The effect of wattle tannin drench or an *acacia* meal supplement on faecal egg counts and total worm burdens of tropical sheep with an experimental nematode infection[1]

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Abstract

Following on from our studies on the potential use of tannins to control intestinal parasites in small ruminants we recently conducted two further trials to investigate the effect of a wattle tannin oral drench (WT), or supplementation of the diet with *acacia* (AMS), on faecal egg counts (FEC) and worm burdens of growing tropical Black Head Persian (BHP) sheep with an experimental intestinal nematode infection. In trial 1, 28 rams infected with a single dose of 1,500 mixed nematode larvae per 20 kg body weight were used. Faecal egg counts (FEC) were monitored regularly throughout the study. On day 30 post-infection, the infected sheep were blocked on the basis of their egg numbers and randomly assigned into two equal groups. For three consecutive days one group received a tannin drench at 1.5 g WT/kg body weight, and the remaining group received a placebo. All sheep were humanely slaughtered on day 42 post-infection and their worm burdens were assessed. Worm burdens and FEC were reduced (P<0.001) by the drench administration. Faecal mucus and water contents were increased. Trial 2 used another 28 infected growing BHP rams and had an identical design to trial 1, except that from day 30 post-infection animals in one group of 14 rams were offered 150–170 g of AMS daily, whereas in the other group they were offered *Pannicum* meal as control. On day 60 all animals were slaughtered for worm burden estimation. There was only a slight reduction in FEC (19 per cent) but none in the worm burdens.

The first trial demonstrated that the WT drench had significant activity against important nematodes of tropical sheep, as opposed to its small effects in infected tropical goats reported previously, suggesting that there are species differences between the two hosts. Feeding the *acacia* meal (trial 2), which is high in tannins, appeared to have little effect.

Introduction

One of the important constraints to small ruminant productivity is infection caused by parasitic nematodes of the gastrointestinal tract (Parkins and Holmes, 1989; Gill and Le Jambre, 1996). These infections cause significant losses in terms of poor growth, reduced

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reproductive performance and mortality. Nematode control has traditionally been through using synthetic anthelmintics. However, over-dependency and even misuse of the anthelmintics has resulted in the emergence and spread of nematode populations that are resistant to most anthelmintics (Prichard, 1994). This has led to increases in the cost of control through higher dosages and frequency of treatments (Over *et al.*, 1992). Moreover, anthelmintics are expensive and not affordable to many resource-poor farmers in the developing countries. There is, therefore, a need to search for cheap and sustainable nematode control alternatives. One such alternative could be the use of plants and plant products with anthelmintic activities. Some field studies in the temperate region have shown that forages rich in condensed tannins (CT) can improve the general performance of sheep with nematode infestations (Niezen *et al.*, 1993). These promising results have stimulated considerable research interest on the effect of tannins on different nematodes, particularly those of small ruminants. Previous studies have shown that dietary inclusion of the CT in quebracho extract (QT) could significantly reduce FEC and worm burdens of temperate sheep infected with *Trichostrongylus colubriformis* (Butter *et al.*, 2000; Athanasiadou *et al.*, 2000). Administration of QT as an oral drench was shown to be effective in reducing worm burdens of temperate sheep infected with *Haemonchus contortus* and *T. colubriformis* (Max *et al.*, 2002). However, similar studies with tropical goats using wattle tannin (WT) (Max *et al.*, 2003) revealed no significant reduction in FEC or worm burdens. It was argued that the apparent differences between sheep and goats could either be a species difference or rather an adaptation of the animals to tannins prior to the experimental study. A survey of local plants was undertaken in Tanzania and *Acacia polyacantha* was found to contain significant quantities of condensed tannin. In another study, (Max *et al.*, 2003) supplementation of worm-infected Small East African (SEA) goats with 130 g of tanniniferous browse (*A. polyacantha*) meal for 20 days gave 27 per cent and 13 per cent reduction in FEC and worm burdens respectively. It was suggested that the anthelmintic effect could be further enhanced if the animals were offered more of the *acacia* leaf meal. Two trials were, therefore, carried out with to investigate the effect of WT drench or *ad libitum* access to *acacia* leaf meal on FEC and worm burdens of growing tropical sheep experimentally infected with mixed nematodes.

Materials and methods

Study site and experimental animals

The two trials were carried out concurrently at Sokoine University of Agriculture, Morogoro, Tanzania. Sixty growing Black Head Persian (BHP) rams, 12 to 14 months of age and weighing 18.7 ± 2.1 kg, were purchased from a ranch in West Kilimanjaro, Northern Tanzania and were housed in individual wooden pens with raised floors. The sheep received a single oral dose of anthelmintic (Levacide®, Norbrook Africa Ltd., Kenya: Levamisole, at 7.5 mg/kg body weight) to clear any nematode infestations. An oral coccidiostat (Trisulmix®, Coophavet Ltd, France: Sulfadimethoxine–trimethoprim, at 20 mg and 4 mg/kg body weight respectively) was given weekly as a prophylaxis since coccidiosis was endemic in the trial area. The sheep were offered *ad libitum* a chopped mixture of *Chloris gayana* and *Brachiaria brizantha* hay and supplemented with 120 g/day of a maize bran-based concentrate (75 per cent maize bran, 24 per cent cottonseed cake and one per cent minerals). Body weights were monitored weekly throughout the trial.

Experimental infection

Faeces from a naturally infested goat were cultured by a standard Baermann technique to give infective stage larvae (L_3). The latter were suspended in distilled water at 4 °C before they were identified to genus level and the proportion of each genus per unit volume of suspension was determined. Four nematode genera were identified as *Haemonchus* (76 per cent), *Oesophagostomum* (16 per cent), *Trichostrongylus* (6 per cent) and *Cooperia* (2 per cent). This proportion agrees well with the epidemiological distribution of these worms within a tropical environment (Urquhart *et al.*, 1987), which indicates *Haemonchus* as the most predominant genus. On a day referred to as Day 1, 56 sheep were orally infected with a single dose of 1500 L3 per 20 kg body weight. The FEC were monitored regularly using the modified McMaster technique from Day 14 to the end of the trial. The sheep were blocked using FEC taken on Day 29 and then randomly assigned to four equal groups (n=14/group). Twenty eight sheep (2 groups) were used for the wattle tannin drench trial and 28 (2 groups) were used for the *acacia* feeding trial. Four sheep were left as an uninfected control that would help to monitor any extraneous source of worm infestation. No eggs were observed in the faeces of the four negative control sheep, a proof that there was no extraneous worm infestation.

Wattle tannin drenching trial (trial 1)

Two groups were used in the WT drench trial. One part of WT powder (Tanzania Wattle Co. Ltd.) was dissolved in two parts of lukewarm water to make a drenching solution. On Day 30, one group (drenched) received the drench orally at 1.5 g WT kg^{-1} body weight daily for three consecutive days, whereas the other group (infected control) received water as a placebo. Any feed refusals were collected and weighed; the data were used for determination of feed consumption. Faecal material consistency and water content were estimated by visual examination and overnight drying of a sample of faecal material respectively. All 28 sheep were humanely slaughtered ten days later (Day 43) for estimation of worm burdens, as described by Dawson *et al.*, (1999).

Acacia meal supplement trial (trial 2)

Starting from Day 30, one infected group (14 sheep) was supplemented daily with a mixture of the concentrate and 150–170 g of dried leaves of *Acacia polyacantha* (CT=175 g/kg DM, determined using the acid butanol method, see Porter *et al.*, 1986). The infected control group (14 sheep) was supplemented with dried green *Panicum trichocladum* grass (CT=3 g/kg DM); the latter was chosen because it contains a very low concentration of CT and its crude protein content is comparable with that of *A. polyacantha*. Feed refusals were collected for determination of feed consumption. Faecal samples were collected for determination of faecal egg counts. The trial was terminated after 30 days of *acacia* meal supplementation (i.e., on Day 60) by slaughtering all the animals, from which samples were taken for estimations of worm burdens.

Data analysis

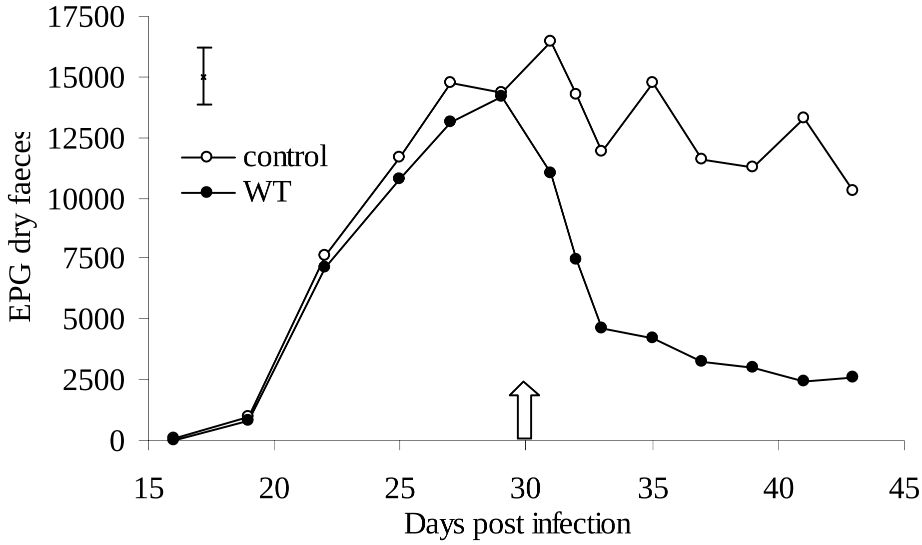
For both experiments, the data were analysed using a statistical package, Genstat 6.1 (Lawes Agricultural Trust, 2002). The FEC were square-root transformed to normalise the data prior to analysis. All the data were analysed as a completely randomised experiment using one-way analysis of variance (ANOVA) for repeated measures where individual animals were used as blocks. The impact of the drench on FEC, feed consumption and faecal water content was measured using data taken a day after the onset of treatment to the day of slaughter inclusively. Where results are presented as percentage change in egg counts, these are based on the back-transformed means from the analyses of the square root transformed data.

Results and discussion

Effect of WT drench on FEC and worm burdens

The effect of WT drench on FEC and worm burdens in the sheep is shown in Figures 1a and 1b, respectively. The FEC of sheep drenched with WT solution was significantly (P<0.001) reduced and the effect was recorded within 24 hours of the first dose and remained low to the day of slaughter (Day 43). Comparison of FEC between the drenched and control group on the day of slaughter showed a reduction of 75 per cent.

a)



b)

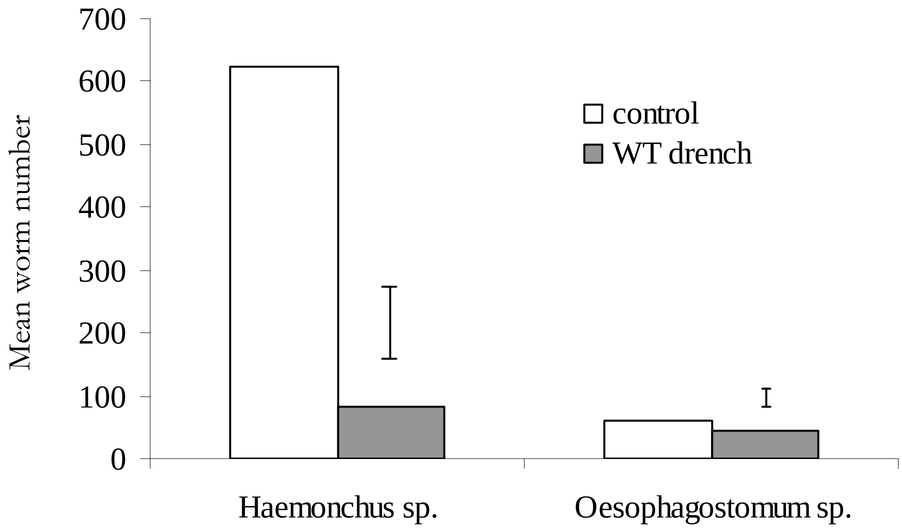
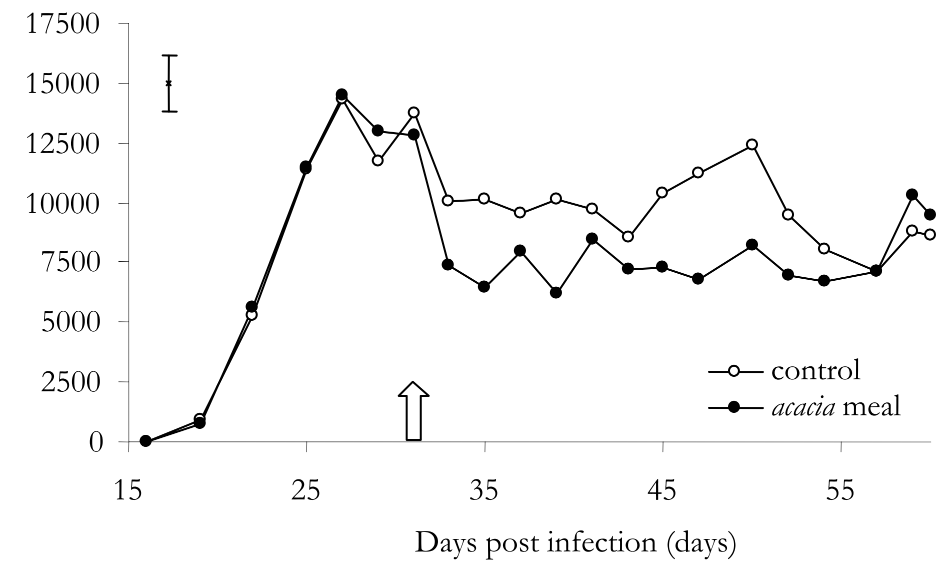


Figure 1 *The effect of wattle (WT) drench on faecal egg counts (FEC) (a) and worm burdens (b) of tropical sheep following its administration for three consecutive days at a dose of 1.5 g WT/kg body weight. The arrow (in Figure a) indicates the onset of WT drench administration on Day 30 post-infection. (Error bars represent s.e.d., EPG=eggs per gram).*

a)



b)

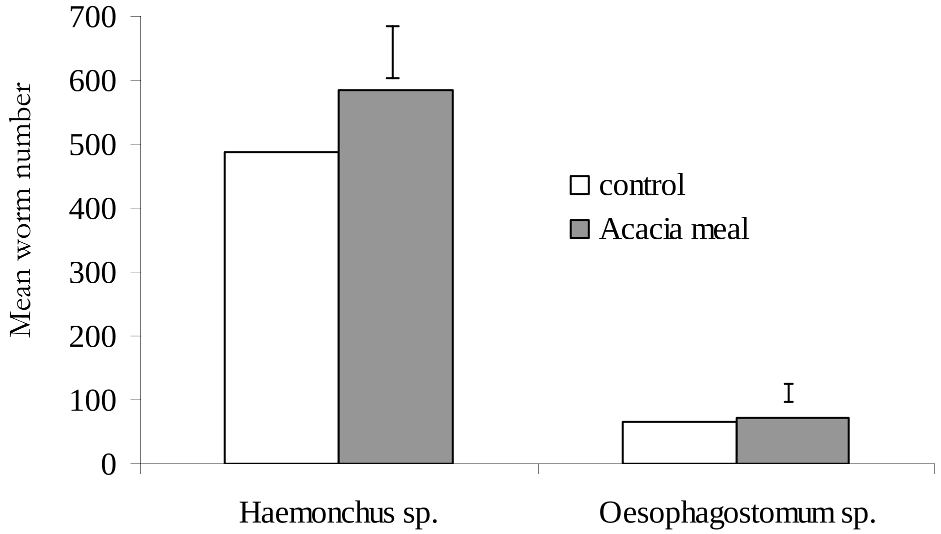


Figure 2 *Effect of acacia meal supplement (150–170g acacia/day for 30 days) on faecal egg count (FEC) (a) and worm burdens (b) of tropical sheep with mixed experimental infections. The arrow (Figure a) shows the onset of treatment on Day 30 post-infection). (Error bars represent s.e.d.; EPG=eggs per gram).*

The effect of the drench on worm burdens was also significant (*P*<0.001); the populations of *Haemonchus* and *Oesophagostomum* were reduced by 87 per cent and 28 per cent, respectively. The recovery of the small intestinal species, *Trichostrongylus* and *Cooperia* was too low for any meaningful statistical comparison (data not shown). The current results contrast with previous findings (Max *et al.*, 2003), in which WT drench did not affect FEC or worm burdens of Small East African (SEA) goats even though a higher WT dose rate was used. This is a strong indication that as far as the effect of tannins on gastrointestinal nematodes are concerned, a species difference does exist between sheep and goats, and differences are not just an adaptation to dietary tannins. Goats, deer and antelopes are among several browsing animal species which can be induced by dietary tannins to produce tannin-binding saliva (Mehansho *et al.*, 1983). This mechanism is lacking, or poorly developed, in other species including sheep, cattle and hamsters (Austin *et al.*, 1989). It has also been suggested that goats are more adapted than sheep to the detrimental effects of CT because of microbial tanninase enzymes in their rumen (Perez-Maldonado and Norton, 1996). Goats are also known to harbour tannin-tolerant bacteria in their rumen; for example *Streptococcus caprinus* have been isolated from feral (Brooker *et al.*, 1994) and SEA goats (Odenyo and Osuji, 1998). The *S. caprinus* was shown to grow in media containing at least 2.5 per cent w/v tannic acid or CT and able to degrade mimosine, tannic acid-protein complexes as well as hydrolysable tannins (Brooker *et al.*, 1994). With these mechanisms in action, it is possible that tannins administered to goats were neutralised or inactivated before they can reach, or act, on the nematodes. As in goats, the WT drench also significantly reduced feed consumption and increased faecal water and mucus contents. This suggests that the effect of drench was not largely resulting from the physiological gut change as previously thought (Max *et al.,* 2003), but rather a direct toxicity to the worms.

Effect of AMS on FEC and worm burdens

Results of the *acacia* meal trial are shown in Figures 2a and 2b respectively. The meal, dried leaves of *A. polyacantha* (CT=175 g/kg DM) was generally accepted by the sheep with the exception of four sheep that consumed an average of 30 to 50 per cent of the supplemented meal throughout the trial. Supplementing the sheep with *A. polyacantha* for 30 days did not significantly reduce (P>0.05) FEC. However, as the egg profiles indicate, sheep receiving the supplement shed fewer eggs than the control sheep; this was equivalent to an average reduction of 19 per cent between days 31 and 60. Unexpectedly, this slight reduction was not reflected by the worm burden results since the *acacia*-fed sheep had, on average, higher worm burdens. This could possibly be an indication that *acacia* meal compromised the egg lying capacity (fecundity) of the worms. The effect of *acacia* meal supplement on nematode infection did not seem to differ much between tropical sheep and goats (Max *et al.*, 2003) despite the fact that the sheep had *ad libitum* access to the supplement for 10 days longer and were very much more responsive to the WT drench.

Conclusions

The WT drench showed significant anthelmintic activity against parasitic nematodes of sheep raised in a tropical environment. When similar studies were conducted previously (Max *et al.*, 2003) with goats little anthelmintic activity was noted. Further on-farm work is required to validate the use of tannin preparations in reducing nematode infections of sheep and other domestic ruminants known to have no tannin-neutralising mechanism, for example cattle. The practical implication of these observations on the effect of tannins on nematodes of small ruminants is that tannin preparations, which are cheap and readily available in the tropics, could be used to supplement the use of expensive drugs to control intestinal nematode infections in sheep.

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