The potential of controlling gastrointestinal parasitic infections in tropical small ruminants using plants high in tannins or extracts from them

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Abstract

A series of previous studies with quebracho tannin (QT) had shown that the tannin was effective at reducing the worm burden of temperate sheep infected with *Haemonchus* and *Trichostrongylus*. The potential of using tanniniferous browse plants available in the tropics as a means to reduce or control nematode infections of small ruminants was, therefore, investigated. Leaves of different browse trees and shrubs were assayed for condensed tannin (CT) concentration using a colorimetric method. Five species with the highest levels were sampled monthly and analysed in order to map their seasonal variation in CT. The level of CT in the leaves ranged between 58 – 283g kg^{-1} DM. Purified quebracho tannin was used as the standard. Seasonal changes in CT levels were affected by the stage of leaf maturity with peak levels occurring after the end of main rain season in June. Anthelmintic activity of crude extracts from leaves of two of the plants shown to have a high tannin content (*Acacia polyacantha* and *Tamarindus indica*) and that of commercial tannin preparation from wattle tree (WT) was then tested *in vitro* against freshly isolated goat nematodes. Time of death of the parasites was recorded. Survival of the nematodes was significantly ($P<0.001$) reduced by both leaf extracts and by the WT.

Acacia sp. leaves were then feed to goats to investigate their effect on faecal egg output and worm burdens of animals with a mixed nematode infection. Twenty-four bucks were infected with a single dose of nematode larvae and faecal egg counts (FEC) were monitored regularly. On day 38 post-infection (p.i.), half of the goats were offered a supplement of dried acacia leaves at 130g per animal for 20 days while the remaining half (control) received a grass supplement with comparable nutritional value but without condensed tannins. All goats were humanely slaughtered at the end of the trial for worm burden estimation. Mean FEC of the acacia-fed group was 27 per cent lower with a slight reduction (13 per cent) in the population of the large intestine worm, *Oesophagostomum columbianum*, compared to the control group. In a second trial, 36 bucks were infected as before and then randomized on the basis of their FEC into three equal groups 30 days after infection. For three consecutive days one group received 1.2g WT/kg body weight, one 2.4g WT/kg and the third group received a placebo drench. All goats were humanely slaughtered on day 42 p.i. Neither FEC nor worm burdens were significantly reduced by the drench administration. This is in contrast to studies with quebracho tannin and temperate sheep conducted earlier.

Studies are also required using sheep reared in the tropics to determine whether the apparent species differences are real rather than due to adaptation of the animals to tannins prior to the experimental study.

It is not possible yet to come to a definitive conclusion on the value of tannins in controlling parasitic infections in tropical small ruminants.

Introduction

Gastrointestinal (GI) parasites are responsible for significant production losses in livestock worldwide (Gill and LeJambre, 1996) particularly under tropical and subtropical climates (Waller, 1997). Marginal levels of nutrition and a climate that favours survival of the parasites in most of the year explains why GI infections are more devastating in these regions (Waller, 1997). Control of GI nematode infections has traditionally been done using anthelmintics (chemotherapy) with best results being obtained when this approach is integrated with proper grazing management and resistant animals. However, in the last 2 - 3 decades there has been over-dependency and even misuse of the chemotherapeutic approach with consequent evolution of anthelmintic resistance (Ngomuo *et al.*, 1990; Prichard, 1994). This is especially true among major nematode species of small ruminants. Apart from anthelmintic resistance, poor availability and affordability of anthelmintics to resource-poor farmers in developing countries have compounded the problem (Hammond *et al.*, 1997). Moreover, there is a growing concern over drug residues in the food chain and the environment. Search for novel anthelmintics that are both more sustainable and environmental friendly is undoubtedly a sensible approach to the control of parasitic infections. One such alternative could be harnessing of the available ethnoveterinary knowledge (Hammond *et al.*, 1997), i.e., the use of medicinal plants with anthelmintic activity. Plant anthelmintics have been known and used in many parts of the world for a long time but little research has been done to validate their use, especially in veterinary medicine. Forages rich in condensed tannins (CT) have been found to improve general performance of parasitised sheep through reduced worm burdens (Niezen *et al.*, 1993; 1998). Furthermore, our recent studies have shown that dietary inclusion of CT in quebracho extract dramatically reduced egg output and worm burdens of sheep infected with *T. colubriformis* (Butter *et al.*, 2000). This has been confirmed by other workers (eg. Athanasiadou *et al.*, 2000). As reported to the Morogoro workshop (Max *et al.,* 2002), an oral drench of QT is effective at reducing both faecal egg counts and worm burdens in sheep infected with *H. contortus*. Some effect was also noted on *T. colubriformis* infection. *In vitro* studies had also shown that the wattle tannin extract available in Tanzania also had antihelmintic properties.

The main objective of the current phase of project (R7424) was to determine whether locally available tanniniferous browse materials or readily available extracts from them could be used to control or reduce the impact of nematode infections in small ruminants under the conditions found in the tropics.

Materials and Methods

Location

The studies were conducted in Morogoro region, which experiences an equatorial type of climate with a bimodal rainfall pattern; a main wet season, usually from March – May and minor wet season from November to January. Browse trees and shrubs constitute the largest proportion of small ruminant feed especially during the long dry season because they are drought tolerant and readily available.

Tannin sources

Wattle (mimosa) extract (WT), a commercial preparation of tannin from the barks of the tropical tree, *Acacia mearnsii* was supplied by The Wattle Tannin Company, Tanzania. Wattle tannin is used in the leather industry. Drench solutions were prepared by dissolving 1 part of WT in 2 parts of lukewarm water (w/v). Solutions of varying concentrations were used in *in vitro* studies to determine the potency of WT against cultured parasites. Freshly prepared crude aqueous extracts from dried leaves of tropical browse trees, *Acacia polyacantha* and *Tamarindus indica*, were also used as another source of CT for the *in vitro* assays.

Test parasites

Three important caprine nematodes, *Haemonchus contortus*, *Trichostrongylus vitrinus* and *Oesophagostomum columbianum* were isolated and maintained as a mixed infection through passage in goats held at the Sokoine University of Agriculture. Faeces from the passage goats were cultured to obtain infective stage larvae (L3). The L3 were suspended in distilled water and kept at 4 – 8 °C in plastic tubes until used (maximum of 2 weeks).

Animals, housing and feed

Small East African (SEA) entire bucks aged between 12 and 14 months were purchased from small-scale goat keepers in Morogoro, Tanzania and housed in individual, raised-floor, wooden pens. The goats were offered a daily allowance of urea- and molasses-treated rice straw and supplemented with 150g maize bran-based concentrate. Once in the experimental house, the animals received a single oral dose of broad-spectrum anthelmintic to clear any gastrointestinal nematode infestations and sprayed with acaricide to rid them of ectoparasites. Due to the endemic nature of coccidiosis in the area of experimentation, a coccidiostat was given regularly as prophylaxis. Body weights were monitored weekly to the end of the trial.

Determination of the concentration and seasonal variation of condensed tannin content of selected browse plants

Browse plants including trees and shrubs from Morogoro were used in the study. Pilot sampling was conducted in January, April and June 2000 to represent the end of the dry season and the onset of the rain season and cool season respectively. These samples were analyzed to shortlist the selected browse plants to five species with the highest tannin content. Briefly, about 1 kg of twigs, 10 - 15 cm from the branch tip were harvested from different branches of a mature tree or a shrub. The leaves and leaflets were separated from the twigs and dried in an oven at 55 °C to a constant weight. The dried leaves were ground to pass through a 1-mm screen and then stored in clean airtight glass jars at room temperature pending laboratory analysis. Concentration of CT in the plant samples was assessed using the acid-butanol method (HCl-butanol-iron) as described by Terrill *et al.*, (1992). Since quebracho tannin was used as a standard, the final concentrations are expressed as quebracho tannin equivalents. It should be noted that due to the complexity of tannin molecules accurate determination of CT is difficult and the acid-butanol method was chosen because its relative simplicity makes it possible to rapidly handle a large number of samples.

***In vitro* anthelmintic activity of browse plant extracts and WT on goat nematodes**

(a) Worm recovery

A goat parasitised with both abomasal and large intestinal nematodes (*H. contortus* and *Oe. columbianum* respectively) was humanely slaughtered each time the survival assay was undertaken. On slaughter, the entire gastrointestinal tract was removed and ligatures were applied to separate the abomasum and the large intestine. Contents of each compartment were processed separately to recover the live worms. The recovered worms were placed on a Petri dish containing lukewarm phosphate buffered saline (PBS). The entire procedure was carried out quickly to ensure that worms were not excessively exhausted prior to incubation.

(b) Preparation of culture media and survival assay

Dried leaves of *A. polyacantha* and *T. indica* were ground to pass through 1-mm mesh. Five grammes of the leaf powder were placed in a 50-ml plastic tube; PBS was added to give a 10 per cent w/v extract solution and left to soak for 90 minutes with regular shaking. The mixture was passed through a coffee strainer to make clear culture media for each plant species. Equal amounts of acacia and tamarind leaf powders were thoroughly mixed and then soaked as before to make a 10 per cent solution of mixed culture medium. Preparation of culture media containing wattle extract was carried out using lukewarm PBS to give 0, 2, 4, 8 and 12 per cent w/v concentrations. To about 20 ml of the culture medium in a Petri dish, known number of male and female worms (approximately 10 - 15 each) were placed, covered and incubated at 38 - 39°C for a period of 10 hours. Survival rates were recorded over different time intervals. Motility and viability of the parasites was assessed by gently prodding the worms using a pointed probe or forceps. The response was recorded as either live or dead. Worms were considered dead when a minimum reaction to touch was observed.

Determination of the effect of tanniniferous browse on FEC and worm burdens of goats with mixed nematode infection

(a) Collection and preparation of supplemental leaves

A. polyacantha leaves were collected in June 2002, approximately a month after the end of main wet season. Leafy twigs, 10 to 15 cm from the tip were removed from main branches and sun-dried within 24 hours. The dry twigs were stamped using sticks and then passed through wire gauze to separate the leaflets from stalks and petioles. The dry leaflets were kept in a cool dry place until needed. To balance for the nutritive value discrepancy between browse-fed and the control group *Panicum trichocladum* (donkey grass) leaves were used. *Panicum* sp. was selected because it contains no condensed tannins and has a crude protein value comparable to that of *A. polyacantha*, it is palatable and was readily available during the trial. The dry *Panicum* sp. leaves were ground to pass through 2-mm screen and kept as before. Samples from the two species were taken for estimation of CT and crude protein content.

(b) Experimental design

After two weeks of acclimatization to the experimental environment, 24 bucks live-weight 15.8 ± 2.4 kg were infected using a single oral dose of the mixed nematode infective stage larvae at a rate of 2000 larvae per 20 kg body weight. Faecal egg outputs were monitored regularly and on day 38 post infection the goats were randomly allocated into two equal groups (n = 12). Animals in one group, the browse-supplemented, received their daily concentrate allowance of 150g/animal/day in which 100g of dry *A. polyacantha* leaves were added. Animals in the control group were supplemented with 100 g/animal/day of dry ground grass leaves (*P. trichocladum*). The browse and control supplements were increased to 130g per day on day 44 post-infection as the goats became more used to eating the leaves. Any refusals were collected and weighed early in the morning before the daily allowance was offered. On day 59, all animals were humanely sacrificed and the abomasa, small and large intestines removed for worm burden assessment as described by Dawson *et al.* (1999).

Investigating the effect of WT drench on FEC and worm burden of goats with experimental mixed nematode infection

The effect of WT drench on FEC and worm burdens of tropical goats was investigates in a very similar experimental design. Thirty-six goats weighing 13.5 ± 2.2 kg were infected as before. Faecal egg counts were monitored regularly from day 16 post-infection to the end of the trial. On day 29 post-infection, the goats were blocked according to faecal egg numbers and randomized into three groups (n = 12). Two groups (LWT and HWT) received low and high doses of WT (1.2 and 2.4 g kg^{-1} body weight), respectively, whereas the third group (control) received a placebo drench (tap water). The goats were drenched for three consecutive days from day 30 to day 32 and allowed to rest for 9 days before they were slaughtered for worm burden estimation on day 42.

Statistical analysis

The effect of tannin concentration on the survival time of the worms was analysed by survival analysis for censored data using a statistical package, Genstat 6, Lawes Agricultural Trust, UK. A linear model was used to predict estimated mean survival times with their upper and lower confidence limits. Faecal egg output data were subjected to ANOVA as repeated measurements with treatment structures and individual animals as blocks. The impact of treatment on faecal egg output was measured using faecal egg counts taken a day after the first dose of treatment to the day of slaughter inclusive. Worm burden data were subjected to one-way ANOVA with individual animals as block structures. Differences were assumed significant at $P<0.05$.

Results and Discussion

Concentration and seasonal variation of foliar CT in selected browse plants

Figure 1 shows concentration and seasonal variation of CT in five browse species, which were selected for their high levels. *Acacia polyacantha* had the highest overall mean CT concentration (282.5g/kg^{-1} DM) whereas the lowest value (58.2 g/kg^{-1} DM) was measured in neem tree (*Azadirachta indica*) samples. The average levels of extractable CT reported here were very comparable to those usually found in tropical herbaceous legumes (Sotohy *et al*., 1997; Getachew *et al*., 2000). Variation in CT levels in the plants followed a regular pattern with the lowest concentrations being recorded in samples collected in November to February; the values increased gradually to a peak in the month of July before declining gradually toward November. Stage of leaf maturity was pointed out as an important factor determining the seasonal changes in CT concentration. The two leguminous tree species, *Acacia polyacantha* and *Tamarindus indica* (Tamarind) are widespread, not only in Morogoro region but country-wide and throughout tropical Africa with several varieties (Mbuya *et al*., 1994). The trees are well adapted to a range of soil types and they are leafy especially towards the end of the main wet season. The remaining three species relatively less abundant but yet widely distributed in the country especially in rural communities where they are used for medicinal (*Azadirachta indica*) and horticultural (*Psidium guajava* (guava) and *Persea americana* (avocado)) purposes (Mbuya *et al*., 1994).

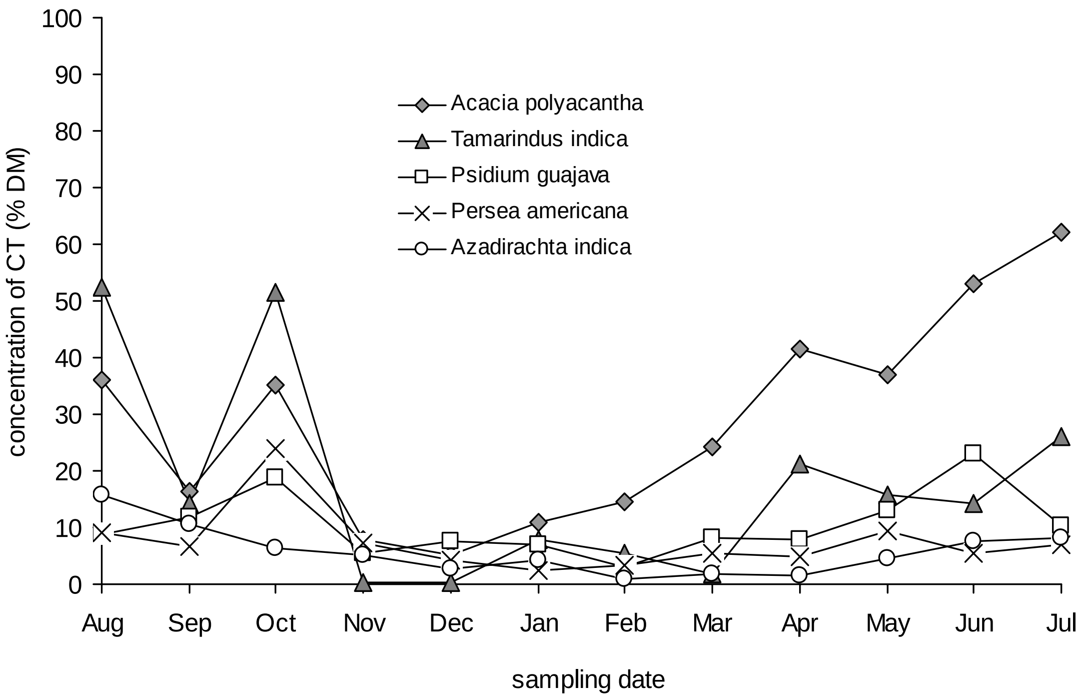

Figure 1 Seasonal change in foliar concentration of condensed tannins in selected browse trees. The values represent a mean of quadruplicate analysis of a pooled sample for the particular month.

It is accepted that the assay used may not have given an absolute value for the tannin content of the various leaves but rather an indication of the seasonal variation.

In vitro activity of crude leaf extracts and WT against goat nematodes

Reports about the use of parasitic stages of target nematodes to study anthelmintic activity of plant extracts *in vitro* are rare due to difficulties in obtaining and maintaining the parasites outside their hosts (Witty, 1999). The current findings indicate that *H. contortus* and *Oe. columbianum* adults can be recovered live and maintained in a simple culture medium for up to 16 hours. The survival of the parasites in culture media containing the crude leaf extracts and WT was significantly ($P<0.001$) reduced (Figures 2 and 3). The results suggest a direct anthelmintic activity of WT and crude extracts from acacia and tamarind. Similar results have been reported using QT against rat nematodes (Butter *et al.*, 2001) and purified tannins from four forages against ovine nematodes (Molan *et al.*, 2000). The possible increase in survival time when the parasites were incubated in culture media containing WT concentrations above 2 per cent might have been due to the astringency of extract which deterred the worms from ingesting the surrounding medium. Although the mechanisms involved in the toxicity of CT to nematodes are not known, recent studies studying the effect of ellagitannin preparations against the tree-living nematode, *Caenorhabditis elegans*, have shown fatal disruption of internal organs including the gonads, uterine wall and the intestines (Mori *et al.*, 2000).

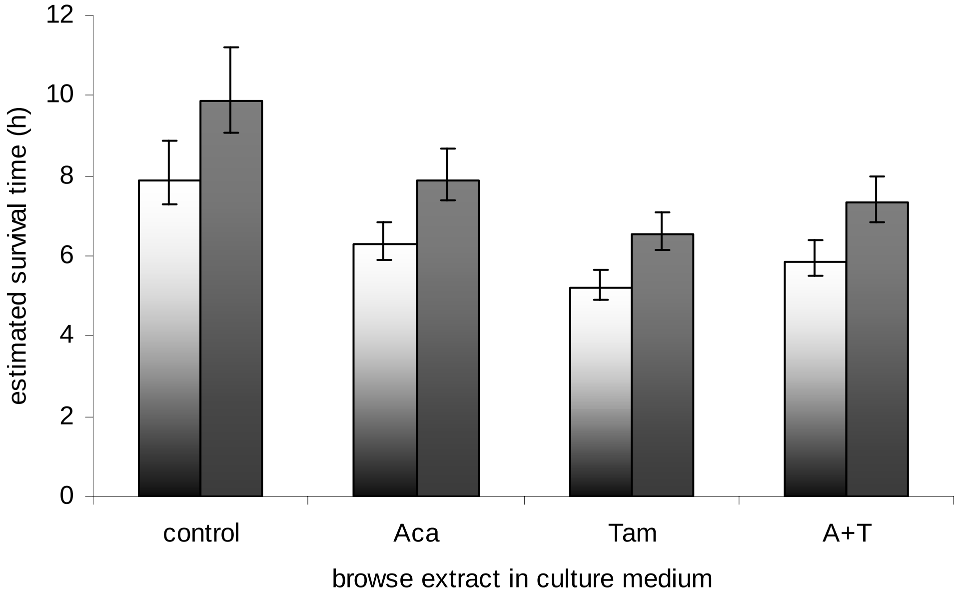

Figure 2 Survival of *H. contortus* in culture media containing 10 per cent (w/v) aqueous crude extracts from various browse plant leaves. (□) Males; (■) females; (Aca) acacia; (Tam) tamarind; (A+T) mixture of acacia and tamarind. Values are mean of triplicate assays.

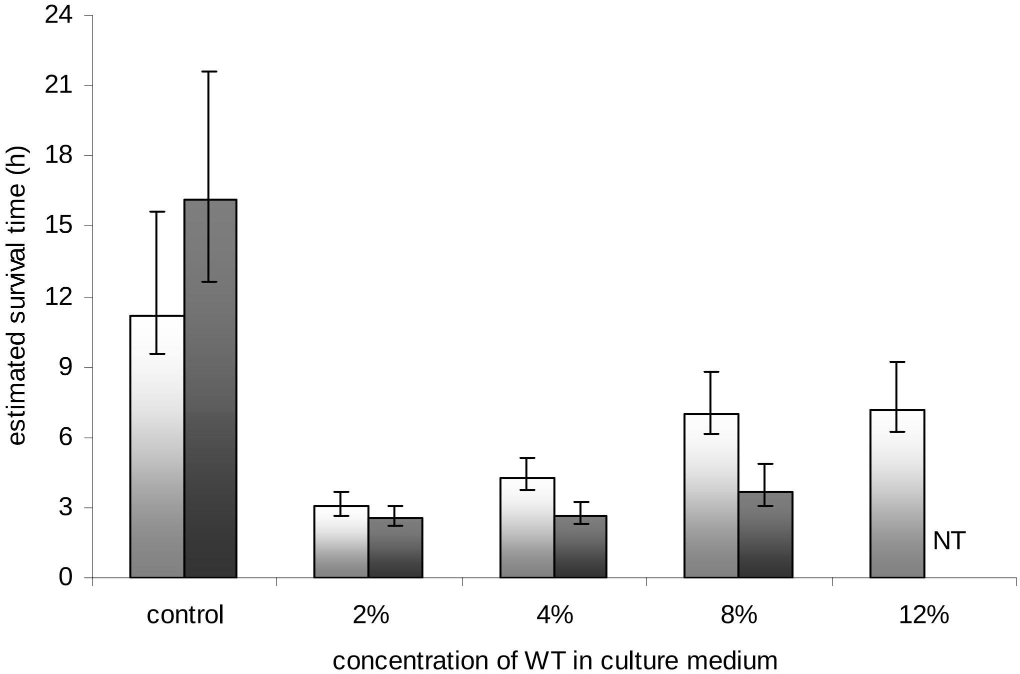

Figure 3 Survival of *H. contortus* (□) and *Oe. columbianum* (■) adults in culture media containing varying concentrations of wattle extract; (NT = not tested). The values are mean of quadruplicate assays; the error bars indicate 95 per cent upper and lower confidence limits.

Effect of a tanniniferous browse supplement on mixed nematode infection in tropical goats

With time, the goats accepted the browse supplement; a few refusals (involving concentrate-browse mixture) were observed in the browse-supplemented group during the first week of treatment introduction. Egg counts were first observed in faeces of most goats on day 21 after infection. Although supplementation of goats with dried *A. polyacantha* leaves for 20 days did not significantly (P>0.05) reduce FEC, the supplemented group had an average of 27 per cent fewer eggs than the control group from day 46 to the day of slaughter (Figure 4). Comparison of egg counts between the two groups on the day of slaughter alone showed a reduction of 33 per cent in the browse supplemented group. Similarly, no significant reductions in total worm burdens of the treated group but a slight reduction (13 per cent) in worm burden of the large intestine dweller, *Oesophagostomum* sp. (means: control vs. supplemented, 273 and 238 worms respectively; pooled SED: 32) was observed (Figure 5). Significant drops in both FEC and worm burdens following consumption of tannin-rich browse have been observed in a similar study (Kabasa *et al.*, 2000) but unlike the current trial, the goats were allowed free access to various browse plants for up to 6 months. In the current study, the control group tended to void wetter faeces than the browse-fed group (treatment x time interaction, P=0.041).

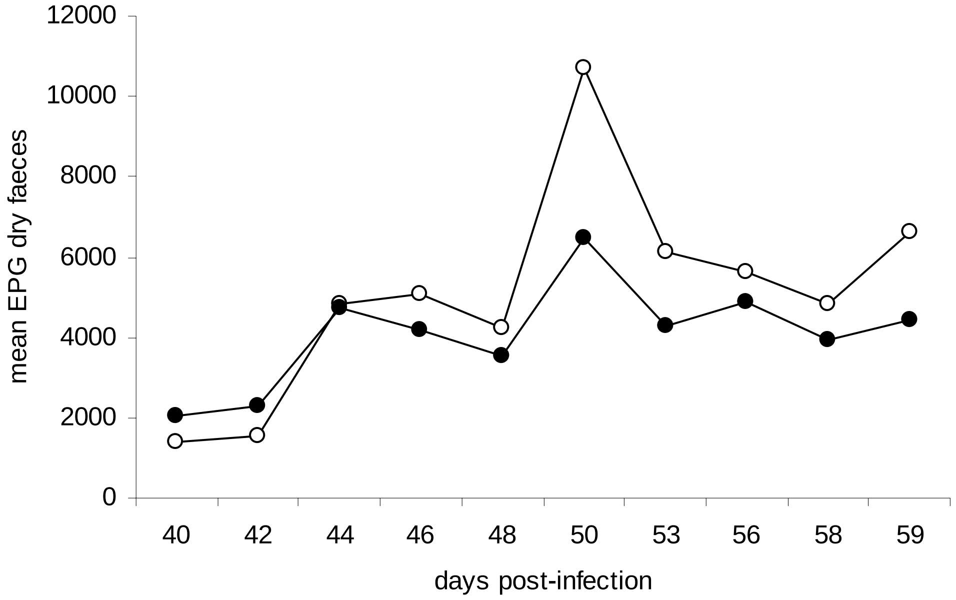

Figure 4: Effect of *Acacia polyacantha* leaf supplement on faecal egg outputs (eggs per gram (EPG) dry faeces) of goats with mixed nematode infection. Each goat in the supplemented group (●) received acacia leaves at 130g/day from day 44 to the end of trial. The control group (○) was offered a similar amount of grass supplement. (SED = 1355, df = 22).

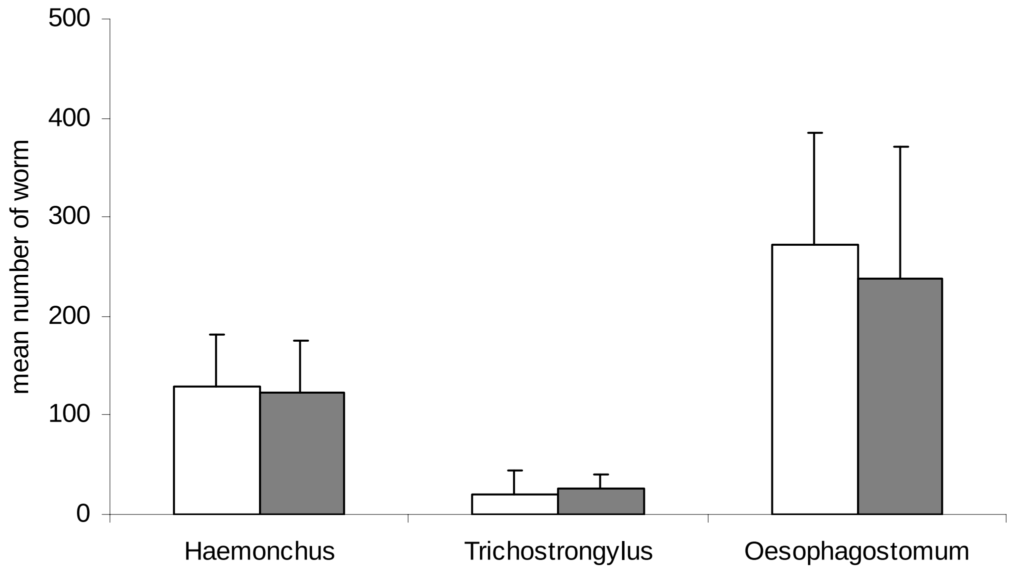

Figure 5 Effect of *Acacia polyacantha* leaf supplement on total worm burdens of goats with mixed nematode infection. Supplemented (■), non-supplemented control (□).

Effect of WT drench on FEC and worm burdens in tropical goats

Following its encouraging *in vitro* activity against goat nematodes and the results of studies with QT drenches of sheep, WT was administered as a drench to investigate its impact on faecal egg output and worm burdens of tropical goats with mixed nematode infection. Surprisingly, neither FEC nor worm burdens were reduced (P>0.05) after three consecutive days of drenching with WT. Unlike drenching temperate sheep with QT, WT induced only slight physiological changes to the goat's GI tract probably due to their adaptation to high tannin feeds. It was, therefore, possible that the *in vivo* anthelmintic activity of commercial preparations is a result of physiological changes in the gut (mucus hyper-secretion and increased faecal water content). These changes could promote dislodgment and expulsion of worms. The present study appears to be the first to report the effects of a commercially available WT extract on parasitised tropical goats. However, the heterogeneous nature of tannins as a group implies that results of one study using a particular type or source of tannin could not be used to generalise their potential as future anthelmintics. The fact that drenching nematode-infected goats with wattle extract, which contains a large proportion of CT, produced poorer results than supplementing them with tanniniferous browse is interesting. McNabb *et al.*, (1998) has stated that the chemical structure of CT may be more important than their concentration. It should, however, be noted that the WT was very effective *in vitro*. In addition it should be accepted that the potency of the extracts (from browse leaves and those produced primarily for the leather industry) may not be directly due to their tannin content *per se* but due to other bioactive components in these preparations.

Conclusion

The work undertaken so far has demonstrated significant *in vitro* anthelmintic activity of commercial tannin preparations (quebracho and wattle extracts) and crude extracts of tanniniferous plants (*A. polyacantha* and *T. indica*) against mice and goat intestinal nematodes. Furthermore, administration of QT as drench to the temperate sheep with mono-specific and mixed nematode infections was found to drastically reduce FEC and worm burdens of *H. contortus* but had less effect on *T. colubriformis*. However, administration of WT drench to tropical goats with a mixed infection did not significantly reduce FEC or worm burdens of any of the nematodes including *H. contortus*. It is suggested that, since the drench was also associated with gut physiological changes in the temperate sheep, but not as marked in the tropical goats, then the *in vivo* anthelmintic activity of the commercial tannin extracts was a result at least in part of gut changes. The reduction in both FEC and worm burden of *Oe. columbianum* following acacia supplementation in goats was an indication that an interaction between specific type of CT, other attributes of the plant and the complex host gut environment might be involved.

While several but not all of our studies have indicated the use of dietary tannins to reduce intestinal parasites in small ruminants, at present it is not yet possible to develop a protocol to exploit these observations in the field. Parasitic nematodes remain as a major problem in small ruminants kept by resource poor farmers in the tropics.

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