Wattle tannins as control strategy for gastrointestinal nematodes in sheep

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Nematode resistance to anthelmintic drugs is affecting small ruminant production in South Africa. This study evaluated the effect of wattle tannins as an alternative nematode control drench. Two experiments (Exp) were conducted to determine the effect of tannin concentration (Exp 1) and frequency of dosing (Exp 2) on nematode parasites. In each experiment gender, egg count (egg per gram, EPG) and initial live weight aided in blocking sheep into groups, within which they were randomly allocated to and drenched with different tannin treatments. In experiment one, 24 sheep (16 females and 8 males, aged 8-9 months) were placed into four treatment: 0, 0.8, 1.6 and 2.4 g tannin/kg BW; they were drenched for three consecutive days in a study that lasted for 21 days. In Experiment 2, 26 sheep (11 males and 15 females aged 9 to 18 months) were placed into three treatments of 9, 9, and 8 sheep each. These treatments were drenched with 1.6 g tannins/kg BW/day for one day, two or three days for the three treatments, respectively. For the two experiments, EPG were counted and L3 larvae cultured and counted in individual faecal samples. In Exp one, for all tannin treatments, EPG decreased (P<0.05) over time and EPG consistently decreased with increasing tannin level, while efficacy increased (P<0.05) with tannin level. Dosing with 1.6 and 2.4 g tannin /kg BW for 3 consecutive days had similar effects on the EPG. Dosing frequency showed that 1.6 g tannin /kg BW for 3 consecutive days was enough to reduce EPG and reduce the degree of pasture contamination.

Keywords: Wattle tannin, GI nematodes, L3 larvae, sheep.

INTRODUCTION

Numerous factors hinder ruminant production in tropical and sub-tropical Africa. These include high temperature, availability and quality of feed which is largely derived by grazing natural pastures, the general inclination in the low-resource systems for limited input, and health. High temperatures and rainfall during spring and summer period favour the development of nematode parasites resulting to increased pasture infestation (Baloyi et al., 2012). Consequently, gastro-intestinal nematodes are known to limit small ruminant production not just in South

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Africa, but in most countries in Africa (Van Wyk et al., 1997), during spring and summer when animals are expected to regain weight lost during winter (Ahmed, 2010). Generally, gastro-intestinal nematodes (GIN) have been and are being controlled using synthetic anthelmintic. However, over-dependency and misuse of the anthelmintic have resulted in the emergence and spread of nematode populations that are resistant to most anthelmintics (Prichard, 1994). Misuse could be related to cost saving tendencies, since anthelmintic are expensive for many poor farmers in the developing countries and the supply is erratic. This necessitates the development of effective and (or) cheaper alternative or complementary gastrointestinal nematode control strategies. Previous studies have shown that forages containing tannins can be used to control gastro-intestinal nematodes (Athanasiadou et al., 2000; Athanasiadou et al., 2001; Min et al., 2003, 2005). Generally, tannins (CT) are phenolic compounds found in forage legumes, trees and stems (Barry and McNabb, 1999). Tannins are widely distributed in legume pasture species such as Lotus corniculatus L. cv. Grassland Goldie and in several Acacia spp (Degen et al., 1995). Tannins have either beneficial or detrimental effects on ruminants, which depending upon their type, level of intake and their structures. The aim of this study was to evaluate the effect of different concentrations and frequency of wattle tannin on egg production by GIN and on L3 larvae in sheep grazing contamination pasture.

MATERIALS AND METHODS

Experiment one

The study was conducted at the Livestock Section of the Ukulinga Research Farm at the University of KwaZulu-Natal in South Africa. Experiments were conducted to determine the effect of tannin concentration on nematode parasites. In Experiment 1, 24 sheep (16 females and 8 males) aged 8 to 9 months; with initial live weight for females (22.6 ± 9.5 kg) and males (26 ± 7.5 kg) were used. The initial faecal eggs (egg per gram, EPG) were determined in rectal faeces samples. The sex, initial EPG and initial live weight (W) were used to place animals into groups of 4 males and 2 females each. Within a group animals were randomly assigned to four tannin treatments (0, 0.8, 1.6, 2.4 g tannins/kg W); these treatments will henceforth be denoted as T0.0, T0.8, T1.6 and T2.4, respectively. Using a stomach tube, tannin solutions were drenched (in 300 ml) between 07:00 and 09:00 h for 3 consecutive days. The use of stomach tube is critical to avoid delivering a tannin solution into the lungs via the trachea.

Experiment two

Experiment 2 determined the effect of dosing frequency of tannin. Twenty six sheep (11 males and 15 females), aged 9 to 18 months and initial live weight (females 34 ± 7.5 kg and males 32.9 ± 8.6 kg) were used. Animals were placed into 3 groups (of 9, 9 and 8 sheep each) according to their initial EPG and initial live weight. Sheep groups were drenched with 1.6 g tannins/kg BW for one day (once), two (twice) or three (thrice) days. This experiment used 1.6 g tannins/kg BW because its effect on EPG was not different from 2.4g tannin /kg W in Experiment 1.

Procedures

Experimental animals were allowed to graze naturally contaminated Kikuyu (Pennisetum clandestinum Hochst. ex Chiov.) pasture containing (kg DM) 241 g NDF and 150g CP. No feed supplement was given to the experimental animals during the 2 experiments. Larval contamination of pasture was determined in four paddocks following the method described by MAFF (1986) and Hansen and Perry (1994). Rectal faecal samples were taken in the morning on days 0, 5, 8, 12, 15, 18 and 21 (Experiment 1) and on days 0, 7, 14 and 21 (Exp2) for determining of EPG and for faecal culture of L3. Mc Master Technique (Hansen and Perry, 1994) was used to evaluate EPG on days of sampling. The efficacy was determined as follows: Efficacy (%) = (EPGt − EPG)/ EPGt; where EPGt is initial EPG. Faeces collected on days 0, 5, 15 and 21 from each treatment were pooled, thoroughly mixed, sub-sampled into three trays and incubated at 27°C for 15 days. During the incubation, samples were kept damp by sprinkling every day at 10 am. For larvae count 9g of faecal culture was used to enumerate nematode larvae according to the Baermann Technique (Hansen and Perry, 1994).

Data of EPG and nematode larvae data were analyzed using General Liner Model (GLM) procedure of SAS (2000). The effect of tannin on EPG and L3 larvae were determined by integrating, for each animal, curves of the form: f(x) = a + b.x + c.x^2 + d.x^3, where f(x) is a polynomial function of EPG and L3 larvae on time x; a, b, c and d are coefficients derived using SAS. Initial EPG and initial weight were declared as covariates.

RESULTS

The larval infestation (L3 larvae/kg of dry herbage) ranged from 115 to 180 with a mean of 138 ± 28.9. In Experiment one faecal egg counts remained fairly stable for the T 0.0 treatment throughout the study, but decreased with time for the rest of the tannin treatments (Table 1). The tannin x time interaction was significant (P < 0.05). However, the univariate analysis indicated that the effect of tannin level was significant (P < 0.05) on days 15, 18 and 21. Consistently, the highest level of tannin (T 2.4) had the lowest EPG. Figure (1) showed efficacies of different tannin drenches. These efficacies were significant (P < 0.05) from day 15, 18 and 20; however the efficacies were linearly related to the tannin dosage. Table (1) showed that total larvae increase with time for T 0.0, but decreased with time for T 0.8, T 1.6 and T 2.4. The univariate analysis pointed out that the effect of tannin level was significant (P < 0.05) on days 15, 18 and 21.

In Experiment two, the results demonstrated the effect of dosing tannin frequency (Table 2). Dosing thrice reduced (P < 0.05) EPG on day 14 and day 21 relative to dosing one or twice (Table 2). Figure 2 showed the percentage of EPG reduction relative to the starting point. In all three sampling days, the % reduction increased with dosing frequency. Total larvae recovered are shown in Table 2. The total larval count decreased with time and with the frequency of dosing.
Table 1. Effect of wattle tannin on nematode egg counts and larvae counts in sheep (Experiment 1).

<table>
<thead>
<tr>
<th>Days</th>
<th>T₀.₀</th>
<th>T₀.₈</th>
<th>T₁.₆</th>
<th>T₂.₄</th>
<th>T₀.₀</th>
<th>T₀.₈</th>
<th>T₁.₆</th>
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<td>0</td>
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<td>7319±251</td>
<td>9275±137</td>
<td>7354±142</td>
<td>302±3.7</td>
<td>261±3.1</td>
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<td>5</td>
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<td>8773±124</td>
<td>7019±152</td>
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<td>8</td>
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<td>4423±152</td>
<td>2590±118</td>
<td>314±3.5</td>
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<td>4796±116</td>
<td>1846±120</td>
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<td>153±1.6</td>
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<td>15</td>
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<td>3317±209</td>
<td>3264±142</td>
<td>1510±123</td>
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<td>139±2.0</td>
<td>116±1.9</td>
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<td>1416±117</td>
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<td>0.5326</td>
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DISCUSSION

The range of larval infestation (L₃ larvae/kg of dry herbage) of 115 to 180 (mean of 138 ± 28.9) is high enough to sustain continued re-infection of sheep over time. This could explain why T₀.₀ sustained high and stable EPG throughout the study. Tembely et al. (1997) reported that numbers of L₃ larvae (between 12-1499 L₃ larvae/kg of dry herbage) recovered from the grass were enough to contaminate the pasture when the weather conditions are stable. FAO (2009) defined a safe pasture is one with few or no parasite larvae that can infect the animal with time. Consequently, a value of 138 L₃ larvae per kg dry herbage is sufficient to ensure continuous infestation.

Predictably, EPG decreased over time in sheep drenched with tannin compared to control group. This is a clear indication that tannins exert a direct and negative effect on gastro-intestinal nematodes. Although, the decrease was greatest in sheep drenched with 2.4 g tannin/kg W, the difference between this level and T₁.₆ was relative small. Declining trends in the EPG that can be ascribed to tannins have been observed in tropical sheep infected with *Haemonchus contortus* and *Oesophagostomum*, and orally drenched with 1.5 g wattle tannin/kg W for three consecutive days (Max et al., 2005). The latter authors have shown that wattle tannin is effective in reducing faecal egg counts by 75% and worm burdens of sheep infected with *H. contortus* and *Oesophagostomum* by 86 and 28%, respectively. Consistent with these authors drenching with tannin attained significant reduction in EPG after 15 days.

Athanasiadou et al. (2001) observed that the ingestion of quebracho extract at a rate of 8% of dry matter intake...
Table 2. Effect of wattle tannin on nematode egg counts and larvae counts in sheep (Experiment2).

<table>
<thead>
<tr>
<th>Days</th>
<th>T₀,₀</th>
<th>T₀,₈</th>
<th>T₁,₆</th>
<th>T₂,₄</th>
<th>T₀,₀</th>
<th>T₀,₈</th>
<th>T₁,₆</th>
<th>T₂,₄</th>
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<td>0</td>
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<td>2931±237</td>
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<td>7</td>
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<td>895±118</td>
<td>304±3.5</td>
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<td>625±120</td>
<td>305±1.9</td>
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<td>0.4321</td>
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</table>

Figure 2 Effect of dosing tannin frequency on % nematodes egg per gram reduction. SED=1.81. Once, twice and thrice stand for dosage once, twice and thrice using 1.6 g tannin/kg live weight.

(DMI) by infected sheep for 7 days caused a decrease in feacal egg count and worm burden compared with parasites of control sheep. Other studies have shown that oral drench with quebracho extract was effective in reducing EPG and worm burdens in temperate sheep infected with *H. contortus* and *Trichostrongylus colubriformis* (Max et al., 2002) and in infected goats (Paolini et al., 2003). Contrary to expectation, Max et al. (2005) observed that drenching with wattle tannin did not elicit a reduction of EPG in tropical goats. Since tropical goats typically browsed tanniferous forages, it is possible that frequent exposure to tannin can in the long term result to a selection of nematodes that are less responsive to tannins. Dosing sheep with 1.6g tannins/kg BW once, twice and thrice was shown to reduce the EPG by 34.6, 72.9 and 83.6 %, respectively, on day 21 relative to the starting point. These results suggest dosing tannin as an oral drench for three consecutive days was likely more effective than dosing for two or one day only. Drenching wattle tannin (1.5 g.tannins/kg BW) for three consecutive days in sheep reduced EPG and warm burden in Black Head Persian sheep in Tanzania (Max et al., 2009).

Tannin efficacy has been demonstrated in and outdoors but this study modelled outdoor experimentation. For instance, Athanasiadou et al. (2000) indicated efficacy of 50% reduction of egg excretion and 30% reduction of worm burden in sheep drenched CT extract. Also a 30% reduction in the EPG has been observed in goats offered condensed tannin-containing forages (Kahiya et al., 2003). The variation in these efficacies is likely due to different experimental conditions such as weather and epidemiological situation of the parasites.

Although, the mechanism by which tannin affect nematodes is yet undefined, it is possible that tannins form complexes with protein at the surface of parasites and as such disturb their metabolism, and/or that a metabolic response by nematodes to tannins will divert nutrients away from reproduction thus reducing fecundity. It is also possible that repeated drenching with tannins would numb, injure the external surface and/or kill the
nematode. In an attempt to explain the toxicity of tannin on nematode parasites Niezen et al. (1998) postulated that condensed tannins may bind and disturb the integrity of the parasites or affect the growth of the parasites. Further to the above observations, there is supplementary benefit of reduced contamination of pasture with infective larvae. These results support the observation that condensed tannins extract from several forages (Molan et al., 2000) inhibited hatching and larval development of *T. colubriformis*. Min et al. (2003) also reported that egg hatching and larval development of *H. contortus* were inhibited in goats grazing Lespedeza which contains condensed tannins. It was postulated that condensed tannins might bind to faecal egg proteins (Min et al., 2003) inhibiting egg hatching and larval development.

It is concluded that a daily dose of 1.6 g wattle tannin/kg W delivered as an oral drench in 300 ml solution over three successive days reduced nematode’s egg production. Thus, reduce the degree of pasture contamination. Dosing for three days is more effective than dosing for less. Further practical might be in need to implement tannin in livestock industry.

**Conflict of Interests**

The authors have not declared any conflict of interests.

**ACKNOWLEDGEMENT**

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**REFERENCES**


