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

A.C.S. Chagas a,*, L.S. Vieira b, A.R. Freitas a, M.R.A. Araújo b, J.A. Araújo-Filho b, W.R. Araguão b, A.M.C. Navarro b

a Embrapa Pecuária Sudeste, Rod. Washington Luiz, Km 234, Caixa Postal 339, 13560-970 São Carlos, São Paulo, Brazil
b Embrapa Caprinos, Estrada Sobral/Groatras, Km 4, 62010-970 Sobral, Ceará, Brazil

Received 14 June 2007; received in revised form 4 October 2007; accepted 4 October 2007

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 log10 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; Matsos 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

* Corresponding author. Tel.: +55 16 3361 5611; fax: +55 16 3361 5754.
E-mail address: carolina@cppse.embrapa.br (A.C.S. Chagas).
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 tympanism 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 hermaphroditia 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).
We submitted the EPG data to log_{10} transformation (EPG +1) and analyzed them statistically by the MXED procedure of SAS (SAS Institute, 2002/2003), considering repeated-measures-analysis (Littell et al., 1996, 1998).

Analysis 1: we followed the model \( y_{ijk} = \mu + \alpha_i + d_{ij} + t_k + (\alpha t)_{ik} + e_{ijk} \), where \( y_{ijk} \) is response from the evaluation of sample \( k \) from animal \( j \) of treatment \( i \); \( \mu \) = overall average; \( \alpha_i \) = fixed effect of treatment \( i \); \( d_{ij} \) = random effect of animal \( j \) in treatment \( i \); \( t_k \) = fixed effect of the collection; \( (\alpha t)_{ik} \) = interaction effect; and \( e_{ijk} \) = random error, reflecting the EPG variations in each animal.

The most suitable structure of the variance–covariance matrix \( R (R = V(e_{ijk})) \), which reflects the variations in the EPG data within each individual, was compound symmetry, chosen by means of Akaike’s information criterion, \( \text{ASR} = -2L_R + 2q \), where \( L_R \) is the logarithm of the maximum restricted likelihood function evaluated at the maximum point (Wolffinger, 1993) and \( q \) is the model’s dimension.

We also analyzed the variance of the 39 sample collections (3 June 2004–21 December 2005), divided into five periods of the year: the start, middle and end of the dry season (SD, MD and ED) and the start and middle of the rainy season (SR and MR). The new model adopted and analyzed by the MXED procedure was Analysis 2: \( y_{ijkl} = \mu + \alpha_i + \alpha_{ij} + e_k + (\alpha e)_{ik} + e_{ijkl}, \) where \( y_{ijkl} \) = response to the evaluation of sample \( l \) from period \( k \) of treatment \( i \) of animal \( j \); \( \mu \) = overall average; \( \alpha_i \) = fixed effect of treatment \( i \); \( \alpha_{ij} \) = random effect of the animal within treatment \( i \); \( e_k \) = fixed effect of period \( k \); \( (\alpha e)_{ik} \) = interaction effect; and \( e_{ijkl} \) = random error.

### 3. Results

The mean percentages of larvae obtained in the fecal cultures did not vary during the entire study. The following genera, in percentage, were identified: *Haemonchus*: 65.58 ± 3.27, *Trichostrongylus*: 15.92 ± 7.38 and *Oesophagostomum*: 18.50 ± 6.22.

Table 1 presents the results of the Type 3 tests of fixed effects of analyses 1 and 2, referring to the EPG counts. In Analysis 1, we found no significant difference \( (P > 0.05) \) in the mean EPG count among the treatments and in Analysis 2 we detected a significant difference \( (P < 0.0001) \) for the collections carried out within the periods established.

The means obtained by least squares with the respective 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. Fig. 1 shows the transformed EPG means in the 39 samples collected. There are fluctuations around a rising trend until collection 16 and strictly increasing values from collection 22 to 29. Since there was no difference among treatments, the fluctuation of the log_{10} values (EPG +1) is identical in all the cross sections, i.e., with rising values in the order of treatments 4, 2, 3, 5 and 1.

Just as for Analysis 1, for Analysis 2 we did not find a statistical difference \( (P > 0.05) \) among the treatments, whose means and standard errors for treatments 1 to 5 were 3.5 ± 0.21; 3.45 ± 0.23; 3.78 ± 0.21; 2.72 ± 0.21 and 3.41 ± 0.22, respectively. We observed a highly significant \( (P < 0.0001) \) effect of the EPG counts among the periods of the year (SD, MD, ED, SR and MR), whose means and standard errors were, respectively, 2.74c ± 0.19; 3.75b ± 0.19; 4.34a ± 0.21; 3.47b ± 0.25 and 2.56c ± 0.25. Fig. 2 shows the behavior of the means of the five treatments in function of the five periods (left graph) and vice versa (right graph). It can be seen that the animals’ contamination increased gradually in the dry season and decreased in the wet season. The highest mean EPG counts occurred in period at the end of the dry season (ED) for all the treatments, and the opposite occurred for middle of the rainy season (MR).

### Table 1

Type 3 tests of fixed effects (Prob > F) for the EPG data submitted to log 10 transformation (EPG +1)

<table>
<thead>
<tr>
<th>Effects</th>
<th>d.f. (numerator)</th>
<th>d.f. (denominator)</th>
<th>F-Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analysis 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>4</td>
<td>33</td>
<td>1.99</td>
<td>0.1184</td>
</tr>
<tr>
<td>Collection</td>
<td>38</td>
<td>1116</td>
<td>11.93</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment × collection</td>
<td>152</td>
<td>1116</td>
<td>1.41</td>
<td>0.0016</td>
</tr>
<tr>
<td><strong>Analysis 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>4</td>
<td>33</td>
<td>1.80</td>
<td>0.1533</td>
</tr>
<tr>
<td>Period</td>
<td>4</td>
<td>1252</td>
<td>24.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment × period</td>
<td>16</td>
<td>1252</td>
<td>0.95</td>
<td>0.5123</td>
</tr>
<tr>
<td>Collection (period)</td>
<td>34</td>
<td>1252</td>
<td>10.04</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
4. Discussion

Studies of the anthelmintic efficacy of leaves of *A. indica* in ruminants have presented contradictory results. The supply of partially dried leaves reduced the EPG in cattle, but the animals presented infection below 600 eggs from the start of the treatment, including the control group (Pietrosemoli et al., 1999). In another experiment, sheep that received 3 g/kg of fresh neem leaves for 6 weeks had a significant reduction ($P < 0.05$) in the number of worms found upon necropsy, but this was not reflected in a reduction in the EPG in relation to the control group (Chan-drawathani et al., 2006).

The lack of efficacy detected in the field in the present study is similar to the results presented in other works. Sheep artificially infected with *H. contortus* and *T. colubriformis* larvae received an ethanolic extract of *A. indica* seeds, at a dosage of 3 mg/kg of live weight. On the 13th day after administration, the efficacy in reducing the EPG was 5.2%, and in reducing adult worms on necropsy it was 0% (Hordegen et al., 2003). In another study (Githiori et al., 2004), groups of five sheep received fresh neem leaves for 3 weeks in doses of 250, 500 and 1000 mg/kg. A reduction of food consumption was observed in the last two treatments and a significant increase in the EPG. In an experiment reported by Chagas and Vieira (2007), no anthelmintic effect was observed of neem at a dosage of 30 g of dried leaves per goat/day given for 5 days. The EPG was monitored daily for 28 days in the treated and control groups, containing 12 female goats each. Quantities above 30 g were found to be difficult to ingest because of the leaves’ bitter taste, causing palatability problems. The control group emptied the trough containing the shredded corn feed in 30 min, while the group that received neem mixed with the feed took nearly 2 h. Another important point to consider is that azadirachtin-A, the substance believed to act against the parasites, is present in the seeds at a concentration of approximately 24.85 mg/100 g, while the leaves only contain 0.59 mg/100 g (Sundaram, 1996).

Some works have also found no anthelmintic action on small ruminants of *Melia azedarach*, a species that also belongs to the Meliaceae family. The inefficacy observed of administering *M. azedarach* seeds in doses of 10 mg/kg to 1 g/kg can be due to the destruction of the active substances by the ruminal flora and other aspects, such as ruminal pH (Pervez et al., 1994).
Various published results indicate that homeopathic products were not effective. The maker affirms that after 4 months of continuous use of the Fator Vermes™ product, the infecting larvae ingested lost their ability to lay eggs (Arenales, 2002). However, an experiment conducted on sheep for 6 months with this product did not find any efficacy (Rocha et al., 2006). In Alberti et al. (2005) the Fator Ovino™ product did not demonstrate efficacy in reducing the EPG in relation to the control during a 12-month experiment. Each group was composed of 15 mixed-breed sheep, and a dosage was tested of 200 g/(animal day), 100 times greater than that recommended by the company. In the study conducted by Cruz et al. (2006), Fator Vermes™ showed efficacy when tested on female goats in an extensive system for 3 months. The conclusions were based mainly on the last collection of the experiment (D84). Chandrawathani et al. (2006) stress the importance of conducting experiments lasting longer to evaluate the effect of chemical products on animals.

A homeopathic remedy based on Artemisia cina was tested on sheep with natural and artificial infection by gastrointestinal nematodes and no statistically significant difference was observed between the treated and control groups (Cabaret, 1996). Homeopathic medicines based on Sulfur, Ferrum phosphoricum, Arsenicum album and Mercurius solubilis, also did not demonstrate anthelmintic effect on lambs, but a significant weight gain was observed in the animals (Cavalcanti et al., 2006). In an experiment conducted on sheep for 6 months, although no significant difference was observed between the EPG result (log 10), there was a significant reduction in the third-stage larvae found in the fecal cultures of the group that received a homeopathic treatment in relation to the control (P < 0.01), suggesting a decrease in larval hatching and control through an increase in the animals’ immunological response. The animals received F. phosphoricum, A. album and Calcarea carbonica, the latter of which is known for its positive nutritional effect (Zacharias, 2004). However, in the present work, where the animals received Fator Vermes™ for 18 months, a decrease in the larval hatching rate would have had a reflection in the contamination of the animals. The commercial product tested contains active substances from parasites (CH12): Bunostomum sp., Haemonchus sp., Strongyloides sp., Trichostrongylus sp., Trichuris sp., Oestrus ovis, Eimeria sp., Fasciola hepatica, Dermatobia hominis, Damalinia caprae, Linognathus stenopsis, Musca domestica and Bixa orellana.

The increase in contamination of the animals during the dry season, which is considered unfavorable to the survival of eggs and larvae in the Brazilian Northeast (Arosemena et al., 1999), is due to nutritional deficit. During the dry season, the sheep do not receive supplementation and the forage in the scrublands becomes scarce. The diet with low raw protein lowers the animals’ resistance to parasites and their ability to survive in the adverse conditions (Coop and Kyriazakis, 2001; Bricarello et al., 2005). This study was carried out under the same conditions normally existing in the Brazilian Northeast. Sheep and goats in the region are nearly always raised in the prevailing “caatinga” vegetation, foraging on the native plants, in the majority of cases in conditions of overgrazing (Pereira Filho et al., 2007).

The treatments evaluated in the present study did not prove effective in controlling gastrointestinal nematodes, in the conditions in which they were tested. Because of technological advances and the importance that examining anti-parasite agents based on phytotherapeutics and homeopathic medicines has gained, it is essential that phytochemical studies of the available plant material be carried out in future experiments.

Acknowledgement

The authors would like to thank Laboratory Arenales Fauna & Flora for supplying the homeopathic product.

References


