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Daily feeding of fresh Neem leaves (*Azadirachta indica*) for worm control in sheep

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Abstract. This study was conducted to evaluate the anthelmintic effect of Neem (*Azadirachta indica*) on nematode parasites of sheep. Twelve Santa Ines cross bred sheep from a government farm were randomly selected and equally divided into control (n = 6) and treated groups (n =6). Faecal egg counts (FEC) using the modified McMaster technique and the FAMACHA score for assessing clinical anaemia were carried out daily and recorded for 6 weeks. At the end of the study all the animals were slaughtered and the total worm count (TWC) was done. The results of FEC showed that there was no significant difference between the control and treated group ($p = 0.081$). However, worm burden estimations showed that the number of parasites was significantly higher in the control group compared to the treated group ($p < 0.05$). This result indicated that feeding Neem had an effect on worm numbers in sheep, but was not reflected in their faecal egg counts. Further work is needed to reconfirm the effect of Neem on helminth infections of sheep.

INTRODUCTION

The 2003 figures from the Department of Veterinary Services, Malaysia recorded the total number of sheep and goat populations as 109,004 and 207,522 respectively. (Department of Veterinary Services, http://agrolink.moa.my/jph/). Thus, their combined numbers, which constitute the small ruminant industry of Malaysia, is an important component to the overall livestock sector for the country. It is one of the objectives of the government in the National Agriculture Policy (NAP3) to increase small ruminant production through the importation of exotic breeds of sheep and goats, increasing the number of female animals and conducting intensive research in small ruminants and their diseases (Department of Veterinary Services, http://agrolink.moa.my/jph/).

Helminthosis is one of the most important causes of mortality and morbidity in goats and sheep in Malaysia (Sani *et al.*, 2004) and elsewhere throughout the humid tropical/subtropical countries of the world (Perry *et al.*, 2002). It is mainly caused by strongyle nematodes such as *Haemonchus* spp., *Oesophagostomum* spp., *Bunostomum* spp., *Cooperia* and *Trichostrongylus* spp. The parasite that causes the most problems to small ruminants is *Haemonchus contortus*, better known as the "barbers pole" worm, and it is generally considered the most pathogenic parasite of small ruminants (Soulsby, 1982).

Sheep and goat farmers rely heavily on anti-parasitic drugs, or anthelmintics, to control internal parasites in their small ruminant flocks. A wide variety of anthelmintics, covering the entire range of chemical groups, are used for the treatment

of nematode parasites of sheep and goats in Malaysia. These chemical groups include: the benzimidazoles (eg. albendazole, fenbendazole etc.), the imidothiazoles (eg. levamisole), the salicylanilides (eg. closantel) and the macrocyclic lactones (eg. ivermectin) (Chandrawathani *et al.*, 1994). In 1999, a survey of 39 sheep farms and 9 goat farms found that the majority had worm populations resistant to all classes of drugs (Chandrawathani *et al.* 1999). From this investigation, it was clear that anthelmintic resistance was rapidly increasing in Malaysia. Anthelmintic resistance is when the drug no longer works because the worms have developed a genetically based tolerance to the drug. This should not be confused with the term 'resistance' when applied to animals, which signifies natural immunity of the animal (generally breed of animal) to parasite infection. Assessment of the presence of anthelmintic resistance in the field is usually based on the faecal egg count reduction test (FECRT). However, due to the serious problem of anthelmintic resistance, particularly in Malaysia (Chandrawathani *et al.*, 2004) and other countries, there is growing demand for alternative non-chemical methods of parasite control to reduce the dependence on these drugs.

The alternatives to chemotherapy that are used in the control of helminthosis of sheep and goats, that are appropriate to Malaysian conditions, include pasture rotation (Chandrawathani *et al.*, 1995), breeding of sheep and goats with natural resistance to nematodes (Sani *et al.* 2000), biological control (Chandrawathani *et al.*,1998; 2002), and the use of medicated urea-molasses blocks (Sani *et al.*, 1995). Monitoring of faecal egg counts, through the services provided by the Veterinary Research Institute and other government veterinary laboratories, is conducted routinely in Malaysia to determine the level of worm infection in small ruminant flocks and to recommend when anthelmintic treatment is advisable.

FAMACHA©, is a new tool introduced by South African parasitologists (Bath *et al.*, 2001) to assess the presence of clinical anaemia in small ruminants. It is a system whereby the lower eyelid of the animal is examined and decisions on whether, or not to treat with anthelmintic, is based on the colour of the ocular mucous membrane. The technique is very easy to conduct and sufficiently reliable once learned under the guidance of a competent instructor and can be used by the farmer. It is used to monitor the anaemia status of animals infected particularly with *H. contortus*. This technique was conducted during the course of the trial outlined below.

Exploitation of the possible anthelmintic potential of medicinal plants such as the Neem tree (*Azadirachta indica)* is also an area of active research interest. Two previous studies by Chandrawathani *et al.* (2000 & 2002) showed indications of an anthelmintic effect of Neem against nematode parasites of small ruminants. In 1992, the National Research Council (NRC) of the United States released a report entitled "Neem: A Tree for Solving Global Problems." The NRC Panel considers the Neem tree to be *sic:* 'one of the most promising of all plants, that it may eventually benefit every person on this planet. Probably no other plant yields as many strange and varied products, or has as many exploitable by-products.' Native to India, the Neem tree is a widely planted and naturalized evergreen tree found throughout Asia (National Research Council, 1992). Preliminary studies done by Chandrawathani *et al.* (2000) showed that feeding Neem foliage is safe, eco-friendly, cheap and palatable to sheep. *Ad libitum* feeding of fresh Neem leaves produced 82% reduction in worm eggs of the animals (Chandrawathani *et al.*, 2000) and a further trial on a limited number of sheep showed that Neem produced a significant reduction in worm burdens (Chandrawathani *et al.*, 2002). However, studies by Githiori *et al.* (2003) did not show any significant reduction of Neem extracts on helminth infections in mice. Thus, this study was designed to further investigate these preliminary observations of the effect of voluntary intake of Neem

leaves as a possible natural anthelmintic for use in small ruminants.

MATERIALS AND METHODS

Experimental Animals

This study was carried out on twelve adult sheep (Santa Ines cross-breeds) of mixed sexes and with an average weight of 16kg (± 1.8 kg), which were obtained from a government farm (PTH Gajah Mati, Kedah). Ten animals had high nematode faecal egg counts (e.p.g. > 1000), but two animals with low faecal egg counts (e.p.g. < 200) were artificially infected with a total of 2,700 *H. contortus* larvae given orally (900 L3, three times on alternate days). Two weeks later, the animals were equally divided into control and treated groups, where each group consisted of six animals and the mean weights and faecal egg counts (FEC) of both groups were almost equal. One artificially infected animal was assigned to each group. The animals were housed throughout the study on raised-floor individual pens.

Fresh Neem leaves were collected from trees in the grounds of the Veterinary Research Institute (VRI) Ipoh. The leaves were separated from the stalks and fed fresh to the treated group daily. All the Neem leaves were weighed specifically for each animal according to individual body weight with the daily allowance of 3g/kg body weight. Palm kernel Cake (PKC) pellet was given to all animals only after the Neem treated animals finished eating their daily allocation. Water was provided for all the animals *ad libitum*.

Laboratory Analysis

Daily faecal samples (5 days / week) were collected from each animal in the morning and this was continued for 6 weeks. These samples were subjected to the McMaster faecal egg counting technique, using 3 g individual faecal samples (Christopher *et al*., 1992). Additionally, faecal cultures were prepared for infective larval recovery and differentiation of the nematode species according to the procedures described by Christopher *et al.* (1992).

FAMACHA monitoring

Daily (5 times/week) examination of the mucous membranes of the eye of each animal was conducted and the FAMACHA score between 1-5 was recorded.

Parasite Recovery and Enumeration

All animals were slaughtered 8 weeks after the commencement of monitoring, to recover their gastro-intestinal tracts. The total contents and washings of the abomasum, small intestine were separately collected in a total volume of 2 l water. Duplicate samples of aliquot size 1:100 (20 ml) were taken from the washings from the abomasum and small intestine from each lamb, following thorough stirring. These sub-samples were preserved by the addition of 2ml of concentrated formaldehyde until being examined under a stereo microscope for parasite species identification and enumeration.

Statistical Analysis

Statistical analysis was done on all faecal egg count and total worm count data using SPSS software. The tests conducted were univariate analysis of variance and the T-test.

RESULTS

Faecal egg Counts (FEC) and FAMACHA estimations

The mean FEC of the Neem treated and control groups remained almost the same until day 11, ranging from 3550 e.p.g to 5100 e.p.g. However, commencing from day 12 onwards, the Neem treated group (1160 – 3733 e.p.g) had lower mean FEC, compared to the control group (2140 – 11117 e.p.g), but these differences were not significant ($p = 0.081$).

In the Neem group, there was one animal that was not eating Neem and had to be replaced on Day 4. The faecal egg

count for this animal was 4700 epg and its FAMACHA score was 2.

One animal from the Control group animal (natural infection) died on Day 5, with a FAMACHA score 4, a faecal egg count of 14700 epg and a *H. contortus* worm count of 3133 parasites. A replacement was made for this animal in the Control group. Additionally on Day 9 another animal (natural infection) in the Control group had a FEC of 1200 epg, a FAMACHA score of 3 and it showed symptoms of clinical haemonchosis (anemia, bottle jaw). It was treated with ivermectin and replaced by another sheep with a faecal egg count of 800 epg and FAMACHA score was 2. Furthermore, in the 6th week of the trial, one animal from the Control group died (FAMACHA score 5) and post mortem indicated anemia and pneumonia. The terminal faecal egg count of this animal was 12600 epg and it had a burden of 6260 *H. contortus*. This animal was still eating and had no symptoms anaemia, hence it was not detected in sufficient time to administer a salvage anthelmintic treatment. Throughout the trial, faecal cultures to produce infective nematode larvae, showed that *H. contortus* was the most dominant species *(*96.2 %), however *Trichostrongylus* spp *(*0.8 %) and *Oesophagostomum* spp. (3 %) larvae were also found, for both the Control and Neem treated groups.

Daily FAMACHA monitoring showed that in the Control group the range score recorded was between 3 to 5 and the Neem treatment group was between 2 to 3.

Total worm counts

H. contortus was the only nematode parasite found in the abomasum of all animals. Statistical analysis of the *H. contortus* counts showed that there were less worms in the Neem group (1004), compared with the Control group (3242), which was highly significantly different (p=0.003). In the small intestine and large intestine, the species of worms recorded were only *Trichostrongylus* spp. and *Oesophagostomum* spp. respectively. Similarly, the differences between the worm burdens of the small intestines (p=0.045) and large intestines (p=0.048)

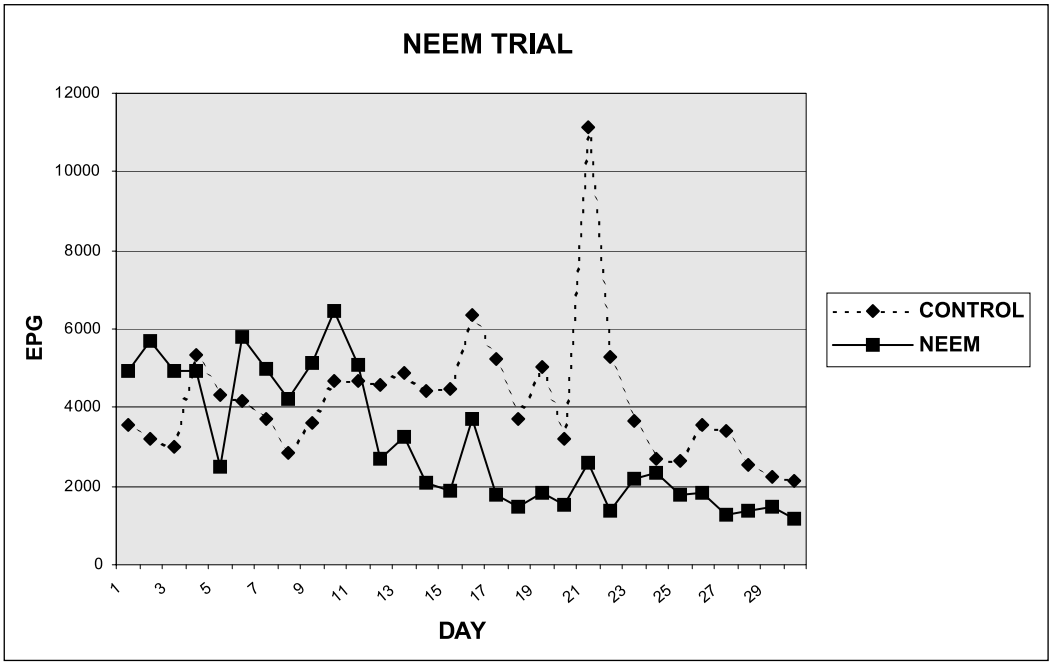

Figure 1. Mean nematode faecal egg counts (e.p.g.) of the Control and Neem fed (Treated) group.

Table 1. Number of worms in each individual animal in the Control and Neem fed (Treated) group

Control Animal no.	ABO. *(H. contortus)*	SI (*Trichostrongylus* spp.)	LI (*Oesophagostomum* spp.)
AE 403	602	11	92
SE 363	336	108	16
SE 332	1012	35	36
AE 431	572	10	4
AE 323	576	172	32
TOTAL	3242*	424*	188*
Neem FedAnimal No.			
AE 281	276	12	12
AE 220	60	0	12
AE 328	468	0	4
AE 257	12	108	0
TOTAL	1004*	120*	28*

* significantly different.

were shown to be significant ($p<0.05$). This finding complies with the larval differentiation done earlier on the faecal cultures. Results are presented in Table 1.

DISCUSSION

The aim of this study was to evaluate the effectiveness of fresh Neem leaves as a possible natural anthelmintic agent for sheep. Previous studies by Chandrawathani *et al.* (2000 & 2002) found that there was a reduction in faecal egg counts and worm burdens in animals fed with fresh Neem leaves, however the number of animals used in these two studies was too few to make categorical judgments. In the study reported here, it was found that although the number of worms were less in animals fed with fresh Neem leaves, there was no statistical differences in terms of the worm egg count in the faeces of these animals, despite the fact that from day 11 onwards the mean egg counts of the Neem group were consistently less than the Controls. The failure to detect a significant difference between the two groups almost certainly would be due to the fact that half of the original animals (3/6) in the Control group succumbed to the effects of haemonchosis (ie. 2 deaths and 1 salvage treatment), and the terminal egg counts and worm burdens of these three animals were the highest recorded in this trial. Other factors which are likely to play a role are the inherent great variability in faecal egg counts in animals infected with *H.contortus*, which results in the difficulty to statistically detect differences, and compensatory egg production whereby there is an inverse relationship between numbers of adult female parasites and their individual egg production which has been reported with *H. contortus* infections in sheep (Brelin, 2002). A study conducted by Khadijah *et al.* (2005) and Wong *et al.* (2005) on the use of fresh Neem, and pelleted Neem, also showed no significant difference in faecal egg counts, compared with control sheep, although the control sheep had higher mean faecal egg counts. Again, the number of animals (6 per group) was small and the experimental animals were not necropsied for worm recovery and enumeration.

The distribution of the worm species in the worm count estimates, correlated with the larvae differentiation from the faecal cultures of sheep used in this trial. Three

species of worms found were *H. contortus*, *Trichostrongylus* spp. and *Oesophagostomum* spp, in order of abundance. In the Neem group, *H. contortus* worm burdens were significantly less than in the Control group ($p = 0.003$). It was also found that the mean number of worms in the small and large intestines of animals fed with Neem were significantly lower ($p<0.05$), compared to the Control group.

herefore from these findings, it can be concluded that fresh Neem foliage fed to sheep has the ability to reduce their worm infections in sheep. Most significantly, it is apparent that the highly pathogenic parasite, *H.contortus* appears particularly sensitive to the intake of fresh Neem leaves by the animal.

Importantly, it was also found that Neem leaves were palatable to the sheep and they readily consumed their daily ration. The variability in faecal egg counts and worm counts within the Neem fed group could be due to differences in terms of physiological conditions of each animal and individual ability to utilize the medicinal properties of Neem. With respect to the FAMACHA score, it was noted that the Control group had higher scores (denoting anaemia), compared to the treated animals. Also the Control animals succumbed more to the effects of haemonchosis, with 2 animals dying and 1 salvage treated with anthelmintic, which had to be replaced in the trial. This shows the severity of haemonchosis in sheep if untreated.

In this study, although we were unable to prove a major anthelmintic effect of Neem (*A. indica*) against both the faecal egg counts and worm counts, it was found that this plant was beneficial against internal parasites of sheep. Pharmacological investigations should be conducted on Neem in order to understand the active anthelmintic principles possessed by this plant. Further studies need to be performed on different cultivars and times of harvesting of Neem leaves to determine whether the results reported in this investigation are consistent. In addition, trials in which Neem constitutes only a very small proportion of the diet, and the effect of long-term, or permanent feeding of Neem to both sheep and goats would be highly worthwhile. There is a degree of urgency with which this work needs to be continued in Malaysia. Current studies show that the prevalence of high level multiple resistance in *H. contortus* is widespread (Chandrawathani *et al.*, 2006). Thus, with the inability of modern anthelmintics to control haemonchosis due to resistance, the small ruminant industry in Malaysia may collapse, unless alternative methods of control, such as herbal remedies, are found to alleviate this situation.

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