep had higher average EPG counts, for all the treatments, at the end of the dry season, and the opposite occurred in the middle of the rainy season.

Keywords: Gastrointestinal nematode; *Haemonchus contortus*; Phytotherapy; Homeopathy; Sheep

1. Introduction

Gastrointestinal nematodes are considered the main impediment to the raising of small ruminants in Brazil. Epidemiological studies in various regions of the country have shown that *Haemonchus contortus* is the predominant species in the parasite populations in these animals (Arosemena et al., 1999; Amarante et al., 2004; Ramos et al., 2004). Although the development of parasite resistance is widely known (Ramos et al., 2002; Melo et al., 2003; Mattos et al., 2004), the main way of controlling gastrointestinal endoparasites in sheep herds continues to be through the use of synthetic anthelmintics. However, access to drugs is often limited by the low purchasing power of small producers and even of communities that survive on raising sheep (Githiori et al., 2004). Besides the high cost of routine vermifuge

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applications on herds, other questions, such as residues in the animal products and environment, have prompted studies of the anthelmintic activity of plants and plant extracts (Vieira et al., 1999).

Phytochemical analyses of plants and controlled experiments, associated with recent knowledge about parasite control strategies, can offer new alternatives for effective and economical control of parasite-borne diseases (Akhtar et al., 2000). *Azadirachta indica* (Meliaceae), whose common name is neem, has been investigated in the control of gastrointestinal nematodes of ruminants, but its real efficacy is still not well clarified scientifically. An aqueous extract of *A. indica* seeds was found to have low efficacy against sheep nematodes (Ahmed et al., 1994), while laboratory studies showed inhibition of 68.3% of larval hatching of *H. contortus* with the use of azadirachtin at 1% obtained from seeds (Pessoa, 2001). In cattle, the consumption of dried leaves caused a reduction in the number of eggs per gram of feces (Pietrosemoli et al., 1999). However, other studies have not found significant results for the ethanolic seed extract on sheep artificially infected with *H. contortus* and *Trichostrongylus colubriformis* larvae (Hordegen et al., 2003) and in sheep treated with dried leaves, in relation to the control group (Githiori et al., 2004; Costa et al., 2006).

Despite the sale of homeopathic products for control of gastrointestinal nematodes, particularly for organic systems, the efficacy of these products still needs scientific demonstration in Brazil. Homeopathic products, if effective, could contribute to better animal health, as well as providing for animal products free of chemical residues (Cruz et al., 2006).

To obtain more solid data on the use of phytotherapeutics and homeopathic remedies in sheep raising, his study examines the anthelmintic activity of neem and the homeopathic product Fator Vermes® in sheep kept in the pasture for a period of 18 months.

2. Materials and methods

The experimental work was performed at the National Goat Research Center in Sobral, State of Ceará, Brazil. Forty sheep of the Morada Nova breed, with ages of approximately 1 year and average weight of 30 kg, were divided into a five treatments of eight animals each. The animals were distributed into each group alternately in descending order of the number of eggs per gram (EPG) of feces.

During the 18-month experiment, each animal received 100 g/day of shredded corn, which could be considered an energy supplement. They did not receive protein supplementation. In treatment 1 (control), the animals received only shredded corn. In treatment 2, they received 1.6 g/(animal day) of the homeopathic product Fator Vermes® (according to the recommendations of its maker, Laboratório Arenales Fauna e Flora), mixed with the shredded corn. For alternating 15-day periods, the animals in treatments 3, 4 and 5 received, respectively, 12.5, 25.0 and 37.5 g/(animal day) of dried and shredded neem leaves added to the same quantity of shredded corn. In Githiori et al. (2004), sheep received fresh neem leaves for 3 weeks in doses of 250 mg/kg (7.5 g in animals with average weight of 30 kg), 500 mg/kg (15 g) and 1.000 mg/kg (30 g). Quantities above 30 g were found to be difficult to ingest because of the leaves' bitter taste, causing palatability problems (Chagas and Vieira, 2007). The neem was given for alternating periods to prevent timpanism problems (Belmiro Pereira das Neves, personal communication) and dried leaves were chosen rather fresh leaves, because the active principles tend to be concentrated with the water evaporation (Chagas and Vieira, 2007).

During this period, each group of animals remained in separate paddocks of roughly 4 ha, constituted of thinned native pasture, where they grazed. The local vegetation, called "caatinga", is composed of bushes such as *Sida* sp. and *Croton campestris*, and grasses like *Aristida setifolia*, *Antephora hermaphrodita* and *Rhychoelitrum roseum* (Araújo Filho et al., 1996). In the dry season the phytomass availability decreases because many plants lose their leaves and the animals choose the plants that are more resistant to drought. Rainfall is seasonal, with January–May being the wet season and June–December the dry (Arosemena et al., 1999).

We made 39 fortnightly collections of feces, taken directly from the rectum of the females, to count the EPG. In this method, 2 g of feces from each animal was mixed in 58 ml of saturated sodium chloride solution and, before homogenization retrieved a sample to count the eggs, using a McMaster's egg counting slide. The total egg count was multiplied by 100 (Ueno and Gonçalves, 1998 adapted from Gordon and Whitlock, 1939). In the fecal culture, 30 g of feces from the animals was mixed with 30 g of sterilized horse feces. This material was placed in an incubator at 27 °C for 7 days to obtain third-stage larvae (Roberts and O'Sullivan, 1950). Every month, we obtained 100 larvae from fecal cultures and identified them by genus to estimate the makeup of the animals' parasite load. The identification was done using morphological characteristics like the shape of the anterior portion as well as caudal and sheath length (Dickmans and Andrews, 1933; Ueno and Gonçalves, 1998).