Condensed tannins and gastro-intestinal parasites in sheep

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Abstract

As the effectiveness of current anthelmintic drenches is being reduced by the emergence of drench resistance and significant production losses may still occur as a result of continuing larval challenge, strategies for nematode control should focus on preventing the build-up of infective larvae on pasture rather than treating the infection. This paper reports the effects of condensed tannins (CT) extracted from *Lotus pedunculatus, Lotus corniculatus*, sulla (*Hedysarum coronarium*) and sainfoin (*Onobrychus viciifolia*) on *in vitro* viability of eggs, first stage larvae (L1) and third stage larvae (L3) of the sheep nematode, *Trichostrongylus colubriformis*. When *in vitro* assays contained CT in a similar concentrations to that in the digestive tract of sheep fed these forages, the CT reduced the development of eggs to L3 larvae as well as the proportion of eggs hatching. The CT also reduced the development of L1 larvae to L3 larvae and decreased the motility of L3 larvae when assessed by the larval migration inhibition assay and this may reduce their infective capacity *in vivo*. If these forages are fed to grazing sheep, then it may be possible to reduce contamination of pasture with infective larvae and reduce our dependence on anthelmintics as the principal method for controlling internal parasites.

Keywords: condensed tannins, drench resistance, egg hatching, larval development, *Lotus*, parasites, sainfoin, sulla, *Trichostrongylus colubriformis*

Introduction

Internal parasites represent a significant economic burden to the animal industries in New Zealand. Proprietary anthelmentic drenches, the current means of internal parasite control, cost New Zealand farmers millions of dollars every year. However, of greater importance than cost is the development of parasite resistance to proprietary anthelmentic drenches (Waller 1994), which has been reported in sheep, goats and cattle in New Zealand (Schetter *et al.* 1989; Vlassoff *et al.* 1994) and the increasing concern about the anthelmintic residues in animal products (Sykes *et al.* 1992).

Alternative strategies for the control of internal parasites are needed and one approach may be to include plants which contain condensed tannins (CT; proanthocyanidins) into the grazing rotation (Robertson *et al.* 1995). Condensed tannins are polyphenolic compounds found in the leaves and stems of a range of forage species (Barry & Manley 1986; Waghorn *et al.* 1990; Terrill *et al.* 1992). Some have multiple benefits such as increasing the liveweight gain, the rate of wool growth and milk production (Wang *et al.* 1996 a, b).

This research programme has measured the effect of purified CT extracted from several plant species on the egg hatching, larval development and larval motility of the sheep nematode, *Trichostrongylus colubriformis* under *in vitro* conditions.

How do tannins affect internal parasites?

Forages which contain CT, notably sulla (*Hedysarum coronarium*) and *Lotus pedunculatus* have been shown to significantly increase the growth rate of parasitised lambs relative to non-CT containing forages, in the absence of anthelmintics (Waghorn & Niezen 1994; Niezen *et al.* 1995; Robertson *et al.* 1995). Infected lambs (Niezen *et al.* 1995, 1998) and deer (Hoskin 1998) exhibited significantly lower faecal egg counts and lower abomasal and intestinal worm burdens when fed sulla compared to their counterparts fed conventional forages without CT.

Low concentrations of CT have been shown to protect plant proteins against rumen degradation and to increase protein flow to the small intestine where they can improve the supply and absorption of amino acids (Waghorn *et al.* 1987). It has been shown that animals given elevated planes of nutrition are better able to resist infection and disease. The expression of disease is more severe in animals having low protein intakes (Coop & Holmes 1996) whereas high protein intakes have been shown to increase the resistance of sheep to *Haemonchus contortus* (Wallace *et al.* 1996) and *T. colubriformis* (Kambara *et al.* 1993). Hence dietary CT may benefit parasitised sheep by improving their protein nutrition which in turn may improve the animals' immune response to the infection. Alternatively, CT may affect parasites directly in the digestive tract.

Results of *in vitro* assays

The egg hatch and larval development assays were carried out as described by Hubert & Kerboeuf (1984, 1992) and were used to evaluate the effects of CT extracted from *L. pedunculatus, L. corniculatus*, sulla and sainfoin (*Onobrychus viciifolia*) on egg viability and larval development of *T. colubriformis*. In the egg hatch assay, the undeveloped eggs were collected from the faeces of experimentally-infected lambs and incubated with a range of CT concentrations (0, 50, 100, 200, 400, 600, 800 and 900 µg/ml) in 200 µl of distilled water in 96-well micro-titre plates. After incubation for 26 hours at 24°C, the numbers of unhatched eggs and first stage larvae (L1) were counted and the proportion of eggs hatched (number of first stage larvae/number of eggs in medium) was calculated. It can be seen from Figure 1 and Table 1 that the proportion of eggs hatched decreased with increasing CT concentrations.

Eggs were incubated with a range of CT concentrations (0, 25, 50, 100, 150, 200, 300, 400 and 500 µg/ml) in an artificial medium for 7 days at 24°C (larval development assay). CT from the four forages were able to disrupt the life cycle of *T. colubriformis* by inhibiting the development of the eggs to third stage larvae (L3) when CT concentrations were 200–400 µg/ml (Figure 2).

The sensitivity of the L1 larvae to CT was investigated by allowing the eggs to hatch in the absence of CT, then exposing them to the same concentrations mentioned above for the reminder of the 7-day incubation period. At the end of incubation period, the numbers of L1, second stage larvae (L2) and L3 larvae were counted and the mean larval development was calculated. The CT reduced the development of both eggs and L1 larvae to the infective stage in a concentration dependent pattern and the eggs were significantly (P<0.01) more sensitive to the action of CT than L1 larvae (Figure 2 and Table 1).

The larval migration inhibition (LMI) assay (Rabel *et al.* 1994), which depends on the ability of the test materials to immobilise and inhibit the migration of the third stage larvae through nylon mesh sieves, was used to show that CT from the four forages had inhibitory effects against exsheathed (the larvae were exsheathed in sodium hypochlorite, Rabel *et al.* 1994) larvae of *T. colubriformis* (Figure 3 and Table 1). Condensed tannins from sainfoin had the highest activity, followed by CT from *L. pedunculatus* , sulla and *L. corniculatus.*

Figure 1 The effect of condensed tannins (CT) extracted from *Lotus pedunculatus, Lotus corniculatus,* sulla (*Hedysarum coronarium)* and sainfoin (*Onbrychus vicifolia*) on the proportion of *Trichostrongylus colubriformis* eggs hatching *in vitro*. Each point represents the mean of triplicates ± SEM.

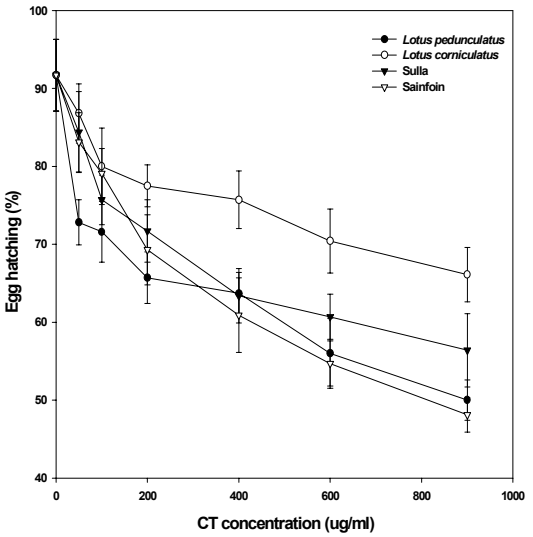


Table 1 The inhibitory effects of condensed tannins (CT) extracted from *Lotus pedunculatus*, *Lotus corniculatus*, sulla (*Hedysarum coronarium*) and sainfoin (*Onobrychus viciifolia*) on egg hatching (EH), larval development (LD; L1 larvae) and larval viability of *Trichostrongylus colubriformis in vitro*. Data are expressed as: EH,% of eggs not hatched; LD,% of L1 larvae which did not develop to L3 larvae and LMI,% of immobilised L3 larvae. Data are means and standard errors.

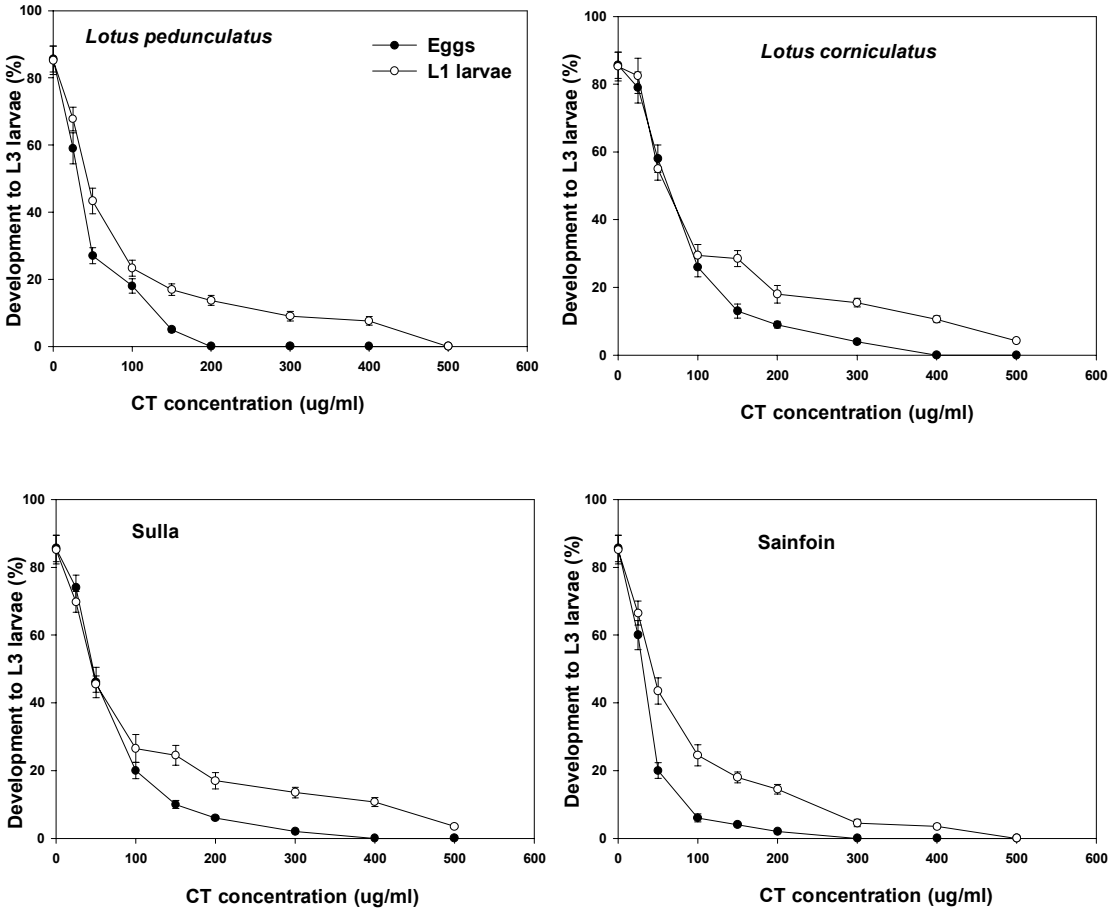
	CT concentrations in media					
	200 µg/ml			400 µg/ml		
	EH	LD	LMI	EH	LD	LMI
L. pedunculatus	34±3.3	86±1.5	21±1.4	37±2.7	91±1.4	34±2.1
L. corniculatus	23±2.7	82±2.6	15±2.0	24±3.7	87±1.3	21±1.7
Sulla	28±4.0	83±2.4	18±1.7	36±3.5	89±1.3	27±2.3
Sainfoin	31±4.5	86±1.4	32±1.4	39±4.8	96±0.7	39±1.2

Table 1 summarises the effects of the three assays for two concentrations of CT. At 400 µg/ml, CT from all the forages significantly inhibited (P<0.001) L1 larvae from attaining full development to L3 larvae and L3 larvae (P<0.001) from passing through the sieves than at 200 µg/ml.

Practical implications

This study showed that CT extracted and purified from *L. pedunculatus , L. corniculatus*, sulla and sainfoin

Figure 2 The effect of condensed tannins (CT) extracted from *Lotus pedunculatus, Lotus corniculatus,* sulla (*Hedysarum coronarium)* and sainfoin (*Onbrychus vicifolia*) on the development of eggs and first larval stage of *Trichostrongylus colubriformis* into infective larvae *in vitro*. Each point represents the mean of duplicate incubations ± SEM.



have the ability to inhibit the *in vitro* development of eggs and first stage (L1) larvae of *T. colubriformis* to L3 larvae. Applying results from this study to a practical situation suggests that CT may be able to break the life cycle of nematodes and may be able to reduce the contamination of pastures with viable eggs. Reduced numbers of infective larvae on pasture may lower our dependence on anthelmintics as the principal method for parasite control.

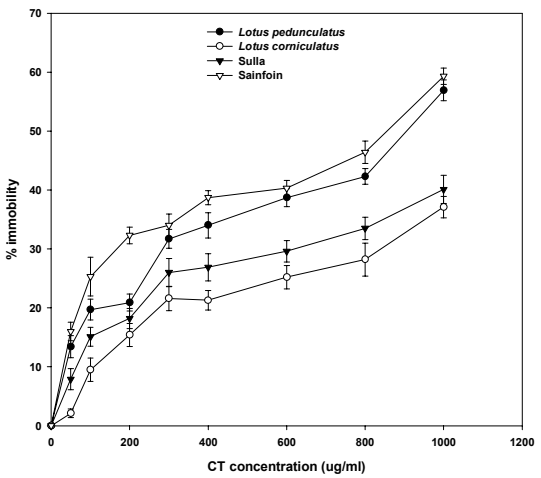
In contrast to anthelmintic drugs which are absorbed from the digestive tract (Sanyal *et al.* 1995), CT are not absorbed (Terrill *et al.* 1994) and leave the body with the faeces. This means that the eggs shed by the worms will be exposed to the effect of CT throughout their development. The concentrations of CT used in these assays are within the range of free CT (about 200–1000 µg/ml) present in abomasal and duodenal digesta and faeces of sheep fed CT-containing forages (Terrill *et al.* 1994).

This study has shown that CT are less potent as inhibitors of larval motility than larval development. For example 400 µg/ml CT from each of the four forages inhibited only 21–39% of exsheathed L3 larvae from passing through nylon mesh sieves (LMI assay; Table 1). At the same concentration, CT inhibited 24–39% of egg hatching and 100% of egg development to L3 larvae. When L1 larvae were exposed to 400 µg/ml 87–96% did not develop to L3 larvae. This indicates that L3 larvae may not be the ideal stage for testing the sensitivity to CT, possibly because L3 larvae and eggs are the only non-feeding stages of the life cycle of *T. colubriformis*. Consequently, the results of the LMI assay may be of less importance than results of the LD assay. Future work should use eggs, L1 and L2 larvae to

test materials able to inhibit the development of these stages to L3 larvae.

This study compared CT extracted from the four forages and indicated small differences in their activity against *T. colubriformis* larvae, with sainfoin being the most active followed by *L. pedunculatus*, sulla and *L. corniculatus*. The cause of differences between CT extracted from these forages is not known, but they do differ in molecular weight and structure (Foo *et al.* 1996, 1997). None of these forages are used widely in New Zealand and they require specialist establishment and management (Waghorn *et al.* 1998).

Figure 3 The effect of condensed tannin (CT) purified from *Lotus pedunculatus*, *Lotus corniculatus,* sulla (*Hedysarum coronarium*) and sainfoin (*Onbrychus vicifolia*) on larval migration inhibition (% immobility) of *Trichostrongylus colubriformis* larvae *in vitro*. Each point represents the mean of quadruplicates ± SEM.



Conclusions

In addition to the other benefits of CT for ruminant nutrition, the *in vitro* results presented here suggest that CT may also be able to disrupt the life cycle of nematodes by reducing the egg viability and larval development. If CT are able to affect helminth parasites through several routes, they offer excellent opportunities for reducing our dependence on proprietary anthelmintic drenches. However, further research will be needed to evaluate the effect of CT or CT-containing forages on larval development under field conditions.

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