

EFFECT OF CONDENSED TANNIN EXTRACTS ON GASTROINTESTINAL NEMATODES OF SMALL RUMINANTS

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Summary

Experiments were carried out to investigate the effect of condensed tannins on gastrointestinal nematodes. In the first two trials (trial 1 and 2) mature and young parasite naïve sheep under artificial haemonchosis were offered a low protein diet containing varying levels of quebracho extract (QT), a model source of condensed tannins. In trial 1, thirty-five mature rams were assigned to five groups ($n = 7$); the control group received no QT whereas the rest received feed containing 1, 2, 4 and 8% w/w QT. Trial 2 had four groups of young rams ($n = 9$), which received 0, 2.5, 5 and 8% w/w QT. All animals in both trials received a trickle dose of infective stage larvae (*L3*; *Haemonchus contortus*) to the end of the experiment. Faecal egg counts (FEC), feed intake and average daily body weight gain (ADWG) were monitored for about 2 months. Dietary inclusion of QT at the 8% level reduced FEC by 36 and 51% in trial 1 and 2 respectively. This was an indication that young and parasite naïve lambs in trial 2 were slightly more responsive to the effect of QT on FEC than mature sheep in trial 1. Although the effect of QT on FEC in trials 1 and 2 was not statistically significant, negative correlations ($R^2 = 0.81$ and 0.64 respectively) between dietary QT level and FEC were observed. Feed consumption and ADWG were significantly reduced ($P < 0.05$) by dietary QT, while faecal water content and faecal output increased significantly ($P < 0.01$).

Two other studies, trials 3 and 4, were carried out to investigate short-term effects of QT on FEC and total worm burdens (TWB) of growing lambs under mono-specific and mixed nematode infections respectively. In the mono-specific infection experiment (trial 3), twenty parasite-naïve ram lambs were trickle-infected daily with 450 *L3* of *H. contortus* larvae. Faecal egg outputs were monitored and on day 22 after the first dose of larvae the animals were randomly allocated into two groups ($n = 10$). The control group received a placebo drench (plain water) whereas the treated group received QT drench at 2.4 g QT kg^{-1} bodyweight (equivalent to 8% (w/w) QT in feed) for three consecutive days. All animals were sacrificed the following day and their abomasa removed for TWB assessment. The effect of QT on mixed nematode infection was investigated in a similar trial (trial 4), whereby 3 groups of sheep ($n = 12$) were challenged daily with *L3* as follows: *H. contortus* alone (Group H), *Trichostrongylus colubriformis* alone (Group T) or a mixture of both parasites (Group HT). FEC were monitored and on day 27 half of the animals in each group ($n = 6$) received the same dose of QT drench for three days and sacrificed as described before. Administration of the drench to sheep mono-specifically parasitised with *H. contortus* significantly ($P < 0.05$) reduced mean FEC of the treated group compared to the control group, a 91% reduction in FEC. Likewise, a significant reduction ($P < 0.01$) in TWB of the treated group, i.e., by 80%, following the three consecutive days of administration was evident. The QT drench was also effective under mixed infection in terms of FEC reduction whereby a significant ($P = 0.016$) drop in each group following QT drench administration was observed. The treatment was

more effective against mono-specific infection of *H. contortus* than that of *T. colubriformis* or under mixed infection. This finding was most conspicuous on the day of slaughter whereby the group x treatment x time interaction was significant ($P = 0.011$). The TWB data revealed an appreciable reduction in the TWB of *H. contortus* ($P < 0.05$) but not *T. colubriformis* ($P = 0.314$).

Direct effects of condensed tannins from QT and wattle extract (WT) to adult nematodes were also assessed under an *in vitro* viability assay. Adult mice nematodes, *Heligmosomoides polygyrus*, were incubated at 38 - 39 °C in either normal saline alone (control) or in normal saline containing varying concentrations of QT or WT. Viability of the parasites was monitored regularly and then expressed as per cent of worms surviving at a particular time interval. Wattle extract was about 4 times more potent than QT in all tested concentrations.

The current findings provide further evidence that condensed tannin preparations are toxic to the nematodes. Prolonged feeding on diets containing high levels of condensed tannins (CT) may be undesirable because of its association with a reduction in growth. Administration of QT as a drench was found to be more practical and offered a quick and best effect. The current QT dose can reduce faecal egg output and TWB of sheep parasitised with the abomasal nematode, *H. contortus*, but less so with the intestinal type, *T. colubriformis*. The latter species may require a higher dose or more days of drenching. The present findings suggest that it may be possible, with limited use of synthetic anthelmintics for use in the tropics, to design appropriate feeding strategies based on tanniniferous forage materials or extracts to alleviate nematode infections.

Introduction

Infections caused by gastrointestinal parasites are among the major drawbacks hindering livestock productivity world-wide (Parkins and Holmes 1989; Sykes 1994; Gill and LeJambre 1996). In tropical and subtropical regions, where the parasites are more abundant due to favourable environmental conditions, helminthiasis is even more devastating (Waller 1997). Moreover extensive grazing on native pastures, together with lack of supplemental nutrients to animals in these areas leads to low plane of nutrition and, therefore, increased susceptibility. Control of helminthiasis is mainly by chemotherapeutic means, with best results being obtained when this is integrated with proper grazing management and resistant animals. However, in the last 2-3 decades there has been over-dependency and misuse of the chemotherapeutic approach with consequent evolution of anthelmintic resistance (Ngomuo *et al.*, 1990; Bjorn *et al.*, 1990; Prichard 1994) especially among major nematode species. Apart from anthelmintic resistance, poor availability and affordability of anthelmintics to small-scale farmers in developing countries have compounded the problem (Hammond *et al.*, 1997). It follows that a search for novel and more sustainable anthelmintics is the best approach to the control of helminthiasis. 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 (Niezen *et al.*, 1993; 1998; Robertson *et al.*, 1995). Furthermore recent studies have shown that dietary inclusion of the CT in quebracho extract (Butter *et al.* 2000; Athanasiadou *et al.*, 2000) dramatically reduces egg output and worm burdens of sheep infected with *T. colubriformis*.

The main objective of the project is to determine whether locally available tanniniferous browse materials or readily available extracts from them can be used to control nematode infections in small ruminants.

Material and methods

Test parasites and condensed tannin source

The Moredun Research Institute, UK supplied the nematode parasites (*H. contortus* and *T. colubriformis*) as infective stage larvae (L3) suspension in distilled water; this was kept in culture tubes at 5 °C until needed. Adult *Heligmosomoides polygyrus* used in the *in vitro* assay were freshly obtained each time from passage mice held at the University of Nottingham. Two sources of CT were used; quebracho extract (QT) powder from the barks of tropical heartwood (*Schinopsis balansae*) which was supplied by Hodgesons Chemicals Ltd, UK, and wattle (mimosa) extract (WT) from *Acacia mearnsii* prepared by Wattle Tannin Co., Tanzania. Both are commercial preparations for use in leather industries. The QT was thoroughly mixed in the feed prior to pelleting or dissolved in lukewarm water or saline depending on the type of experiment. The WT was dissolved in saline.

Housing and feed

Animals were kept in individual raised pens with slatted floors in a house maintained at an ambient temperature of 15 °C, with water *ad libitum*. The animals were offered a relatively low-protein (97g CP/kg) pelleted grass meal, to restrict live-weight gain.

Experimental

I Feeding trials

Two feeding experiments were conducted to investigate the effects of dietary condensed tannins in quebracho extract on faecal worm egg output and growth performance of sheep receiving a trickle infection of the intestinal parasite, *H. contortus*. Both experiments were similar except for the age of the animals and the daily feed allowance given. Experiment 1 used older sheep that have been to pasture and possibly had experience of intestinal worm infections whereas parasite-naïve growing lambs were used in the second experiment. In experiment 1 the feed allowance was offered at 4% of body weight whereas in experiment 2 it was set at 3% of body weight.

In experiment 1, thirty-five castrated rams (Charollais x Mule), initial live-weight 39.5 ± 0.5 kg, were randomly assigned into 5 groups ($n = 7$) and offered feed containing no QT (control), 1, 2, 4 or 8% w/w QT. Three weeks after the introduction of the experimental diets all sheep received a daily oral dose of 500 infective stage (L3) *Haemonchus* sp. larvae to the end of the trial. Feed consumption, body weight gain and faecal egg outputs were monitored. Experiment 2 used 36 young and parasite naïve (Finn x Dorset; mean live weight 23.6 ± 0.5 kg) ram lambs in 4 groups ($n = 9$) which received feed with no QT, 2.5, 5 or 8%w/w QT and were trickle infected daily with 400 L3 (*Haemonchus* sp.). Parameters were monitored as before.

II Drenching trials

Short-term effects of quebracho extract on FEC and total worm burdens of growing lambs under mono-specific or mixed gastrointestinal nematode infections were investigated in two separate experiments (trial 3 and 4). In the mono-specific infection experiment (trial 3), twenty parasite-naïve (Finn x Dorset) ram lambs, about 4 months old (41.9 ± 1.1 kg) were trickle-infected daily with 450 L3 larvae of *H. contortus*. Faecal egg outputs were monitored and on day 22 after the first dose of infective stage larvae the animals were randomly allocated into two groups. The control group ($n=10$) received a placebo drench (plain water) whereas the treated group received QT drench at 2.4 g QT kg⁻¹ bodyweight (equivalent to 8% (w/w) QT in feed). After 3 days of drenching i.e., days 22, 23 and 24, all animals were humanely slaughtered on day 25 and worm burdens recovered from the abomasum for TWB estimation.

Effect of QT drench on sheep with mixed nematode infections (trial 4) was carried out in a similar manner. Thirty-six young (Mule x Charollais) rams, about 3 months old (40.6 ± 3.1 kg) were assigned into 3 groups ($n = 12$) and challenged daily as follows: *H. contortus* alone (Group H; 500 L3), *T. colubriformis* alone (Group T; 4000 L3) or a mixture of both (Group HT; 500 and 4000 L3 respectively). Faecal egg outputs were monitored and on day 27 after the onset of trickle infection animals in each group were blocked by FEC numbers and further subdivided into 2 groups ($n = 6$), i.e., control and treated. Quebracho extract drenching, slaughter and sampling were performed as for trial 3.

III *In vitro* studies

The *in vitro* studies were aimed at investigating the direct effects of condensed tannins upon survival of various stages of GI nematode parasites. Initially ensheathed infective stage larvae of *H. contortus* were incubated at 37 - 39 °C in phosphate buffered saline containing different concentrations of QT. Since survival of the larvae was not effected at all tested concentrations, the adult mice nematode *H. polygyrus* was used instead. It was not possible to routinely get a supply of the unsheathed *H. contortus* without killing an infected sheep each time.

Freshly obtained adult worms were incubated on petri dishes (about 10 -15 male and females) at 37 - 39 °C in QT or WT solutions of varying strengths, for a period of 48 hours. Quebracho extract solutions at 0, 0.5, 1, 2, 4, 8 and 12% (w/v) were tested and survival rates were recorded over different time intervals. Motility and viability of the parasites were 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, virtually, no reaction to touch was observed.

In an attempt to distinguish between the effects due to CT *per se* or to other compounds in the extract, polyethylene glycol (PEG; molecular weight 3000 - 4000, Fisons, UK) was added into the solutions. PEG is known to bind and inactivate condensed tannins so its addition into the culture solutions was expected to reverse any anthelmintic activity due to condensed tannins.

Statistical analysis

The data were analysed by Genstat 5 (Lawes Agricultural Trust, Rothamsted) statistical package. Faecal egg count data were analysed as a completely randomised design (split plot model) using one way analysis of variance (ANOVA) with treatments, i.e. groups, as factors and sheep as blocks. Linear and quadratic trends were also fitted using polynomial function to compare profiles among groups. Live-weight gain, faecal output and dry matter content were analysed as a completely randomised design with treatments and sheep as factors and block respectively. Worm burdens in the mixed infection trial were categorised as abomasal or intestinal worms and analysed separately.

Results

Effect of dietary QT on faecal egg output and growth performance of sheep during artificial haemonchosis (experiments 1 and 2 respectively).

Feed consumption and live weight

There was evidence of decreasing feed consumption ($P < 0.05$) especially for animals on QT containing feed in both experiments. This tendency appeared to be dose dependent as more

refusals were associated with feed containing 8% QT (Tables 1 & 2). Although mean live weights were similar among groups, average daily weight gain (ADWG) of animals on 8% QT, towards the end of experiment 1, was significantly lower ($P < 0.05$) than other groups. In experiment 2, both initial and live weight prior the introduction of experimental diets were similar ($P > 0.05$), this soon changed as a result of hyporexia in response to the presence of QT in feed. The ADWG were divided into three phases: the period from onset of experiment to introduction of experimental diets (phase 1); from introduction of the diets to commencement of trickle infection (phase 2); and finally the post infection period (phase 3). Live weight gains were similar ($P = 0.17$) during phase 1 in all groups but remained significantly different ($P < 0.001$) in phases 2 and 3, with QT8 having the lowest value. There was a negative linear correlation ($R^2 = 0.92$), during phase 2, between ADWG and dietary QT inclusion level, i.e., ADWG decreased with increasing level of QT above 2.5% in feed.

Table 1 Effect of dietary QT on feed consumption, faecal egg output and growth performance of mature sheep during artificial haemonchosis (Experiment 1).

Parameter	QT0	QT1	QT2	QT4	QT8	SED (30 d.f)	P value	vs.QT %*
(%) Feed consumed	87 ^a	92 ^a	93 ^a	88 ^a	79 ^b	3.491	0.004	-0.78
Total faecal outputg DM day ⁻¹	502 ^a	644 ^b	880 ^{c,d}	752 ^{c,d}	691 ^b	65.44	0.002	0.46
Faecal DM (%)	30.4 ^a	29.6 ^a	27.9 ^b	29.4 ^a	27.7 ^b	0.685	0.001	-0.71
ADWG g day ⁻¹								
Phase 1	170.2 ^a	168.2 ^a	152.2 ^a	152.9 ^a	148.1 ^a	15.2	0.481	
Phase 2	207.3 ^a	208.4 ^a	208.8 ^a	200.0 ^a	196.3 ^a	52.9	0.999	-0.92
Phase 3	232.9 ^a	229.9 ^a	223.4 ^a	217.8 ^a	140.9 ^b	32.9	0.047	-0.94
All phases	203.5 ^a	202.2 ^a	194.8 ^a	190.2 ^a	161.8 ^a	25.52	0.487	-0.98
Initial liveweight (kg)	30.6 ^a	31.3 ^a	31.6 ^a	32.2 ^a	33.0 ^a	1.298	0.41	
After expt diet (kg)	46.0 ^a	47.3 ^a	46.7 ^a	47.2 ^a	46.6 ^a	1.762	0.953	
Final liveweight (kg)	60.9 ^a	62.1 ^a	61.5 ^a	61.0 ^a	55.2 ^b	2.951	0.161	-0.89

The results are means of 7 animals in a group, different superscripts within a row are significantly different ($p < 0.05$); * is a correlation value between a given parameter and dietary level of QT. Phases were considered as a period before introduction of experimental diets (phase 1), from the experimental diets to commencement of trickle infection (phase 2) and finally the post infection period (phase 3). Means with different superscripts within a row are significantly different ($p < 0.05$)

Faecal egg output

Faecal egg profiles of the sheep looked similar for both trials and are shown in figures 1 & 2. Eggs were first observed 16 days after the first dose of larvae and outputs continued to increase reaching a peak about two weeks later. Egg outputs decreased gradually after peak but became irregular about day 40; this irregularity was marked by small surges in egg profiles regardless of treatment. Statistically, dietary inclusion of QT did not significantly ($P > 0.05$) reduce faecal egg outputs but diet x time interaction together with linear trends were significant ($P < 0.05$) indicating that as dietary load of QT increased, some points along the faecal egg profiles were significantly different.

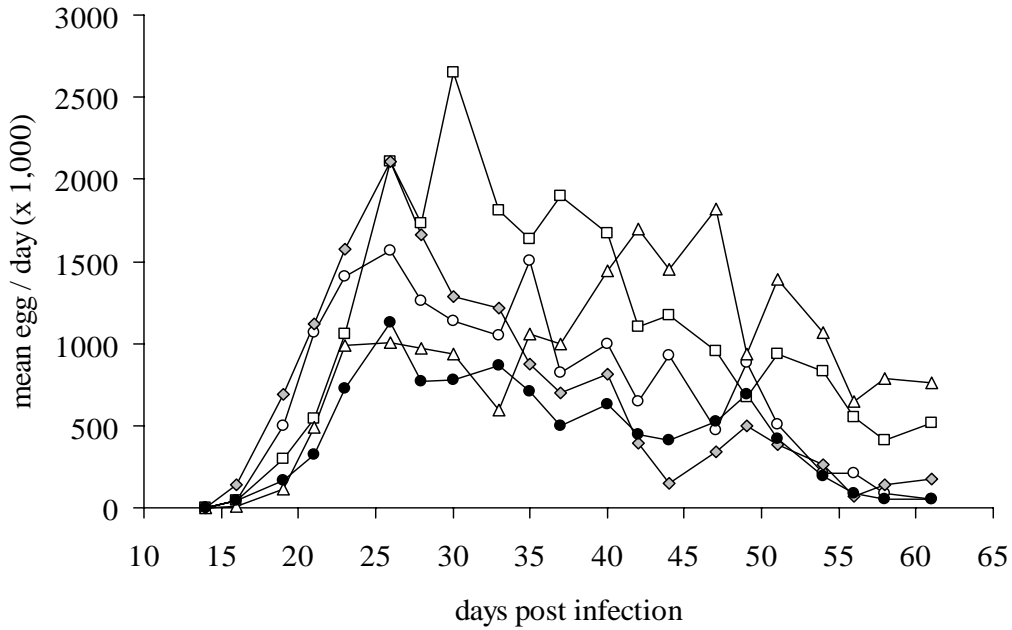


Figure 1 Total daily faecal egg output (eggs per day) of sheep artificially infected with *H. contortus* and fed various levels of quebracho extract. 0% (--○--); 1% (--□--); 2% (--△--); 4% (--◇--); and 8% (--●--) w/v quebracho extract. SED for comparing treatments = 42.7×10^3 ; df = 30. (Experiment 1)

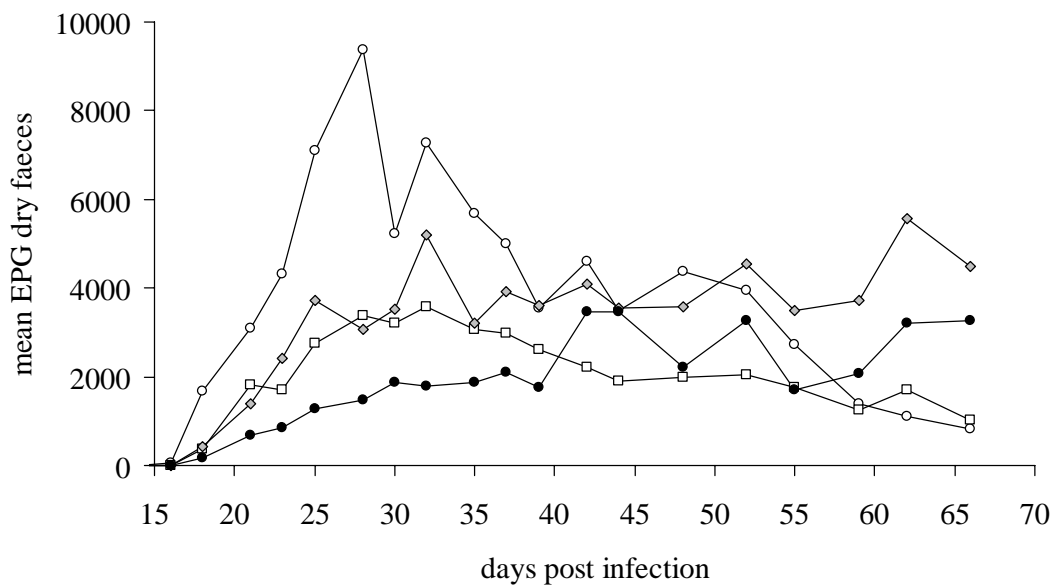


Figure 2 Faecal egg profiles (egg per gram dry faeces) during artificial haemonchosis, of parasite naive ram lambs maintained on different dietary levels of quebracho extract; control (--○--); 2.5% (--□--); 5% (--◇--); and 8% (--●--) w/v quebracho extract. SED for comparing treatments = 1800, df = 32. (Experiment 2)

Faecal consistency

Dietary QT also increased water content of the faeces. The control groups had significantly lower ($P < 0.001$) faecal water content compared to the groups maintained on QT-containing diets (see faecal DM data, Tables 1 & 2). Moreover, visual examination revealed a tendency for loose brownish mucus-laden faecal material in the sheep receiving QT in a dose dependent manner. Dirty reddish-brown urine was a frequent observation that characterized groups on tannin rich diets.

Table 2 Faecal egg counts and growth parameters of parasite naïve lambs daily infected with 400 L₃ *H. contortus* and offered diet with varying levels QT (Experiment 2)

Parameter	QT0	QT2.5	QT 5	QT 8	SED (32 d.f.)	P value	vs.* QT%
Feed consumption (%)	99.8	100.0	100.0	93.5	1.444	< 0.001	-0.79
Faecal dry matter content (%)	32.8 ^a	27.4 ^b	26.9 ^b	27.9 ^b	1.6	0.03	-0.90
ADWG (g day ⁻¹)							
Phase 1	196.1 ^a	263.1 ^a	241.9 ^a	227.6 ^a	29.7	0.17	
Phase 2	198.4 ^a	215.8 ^a	199.9 ^a	30.1 ^b	39.3	< 0.001	-0.80
Phase 3	196.2 ^a	137.0 ^b	124.6 ^b	117.2 ^b	13.5	< 0.001	-0.88
All phases combined	196.9 ^a	205.3 ^a	188.8 ^a	125.0 ^b	14.2	< 0.001	-0.84
Initial liveweight (kg)	24.3 ^a	23.2 ^a	23.4 ^a	23.7 ^a	1.15	0.803	
After 12 days on experimental diet (kg)	30.1 ^a	31.2 ^a	31.6 ^a	27.1 ^b	1.36	0.008	-0.58
Final liveweight (kg)	44.9 ^a	42.5 ^a	41.2 ^a	36.4 ^b	1.96	0.001	-0.98

The results are mean of 9 animals per group. Phases were considered as period from onset of experiment to introduction of experimental diets (phase 1), from the diets to commencement of trickle infection (phase 2) and finally the post infection period (phase 3). Means with different superscripts within a row are significantly different ($p < 0.05$).

Effect of QT drench on faecal egg output and total worm burdens of sheep during mono-specific and mixed nematode infections (experiments 3 and 4 respectively)

Faecal egg counts

Worm eggs were first observed in the faeces 17-18 days after the first dose of infective stage larvae. Experiment 3 showed two distinct egg profiles ($P < 0.01$) (Fig. 3); while the FEC of the control group increased progressively throughout the 10-day (day 16-25) monitoring period, the treated groups started to decline two days after the first QT dose. There was a significant reduction ($P < 0.05$) in mean FEC of the treated group on the day of slaughter (day 25), this was shown to be 91% lower than the control group. Experiment 4 revealed that QT drench had a significant reduction in FEC of sheep infected with *Haemonchus* sp (group H) but not with *Trichostrongylus* sp. (group T) or both (group HT). Comparisons of mean FEC between control and treated animals within each group revealed a significant reduction ($P < 0.05$) in groups H and HT but not in group T ($P = 0.181$). Percentage FEC reductions, as calculated on the day of slaughter (day 30), were 77, 35 and 23% for groups H, T and HT respectively. A peculiar trend whereby FEC dropped markedly a day after the drench and then started to increase towards the day of slaughter was observed in group T and was more conspicuous in group HT (figure 4a – c).

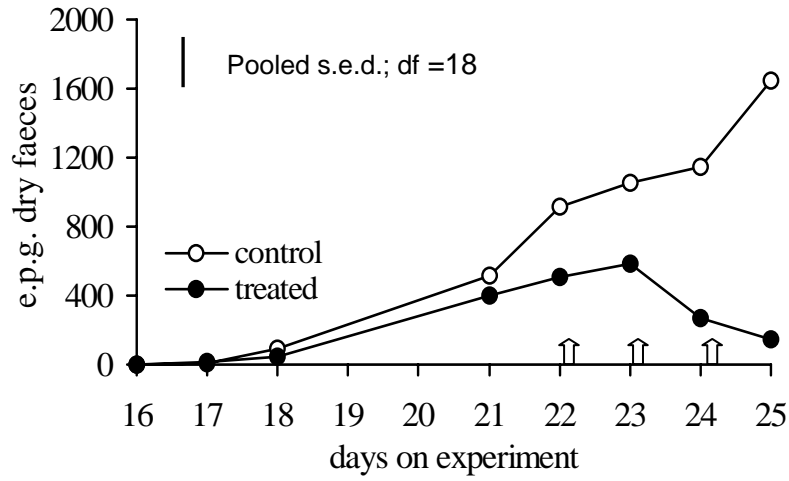


Figure 3 Effect of quebracho extract drench on faecal egg outputs of sheep parasitised with *Haemonchus contortus* alone, arrows show days of drench administration. (Experiment 3)

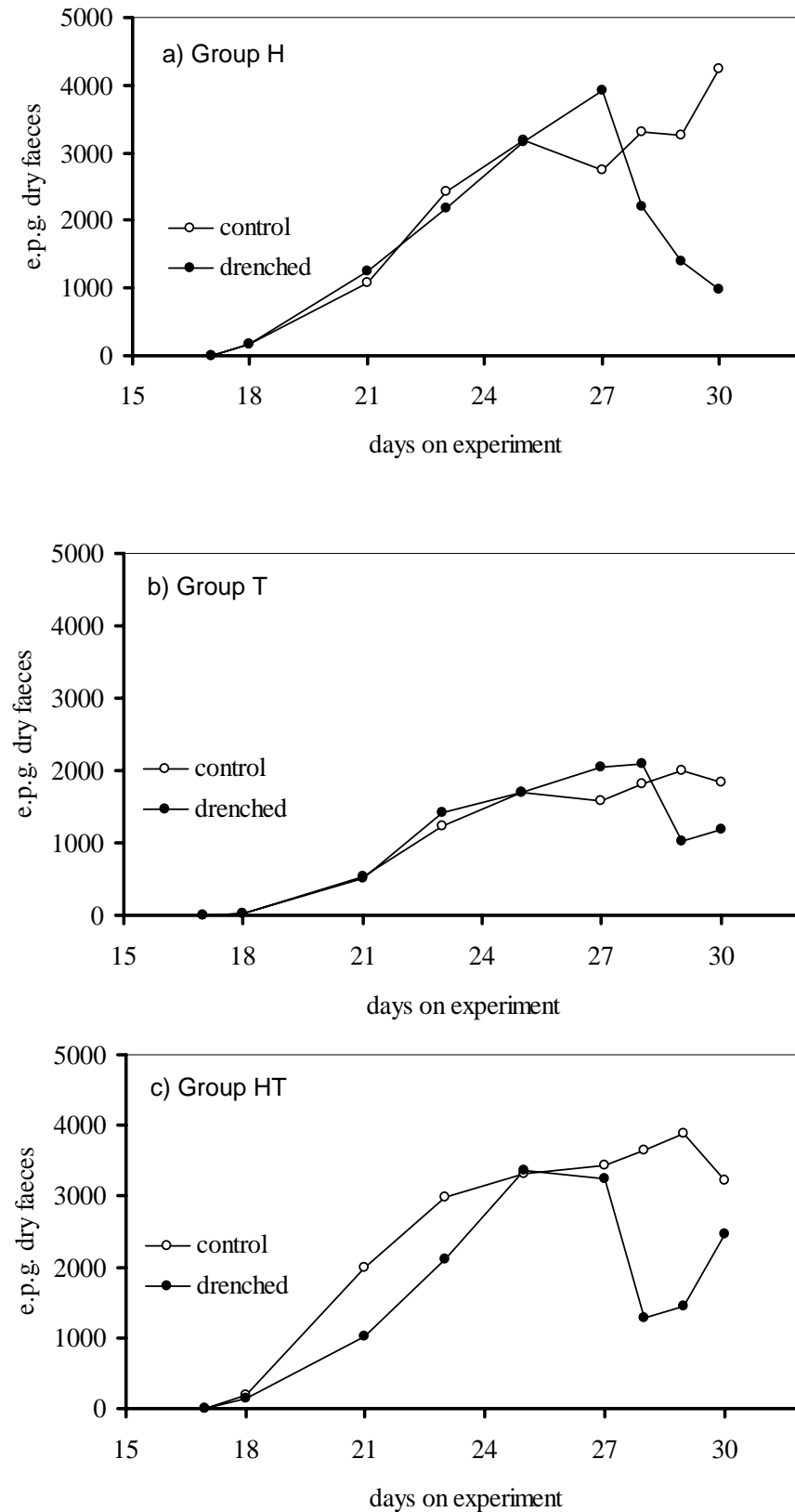


Figure 4a - c Faecal worm egg profiles of the three groups of sheep infected with either *H. contortus* alone (a), or *T. colubriformis* alone (b) or both species (c). Control [-o-] or drenched with QT solution at 2.4g kg⁻¹ body weight [--•--]. SED for comparing treatments = 587, df = 30. (Experiment 4)

Total worm burdens

Total worm burden results (TWB) for mono-specific infection are shown in figure 5; there was a significant difference ($P < 0.01$) in the TWB, i.e., males, females and immature worms, between the control and treated groups. Comparison of mean TWB between the two groups showed a reduction of 80% after three consecutive days of drenching with QT extract. Treatment was not effected by sex of the worms as the treatment x sex interaction was not significant ($P = 0.61$), male-female ratios were 0.96 and 1.11 for control and treated groups respectively. The TWB results for the mixed infection are shown in Figure 6 and 7. Drenching with QT extract reduced TWB of *H. contortus* in group H by 33% ($P = 0.234$) and in group HT by 99% ($P = 0.015$). On the other hand the drench reduced TWB of *T. colubriformis* in group T by 39% ($P = 0.216$) but by only 12% ($P = 0.37$) in group HT. Although TWB reduction was observed in all drenched animals and affected both worm species, it was only with *H. contortus* under mixed infection (group HT) in which the treatment was shown to be statistically significant.

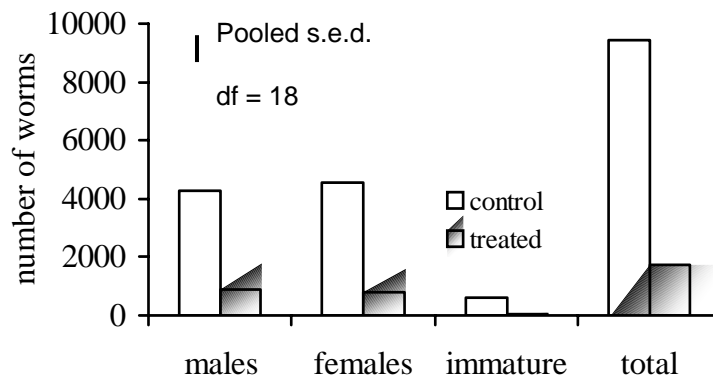


Figure 5 Effect of quebracho extract drench on total worm burdens (TWB) of sheep mono-specifically parasitised with *Haemonchus contortus*. (Experiment 3)

Changes in the gastrointestinal tract (GIT)

Reduced appetite and loose mucous faeces were a frequent observation on the day following the first dose of QT in all drench trials. Slight reddening of the abomasal-ileal region and tiny dark spots on Peyer's patches were evident in drenched animals on necropsy.

Direct effect of QT and WT solutions on different nematode stages (*in vitro* studies)

Incubation of L3 larvae of *Haemonchus contortus* in QT solutions of up to 37% did not show any toxicity to the larvae, however, this was not the case with *Heligmosomoides polygyrus* adults. The latter had its viability compromised at all levels of QT tested. The most effective concentration was 2% QT, which killed 50% and 100% of the worms within 18 and 36 hours respectively (Figure 8). The least effective solutions were 8 and 12% QT, which required about 35 hours for 50% of the worms to die and with both, about 15% of the parasites surviving the 48-hour incubation period. Males were more susceptible to the toxic effect of condensed tannins in QT than females. The addition of PEG in the QT solutions did not improve viability of the parasites.

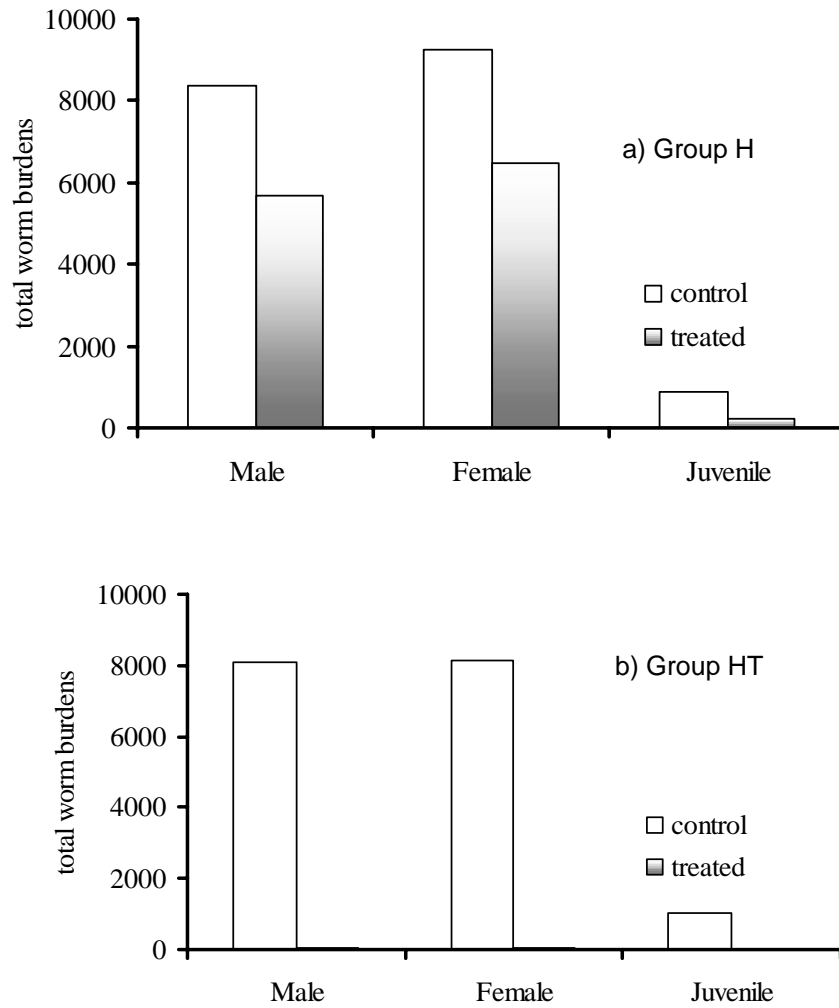


Figure 6a & b Total worm burdens of *H. contortus* recovered from animals of groups H (a) and HT (b) at slaughter. SED =154, df 20. (Experiment 4)

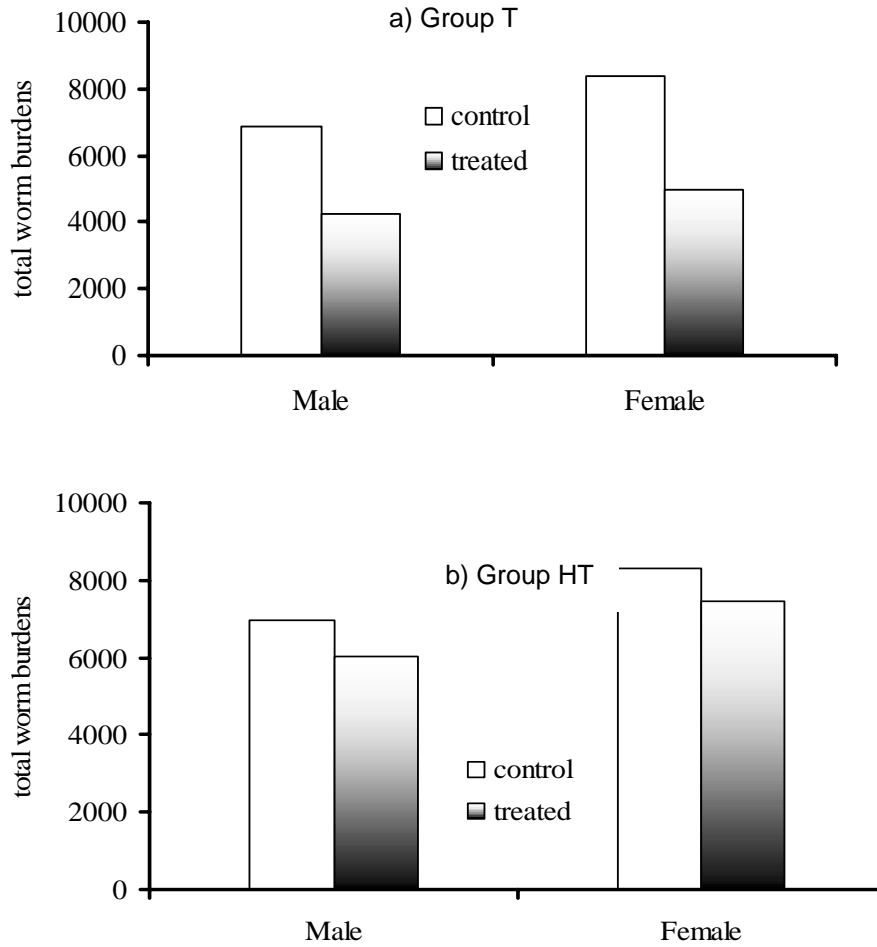


Figure 7a & b Total worm burdens of *T. colubriformis* recovered from animals of group T (a) and of group HT (b) on slaughter. SED = 1895, df = 20. (Experiment 4)

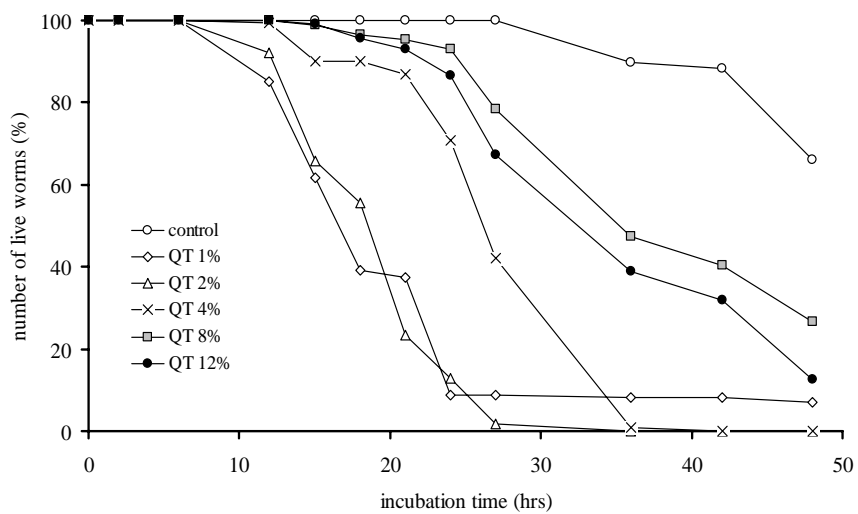


Figure 8 Survival of *H. polygyrus* in culture solutions containing different concentrations of quebracho tannin extract (QT) (mean of 3 experiments)

Wattle extract

The effects were similar to those of QT with the exception that WT was about 4 times more potent than QT (Figure 9). While it took more than 48h for all worms to die in most QT solutions, no worm survived the 10-h incubation in WT solutions. Addition of PEG to the WT test solutions only slightly affected the worm survival. On average PEG increased the time required to kill 50% of incubated worms (t₅₀) by only an hour, i.e., it shifted the t₅₀ from 2 - 3 hours to 3 - 4 hours. Only 10% of the parasites in WT solutions managed to survive the 10-hour incubation period as a result of PEG addition. This was similar to the result seen with QT. This may indicate that the WT and the QT preparations contain materials other than tannins that were toxic to worms.

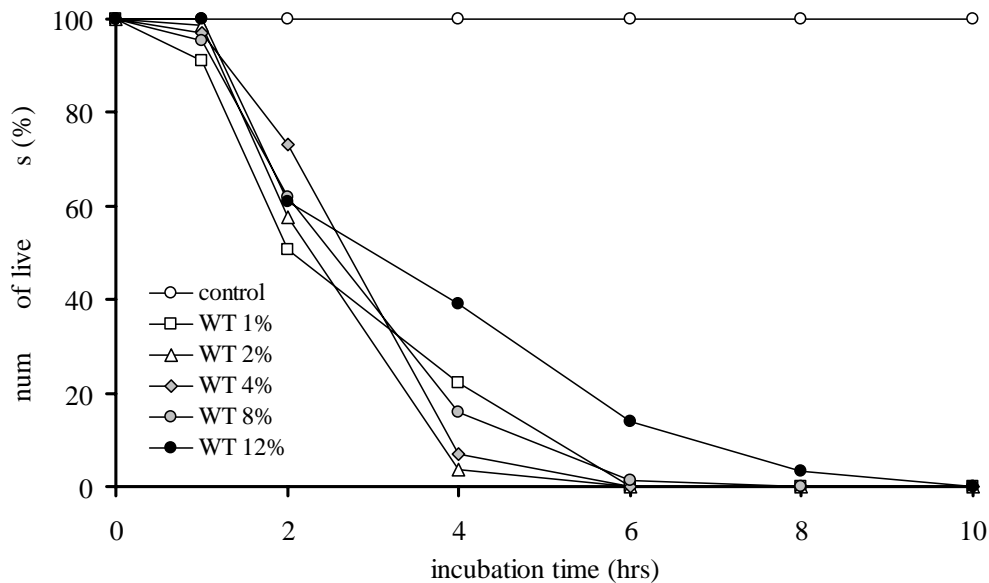


Figure 9 Survival of *H. polygyrus* in culture solutions containing different concentrations of wattle (mimosa) tannin extract (WT) (mean of 3 experiments)

Discussion

The current results are some further evidence of anthelmintic activity of condensed tannin preparations. Dietary inclusion of QT (experiments 1 and 2) appreciably reduced FEC of sheep with haemonchosis but surprisingly this changed towards the end of trial (Fig 1 and 2) suggesting that prolonged feeding with relatively high levels of CT may result in resistance by the worms. Animals on 8% QT feed had lower ADWG than others but again this was a result of anti-nutritional effects of CT as explained above. Drenching trials were carried out to address some of the shortcomings of the feeding experiments. The QT drench caused a significant reduction in both FEC and TWB of sheep infected with *H. contortus* alone (experiment 3). Reduction with *H. contortus* was also seen in experiment 4, although the magnitude was different between the single and the mixed infections. Larvae of *T. colubriformis* showed only a little response to QT drench (experiment 4). When a similar trial was carried out in sheep (Athanasiadou *et al.*, 2001), the same dose of QT drench was found to be effective against *T. colubriformis* but not *H. contortus*, i.e. opposite to the current observations. It should also be noted that tannins are very heterogeneous compounds and differences in these data indicate that their biological effect might also be variable. It should also be noted that addition of PEG did not greatly reduce the toxicity of the tannin preparations *in vitro*; an indication that compounds other than CT in the extract might be responsible for observed toxicity.

Physiological changes in the GIT following administration of QT drench, i.e., increased faecal water content and mucus secretion, were undesirable but they might have contributed

towards dislodging and eventual expulsion of worms. The QT dosage can be adjusted to reduce some of the deleterious effects.

The studies presented here support the current view that tannin preparations have anthelmintic properties but further work is still required to confirm this and to devise the appropriate dose levels. The possibility of using feeds containing plant materials high in tannins needs also to be explored.

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