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Effect of dietary tannin and protein concentration on nematode infection (*Trichostrongylus colubriformis*) in lambs

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SUMMARY

The aim of this study was to determine whether the inclusion of a condensed tannin (quebracho tannin, QT) and/or the elevation of dietary protein could reduce *Trichostrongylus colubriformis* establishment and existence in the small intestine of lambs. Thirty-six lambs (mean liveweight 32·6 ± 3·9 kg) were randomly allocated to one of six experimental groups, groups 1–5 were parasitized with a trickle infection of 3000 infective *Trichostrongylus colubriformis* larvae daily, whilst group 6 remained as uninfected controls. Experimental diets were formulated to contain 222 g CP/kg (high protein) or 97 g CP/kg (low protein) with or without the inclusion of 50 g QT/kg. All six animal groups were fed the low protein diet, group 2 fed low protein diet + QT, for one month prior to infection (groups 1–5). Once nematode eggs were observed in the faeces, diets were abruptly changed in three experimental groups. Group 1 remained on the low protein diet, group 2 remained on the low protein + QT diet, group 3 changed to the high protein diet, group 4 changed to the high protein + QT diet, group 5 changed to the low protein + QT diet and group 6 remained uninfected and fed the low protein diet. Production, haematological and parasitological parameters were monitored at regular intervals. Results show that parasitized animals fed the high protein diet achieved growth rates similar to those of uninfected low protein-fed lambs. Inclusion of dietary QT did not depress liveweight gain. Total daily faecal egg counts declined after feeding the high protein diet. Inclusion of QT into the low protein diet also reduced faecal egg counts to similar levels observed in the high protein-fed lambs. The inclusion of QT into the high protein diet did not further reduce faecal egg counts. No significant differences in the haematological parameters measured were observed between infected animals (groups 1–5), suggesting that the beneficial effect of dietary QT in the low protein diet is unlikely to be mediated through an immune response. These data suggest that the inclusion of QT in low protein diets may be an alternative to feeding high protein diets to reduce nematode burden in lambs.

INTRODUCTION

The effects of gastrointestinal parasites in ruminant animals are well documented (Parkins & Holmes 1989; Poppi *et al.* 1990; Sykes 1983, 1994; Coop & Holmes 1996). Subclinical infection depresses liveweight gain, feed intake, milk and wool production and can impair soft tissue deposition and skeletal growth. Endoparasitic control is still heavily reliant on the use of anthelmintic drugs, although the frequent administration and misuse of these drugs is leading to ever-increasing anthelmintic resistance (Pritchard 1994; Waller 1997). In tropical and subtropical regions of the world, where marginal levels of nutrition lead to greater susceptibility to infection, deaths due to nematode infections are still widely apparent (Anon 1991; Waller 1997). In many of these regions anthelmintics are either unaffordable, of inferior quality or so intensively used that extensive multiple resistance has made these drugs ineffective. Consequently, alternative methods of parasitic control are required that are practical and realistic for introduction into farm production systems. One such possibility could be the exploitation of forage species capable of reducing infection levels solely, or in conjunction with limited drug use.

Sheep infected with gastrointestinal nematodes are

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capable of mounting an immune response to the parasite, expelling the adult worms from the gut and retaining immunity against subsequent challenges. The dietary status of the host does not affect innate or initial immunity against parasitic infection (van Houtert & Sykes 1996), however, the development of immunity during challenge can be promoted by improved protein supply to the infected animal, reducing production losses and enhancing the host's resistance to further infection (Wagland *et al.* 1984; Abbott *et al.* 1986, 1988; Bown *et al.* 1991; Kambara *et al.* 1993). The presence of low concentrations of some condensed tannins in ruminant diets has been reported to reduce protein degradation in the rumen, thus increasing the protein supply to the lower gastrointestinal tract (Waghorn *et al.* 1994). The aim of this study was to determine whether the inclusion of a condensed tannin (quebracho tannin, QT) and/or the elevation of dietary protein could reduce *Trichostrongylus colubriformis* establishment and existence in the small intestine of lambs.

MATERIALS AND METHODS

Animals and diets

Thirty-six parasite-free castrate male lambs (Charolais × mule) were individually housed in raised slotted-floor pens and exposed to an 18 h light and 6 h dark cycle with the hours of darkness between 23.00 and 05.00 h. Throughout the trial the lambs were fed either a low (97 g/kg) or high (222 g/kg) protein diet with or without quebracho tannin (QT; type ATO, Hodgesons Chemicals Ltd, Beverly, Hull). The composition of the low and high protein diets are shown in Table 1. Feed was offered to the animals once daily at 08.00 h at 4% bodyweight (Kyriazakis *et al.* 1996*a*). Where appropriate, QT was incorporated into the diet at a level of 50 g/kg. The tannin was assumed to be nutritionally inert. Consequently, lambs fed the tannin-containing diets were fed proportionally (0·05) more than controls to maintain approximately constant nutrient presentation to all lambs. Any feed refusals were collected prior to the morning feed and weighed. Liveweights were recorded twice weekly and the quantity of feed adjusted after each weighing. Free access to water and mineral licks were available at all times and an ambient temperature of 15 °C was maintained throughout the trial period.

Experimental design

The lambs were maintained on a pelleted diet of dried grass until 19 weeks of age when the experimental period began (mean liveweight 32·6 ± 3·9 kg). The animals were then randomly assigned to one of six groups and fed the low protein diet either without (groups 1, 3, 4, 5 and 6) or with QT (group 2) for 31 days prior to the infection period. At 23 weeks of age, groups 1–5 were trickle infected with infective stage *Trichostrongylus colubriformis* larvae for 10 weeks (see below). Group 6 remained as uninfected controls. Following nematode establishment and maturation, as judged by the presence of eggs in the faeces (23 days post infection (p.i.)), diets were abruptly changed in groups 3, 4 and 5 to investigate the effect of increasing dietary protein and/or QT inclusion on the ability of the animals to express resistance to infection (Table 2).

Table 1. *Diet composition* (*g/kg fresh weight*)

	Low protein (L)	High protein (H)
Dried grass	50·0	397·7
Wheat straw + NaOH	77·1	47·4
Molassed beet	350·0	72·0
Barley	500·0	290·0
Fishmeal	0·0	170·0
Vitamins/minerals	22·9	22·9
Estimated crude protein	97	222
Estimated metabolizable energy (MJ/kg)	10·5	10·5

For diets containing quebracho tannin (QT), 50 g QT/kg was added to the diets prior to pelleting to give the corresponding low protein + QT and high protein + QT.
Estimated crude protein and metabolizable energy are based on figures reported in Agricultural and Food Research Council (AFRC 1993).
Vitamins/minerals

Ammonium Chloride	100 g/kg
Calcium	220 g/kg
Phosphorous P	20 g/kg
Sodium	98 g/kg
Cobalt	80 mg/kg
Iodine	200 mg/kg
Manganese	2000 mg/kg
Selenium	8 mg/kg
Zinc	2000 mg/kg
Vit A	320000 i.u./kg
Vit D3	80000 i.u./kg
Vit E	800 i.u./kg

All diets fed during the infection period (days 0 to 71 p.i.) contained 0·5 g chromic oxide (Cr_2O_3)/kg feed (Fisher Scientific UK, Loughborough, Leicestershire) as a faecal output marker (Kotb & Luckey 1972). Faecal samples (approximately 15 g) were obtained by sampling directly from the rectum at regular intervals for determination of egg counts and chromium content. Blood was collected weekly for determination of the number of eosinophils in whole blood and plasma albumin and total protein concentrations. To measure immune response capacity, animals were injected with ovalbumin (Grade V, Sigma Chemicals Co., Dorset) in the adjuvant Quil-A (Superfos Biosector a/s, Frydenlundsvey 30, DK-

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Table 2. *Dietary groupings of lambs*

Group	1 (L-L)	2 (LQT-LQT)	3 (L-H)	4 (L-HQT)	5 (L-LQT)	6 (Uninfected)
Initial diet (age 19–23 weeks)	Low	Low + QT	Low	Low	Low	Low
Diet after nematode establishment (23 days post infection)	Low	Low + QT	High	High + QT	Low + QT	Low

Low = low protein: 97 g/kg; High = high protein: 222 g/kg; + QT, quebracho tannin added at 50 g/kg.

Vedbaek, Denmark) to raise specific antibodies. The animals were subcutaneously injected at 50 days p.i. (1 mg ovalbumin and 500 µg Quil-A in 2 ml of Dulbecco's phosphate buffered saline (PBS; Sigma Chemical Co., Dorset) with a booster injection administered 10 days later. The antigen was injected at four individual sites (0·5 ml/site) along one side of the back, the booster injections being given on the other side of the back. Serum was collected 6 days after the booster injection.

Samples and techniques

Infective larvae and faecal output

Trichostrongylus colubriformis used was maintained at the Moredun Research Institute, Edinburgh. Each lamb was infected orally on 5 days/week with doses of 6000, 3000, 3000, 3000 and 6000 infective larvae given in 10 ml distilled water.

Faecal egg counts were monitored twice weekly or every second day around peak egg output from 18 days p.i. to 71 days p.i. The number of eggs per gram of faeces (EPG) was determined by the method of Christie & Jackson (1982), following centrifugal flotation in saturated NaCl using collapsible cellulose acetate tubes to separate the egg layer. The eggs recovered were expressed on a faecal dry weight basis. Total daily egg output was calculated using estimations of daily faecal output from chromic oxide concentration in the remaining grab sample. Both feed and faeces samples were dried to constant weight in a 70 °C vacuum oven, finely ground and passed through a 1 mm screen. Chromium content was determined in these samples after acid hydrolysis by atomic absorption spectrometry using a nitrous oxide-acetylene flame (Siddons *et al.* 1985). Total faecal collection, using dungbags, was undertaken on two animals from each infected group (groups 1–5) during the period of faecal sampling for egg counts to validate the estimates of total faecal output calculated from the recovery of chromium in the faeces.

Blood collection and analysis

Peripheral blood was taken post-feeding at approximately weekly intervals by jugular venipuncture. For isolation of plasma, 10 ml whole blood samples were mixed with heparin [2500 i.u./ml, Multiparvin, CP Pharmaceuticals, Wrexham] and kept on ice. An aliquot (100 µl) of heparinized whole blood was retained for eosinophil counting (Dawkins *et al.* 1989), the remaining blood was centrifuged to obtain plasma and stored at −40 °C until analysed for total protein and albumin concentrations (Burtis & Ashwood 1994). Globulin concentrations were calculated by difference. Whole blood (10 ml) was also collected into untreated tubes to obtain serum. The IgG response to injected ovalbumin was measured using a standard ELISA. In brief, flexible microtitre plates were coated with ovalbumin (5 µg/ml) in carbonate:bicarbonate buffer (pH 9·6, 200 µl/well) and incubated overnight (4 °C), then washed with PBS-Tween 20 (0.05% (v/v)). The plates were then blocked with 3% (w/v) BSA [dialysed Fraction V powder, essentially fatty acid free, Sigma Chemicals Co., Dorset] in PBS-Tween 20 for 1 h at room temperature and then washed with PBS-Tween 20. Sera were diluted 1:1600 in PBS-Tween 20 and added to triplicate wells (50 µl/well) and incubated at room temperature for 90 min before being washed with PBS-Tween 20. Fifty microlitres of donkey anti-sheep IgG conjugated to alkaline phosphatase [Sigma Chemicals Co., Dorset] diluted 1:2000 with PBS-Tween 20 were added to each well and incubated at room temperature for 90 min. Plates were then washed twice with PBS-Tween 20 before alkaline phosphatase substrate was added and the colour developed for approximately 20 min. The reaction was measured at 410 nm using a Dynatech MR5000 multiwell plate reader (Dynatech, Guernsey, Channel Islands). A standard positive serum sample was included in each ELISA plate and results were adjusted to that sample having an optical density of 1·0. A negative sample using serum from an uninfected animal obtained prior to ovalbumin injection was also included on all plates. All results expressed as optical density units were means of triplicate assays.

Statistical analysis

Data were analysed as a six-treatment completely

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randomized experiment, with six replicate animals in each treatment, using one-way analysis of variance (Genstat 5, Release 4.1; Lawes Agricultural Trust, Rothamsted). Repeated measures were used to analyse changes with time within–treatments. The Greenhouse–Geisser epsilon factor obtained from repeated measures analysis was used to adjust the degrees of freedom for time × diet interactions (Winer *et al*. 1991). Data were further partitioned into linear, quadratic and cubic trends.

Variation between the six treatments was further partitioned for the effect of parasitism (uninfected lambs (group 6) *v*. infected animals (groups 1–5)) and a 2×2 factorial was used to compare dietary protein content (high and low protein) ± QT. *t*-tests using the pooled standard error of difference between means were used to compare the effect of including QT before and after nematode infection (group 2) with those changed from the low protein diet to low protein + QT once eggs were passed in the faeces (group 5). Data were blocked for sheep. Differences were assumed to be significantly different at $P < 0{\cdot}05$.

RESULTS

Liveweight and feed intake

The mean growth rates of lambs in each dietary group over the experimental period are shown in Fig. 1. There were no statistically significant differences ($P > 0{\cdot}05$) between the mean liveweights of the animals in the six treatment groups between day 0 ($38{\cdot}1 \pm 2{\cdot}9$ kg) and day 23 p.i. ($42{\cdot}9 \pm 3{\cdot}1$ kg). Infection tended to reduce mean liveweight compared to the uninfected controls (group 6) over the period from diet change until the end of the experiment (day 23 p.i. to day 71 p.i.). Mean liveweights at the end of the trial were highest in uninfected controls (56 kg liveweight) while infected lambs maintained on the low protein diet ± QT (groups 1, 2 and 5) weighed 45·1, 45·0 and 47·7 kg respectively ($P < 0{\cdot}05$). Increasing dietary protein content from 97 to 222 g/kg ± QT (groups 3 and 4) increased mean liveweight of infected animals with the final liveweights of the high protein ± QT fed lambs (groups 3 and 4) being similar to uninfected controls (53·9 and 52·1 kg for groups 3 and 4 respectively compared with 56 kg for group 6, $P > 0{\cdot}05$, S.E.D. 3·8). The inclusion of QT in the diet did not alter the mean liveweights of the animals fed the same protein diet during the period from diet change to the end of the experiment.

Infected animals all showed some degree of inappetence during the trickle infection but no further reductions in intake were observed in lambs fed tannin-containing diets. The mean daily feed intake

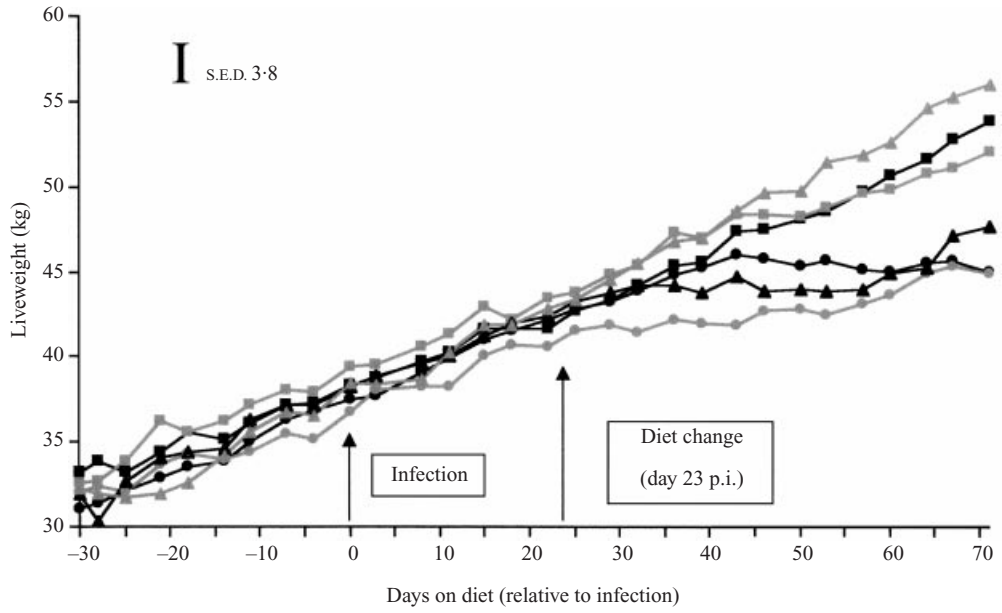

Fig. 1. Growth rate of lambs in each dietary group. Values are means of six animals in each dietary group. Lambs in groups 1, 3, 4 and 5 were maintained on the low protein diet (L, 97 g CP/kg diet) for one month prior to trickle infection with *Trichostrongylus colubriformis* (day 0). Group 2 were maintained on the low protein diet + QT (LQT). Once nematode eggs were first present in the faeces (day 23 post infection) the diet was abruptly changed in group 3 to high protein (L-H, 222 g CP/kg diet); group 4 to high protein + QT (L-HQT) and group 5 to low protein + QT (L-LQT). Group 6 remained uninfected and fed the low protein diet (L) throughout the trial. Group 1 (L-L) ●; group 2 (LQT-LQT) ●; group 3 (L-H) ■; group 4 (L-HQT) ■; group 5 (L-LQT) ▲; group 6 (uninfected) ▲.

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Table 3. *Mean daily feed intake of lambs in each dietary group* (*g feed/kg bodyweight*), *pre-infection and post infection* (*p.i.*)

Group	1 (L-L)	2 (LQT-LQT)	3 (L-H)	4 (L-HQT)	5 (L-LQT)	6 (Uninfected)	S.E.D. (30 D.F.)
Pre-infection	38·3	38·8	38·1	38·7	38·5	37·0	1·99
Days 0–23 p.i.	38·7	38·4	39·5	38·4	38·7	38·1	0·52
Days 23–71 p.i.	$31·3^{a,b}$	$30·2^{a}$	$36·4^{b,d}$	$36·0^{b,c,d}$	$31·1^{a,c}$	$38·1^{d}$	2·53

Means with different superscripts within a row are significantly different ($P<0·05$).

(Table 3) of the lambs were similar in all groups pre-infection ($P>0·05$). Feed refusals were first observed once the nematodes had established in the small intestine, after which, infected lambs maintained on the low protein diets ± QT (groups 1, 2 and 5) consumed less feed compared to those fed high protein diets ± QT (groups 3 and 4, $P<0·05$). Lambs fed the high protein diet ± QT maintained a similar feed intake to the uninfected controls.

Faecal egg counts

The faecal egg outputs of the infected groups are shown in Fig. 2. Eggs were first seen on day 22 p.i. The following day was taken as the time point for diet change in groups 3, 4 and 5. Egg counts continued to increase up to a peak around day 35 p.i. and declined thereafter. Lambs in group 1 which were maintained on the low protein diet throughout the study had a significantly higher mean number of eggs per gram of dry faeces (EPG) after the diet change (days 23–71 p.i., Table 4) than other treatment groups. The inclusion of QT into the low protein diet from day 23 p.i. onwards (group 5) resulted in these lambs passing the fewest nematode eggs, primarily due to a lower peak egg output (Fig. 2). The addition of QT to the diet prior to nematode infection (group 2) did not further reduce egg output. Increasing the protein content of the diet from 97 to 222 g/kg significantly reduced ($P<0·001$) egg output compared to the low protein fed animals (group 1). However, including tannin in the high protein diet (group 4) did not result in any further reduction in the EPG (Table 4). The uninfected controls (group 6) remained parasite-naive throughout the experiment.

All infected lambs showed an erratic pattern of excretion of soft faeces from week 4 p.i. and egg output was therefore calculated on a dry faecal basis. Total daily faecal output shown in Table 4 was estimated from the chromium concentration in the same faecal grab samples collected for egg counting. Faecal output varied between animals and was highly dependent on the level of inappetence observed in the days prior to faecal collection, and also on the diet fed. Total faecal output using dungbags indicated that the average feed intake in the two days prior to the collection of faecal material produced faecal output estimates from chromium recovery that most closely reflected those from total collection. The inclusion of tannin in the high protein diet (group 4) significantly increased ($P<0·001$) faecal dry matter output, QT did not increase faecal dry matter output in the low protein-fed lambs. Table 4 shows the estimated total daily egg outputs. The total daily nematode egg output was reduced by $>50\%$ ($P<0·01$) due to QT inclusion, both when included throughout (group 2) or introduced after worm establishment (group 5). Total daily egg output was also decreased (47%, $P<0·05$) by increasing the protein content of the diet (group 3). Interestingly the inclusion of tannin in the high protein diet (group 4) had no effect ($P>0·05$) on total egg output.

Eosinophilia

Peripheral eosinophil values remained low in all groups until day 38 p.i. and then increased with time in infected animals (groups 1–5, Fig. 3). Eosinophilia in uninfected controls (group 6) remained low throughout. Lambs fed the high protein diet + QT (group 4) showed the greatest response. The presence of QT in both high and low protein diets tended to elevate the number of eosinophils circulating in peripheral blood of infected animals (Table 4). Of infected animals, lambs fed the low protein diet throughout (group 1) had the lowest eosinophil concentration at all times.

Plasma profiles

Mean concentrations of total protein, albumin and globulin were determined for each animal at weekly intervals between 23–71 days p.i. (Table 5). An increase in total protein concentration occurred around day 18 p.i. when the worms were establishing in the gut, the concentration then declined again before increasing from day 33 p.i. onwards (as faecal egg counts began to decline). Mean total protein concentration tended to be elevated in the infected animals (groups 1–5) compared to uninfected controls

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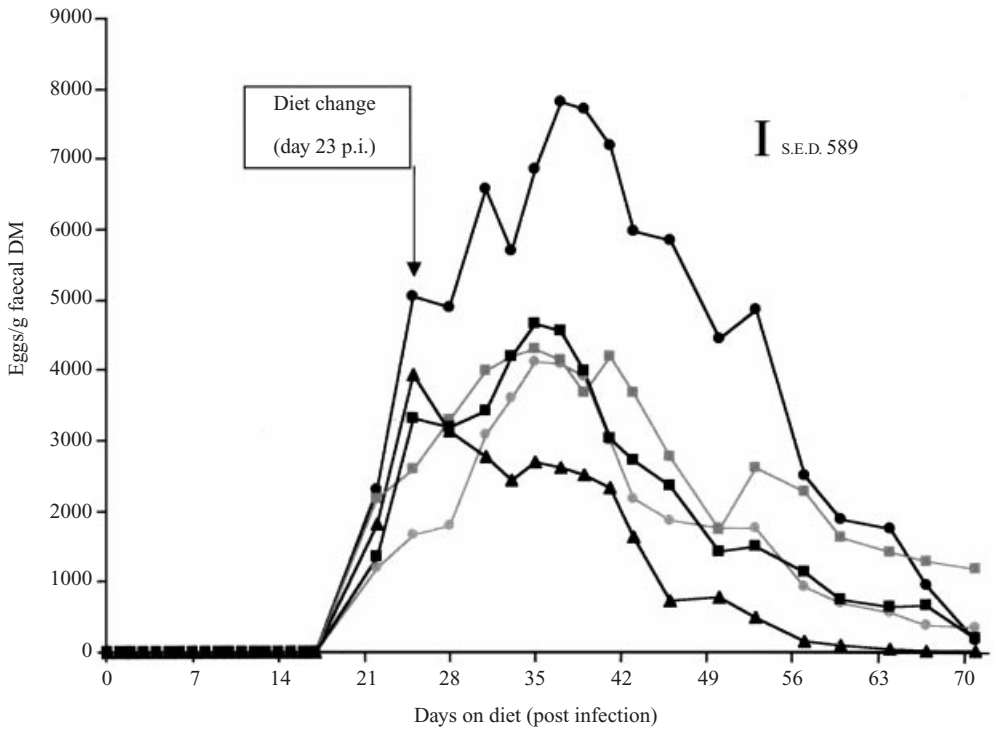

Fig. 2. Nematode eggs/g faecal DM of infected lambs (groups 1–5). Values are means of six animals in each dietary group. Lambs in groups 1, 3, 4 and 5 were maintained on the low protein diet (L, 97 g CP/kg diet) for one month prior to trickle infection with *Trichostrongylus colubriformis* (day 0). Group 2 were maintained on the low protein diet + QT (LQT). Once nematode eggs were first present in the faeces (day 23 post infection) the diet was abruptly changed in group 3 to high protein (L-H, 222 g CP/kg diet); group 4 to high protein + QT (L-HQT) and group 5 to low protein + QT (L-LQT). Group 1 (L-L) ●; group 2 (LQT-LQT) ●; group 3 (L-H) ■; group 4 (L-HQT) ■; group 5 (L-LQT) ▲.

Table 4. *Mean excretion of faeces and nematode eggs days 23–71 post infection* (*p.i.*)

Group	1 (L-L)	2 (LQT-LQT)	3 (L-H)	4 (L-HQT)	5 (L-LQT)	S.E.D. (25 D.F.)
Eggs per gram (faecal DM)	4716[a]	2077[b,c]	2454[b,c]	2904[b,c]	1575[b]	589·2
Faecal output (g DM/d)	456[a]	420[a]	429[a]	677[b]	474[a]	54·3
Daily egg output ($\times 10^4$ DM)	212·3[a]	104·8[b]	112·2[b]	204·0[a]	83·3[b]	39·52

Means with different superscripts within a row are significantly different ($P < 0{\cdot}05$).
Values represent the average of 17 time points taken from day 23–71 p.i. for 6 animals in each infected group (1–5).

(group 6). Levels of albumin present in plasma tended to decrease in the infected groups (1–5) from day 38 p.i. onwards. Mean albumin concentrations of the uninfected animals (group 6) were significantly higher than infected lambs fed the low protein diet ± QT (groups 1, 2 and 5; $P < 0{\cdot}05$). High protein ± QT fed lambs (groups 3 and 4) tended to have higher albumin concentration than the infected low protein ± QT fed lambs, giving concentrations that were significantly similar to uninfected controls (group 6). Globulin concentrations in the plasma tended to increase in parallel with those observed for total protein, the increase in plasma globulin concentrations between infected animals (groups 1–5) compared to uninfected controls (group 6) being most distinct following peak egg production ($P < 0{\cdot}01$). No difference was seen between the infected groups.

IgG response to ovalbumin

All groups made IgG responses to ovalbumin (Table 6), but overall there was no significant difference

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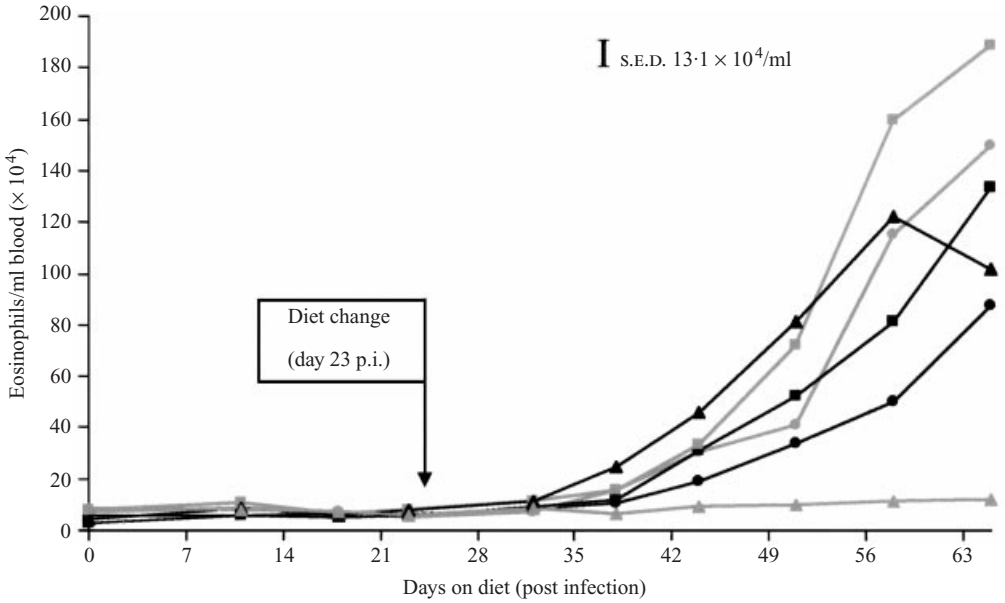

Fig. 3. Mean eosinophil concentrations of lambs in each dietary group. Values are means of six animals in each dietary group. Lambs in groups 1, 3, 4 and 5 were maintained on the low protein diet (L, 97 g CP/kg diet) for one month prior to trickle infection with *Trichostrongylus colubriformis* (day 0). Group 2 were maintained on the low protein diet + QT (LQT). Once nematode eggs were first present in the faeces (day 23 post infection) the diet was abruptly changed in group 3 to high protein (L-H, 222 g CP/kg diet); group 4 to high protein + QT (L-HQT) and group 5 to low protein + QT (L-LQT). Group 6 remained uninfected and fed the low protein diet (L) throughout the trial. Group 1 (L-L) ●; group 2 (LQT-LQT) ●; group 3 (L-H) ■; group 4 (L-HQT) ■; group 5 (L-LQT) ▲; group 6 (uninfected) ▲.

Table 5. *Mean plasma profiles days 23–71 post infection (p.i.)*

Group	1 (L-L)	2 (LQT-LQT)	3 (L-H)	4 (L-HQT)	5 (L-LQT)	6 (Uninfected)	S.E.D. (30 D.F.)
Total protein (g/l)	$6{\cdot}33^{a,b}$	$6{\cdot}56^{a,b}$	$6{\cdot}51^{a,b}$	$6{\cdot}49^{a,b}$	$6{\cdot}60^{a}$	$6{\cdot}11^{b}$	0·227
Albumin (g/l)	$2{\cdot}53^{a}$	$2{\cdot}59^{a}$	$2{\cdot}65^{a,b}$	$2{\cdot}67^{a,b}$	$2{\cdot}59^{a}$	$2{\cdot}85^{b}$	0·106
Globulin (g/l)	$3{\cdot}80^{a}$	$3{\cdot}98^{a}$	$3{\cdot}86^{a}$	$3{\cdot}82^{a}$	$4{\cdot}04^{a}$	$3{\cdot}26^{b}$	0·212
Eosinophils ($\times 10^4$/ml)	$30{\cdot}9^{a,b}$	$52{\cdot}0^{b,c}$	$46{\cdot}5^{a,b}$	$69{\cdot}7^{c}$	$56{\cdot}6^{b,c}$	$9{\cdot}0^{a}$	18·58

Means with different superscripts within a row are significantly different ($P < 0{\cdot}05$).
Values presented for total protein, albumin and globulin represent the average of 9 time points taken from days 23–71 p.i., values presented for eosinophils represent the average of 7 time points taken from days 23–71 p.i. for six animals in each group.

Table 6. *Mean IgG response to ovalbumin*

Group	1 (L-L)	2 (LQT-LQT)	3 (L-H)	4 (L-HQT)	5 (L-LQT)	6 (Uninfected)	S.E.D. (30 D.F.)
OD* (410 nm)	$3{\cdot}49^{a}$	$3{\cdot}33^{a}$	$2{\cdot}66^{a,b}$	$1{\cdot}84^{b}$	$2{\cdot}93^{a,b}$	$2{\cdot}59^{a,b}$	0·607

* Optical density scaled to a positive control having an optical density of 1·00.
Means with different superscripts within a row are significantly different ($P < 0{\cdot}05$).

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between the infected lambs and uninfected controls. Infected lambs fed the high protein + QT diet (group 4) produced the least specific IgG than any other group. The highest values were seen in the lambs fed the low protein diet ± QT diets (group 1, 2 and 5).

DISCUSSION

Liveweight gain and feed intake

The results of this study support previous observations that subclinical infections of *Trichostrongylus colubriformis* in sheep maintained on low planes of nutrition reduce performance (Poppi *et al.* 1990; Kyriazakis *et al.* 1994; van Houtert *et al.* 1995). In the present study infected animals fed a low protein diet (97 g/kg) throughout (group 1) finished the trial some 11 kg lighter than uninfected lambs fed the same diet (group 6). This was at least partly due to a reduction in feed intake in infected lambs that occurred following worm establishment in the small intestine (Kyriazakis *et al.* 1994, 1996*a*). Endogenous protein losses are also elevated in infected animals through increased mucoprotein secretion and gut tissue turnover to repair damage caused by gastrointestinal parasites (Steel *et al.* 1980; Poppi *et al.* 1986, 1990; Kimambo *et al.* 1988; Holmes & Coop 1994). Consequently there is a greater requirement for dietary protein by parasitized animals and the effect of this is accentuated by the reduction in feed intake. If dietary protein intake is not sufficient to accommodate the nutritional penalty of infection-associated endogenous losses, energy and protein are diverted away from growth. The increase in dietary protein concentration from the low protein diet at 97 g CP/kg diet to the high protein diet (222 g CP/kg diet) compensated for the fall in production due to internal parasitism in the present trial. Lambs abruptly changed to the high protein diet ± QT (groups 3 and 4, day 23 p.i.) maintained liveweight gain similar to those animals in group 6 which remained uninfected and fed the low protein diet.

Faecal egg counts

Dietary protein content is believed to have little influence on initial worm establishment in the naive host (Coop 1998) but increased levels of dietary protein are reported to be beneficial to animals with established gastrointestinal infections, promoting nematode expulsion (Coop 1998), reducing liveweight losses and enhancing the host's resistance to further infection (Wagland *et al.* 1984; Abbott *et al.* 1986, 1988; Bown *et al.* 1991; Kambara *et al.* 1993). Data from the present study support these observations. Reduced nematode burdens have also been reported in sheep grazing on forages containing condensed tannins (Niezen *et al.* 1995; Robertson *et al.* 1995). This has usually been attributed to an improved protein supply in tannin-fed animals, although it has also been suggested that the condensed tannin may be acting directly against the nematodes (Niezen *et al.* 1993, 1995). The data reported here showed that both increased dietary protein concentration and tannin inclusion reduced nematode burden but the effects were not additive. Indeed, in lambs fed the high protein diet + QT (group 4), total daily faecal egg output was similar to control animals fed the low protein diet without QT suggesting that in combination the beneficial effects of tannin and protein were neutralized. One group of lambs received QT before and after infection to determine whether condensed tannins affected worm establishment (group 2). Compared to animals fed QT after establishment (group 5), those animals that received dietary QT throughout had a depressed faecal egg count up to day 29 p.i. but no further benefit was seen thereafter suggesting that the tannin had only a slight effect of reducing worm establishment.

Condensed tannins form strong complexes with proteins and other dietary nutrients such as carbohydrates, vitamins and minerals (Makkar *et al.* 1987). While this can result in reduced nutrient availability, in ruminant animals this property is believed to be beneficial in protecting protein from degradation in the rumen, thereby increasing protein supply to the small intestine (Waghorn *et al.* 1994). It has been proposed that the improved amino acid supply in tannin-fed sheep enables them to overcome some of the effects of nematode infection. The results obtained here demonstrate that the inclusion of dietary QT in a low protein diet enhanced expulsion in terms of reduced faecal egg counts. However no improvement in liveweight gain was observed in the tannin-fed animals suggesting that protein availability had not been increased. Some studies have shown that as little as 55 g condensed tannin/kg DM can result in reduced feed intakes (Waghorn 1990), although no reduction was observed in tannin-fed animals in the present study. Some tannins, including QT, have also been reported to inhibit microbial activity in the rumen (Chiquette *et al.* 1988; Makkar *et al.* 1988; Bae *et al.* 1993; Jones *et al.* 1994). Thus at high concentrations of dietary tannin, any benefit of increased protein flow to the duodenum may be negated by reduced fibre digestion in the rumen. It is assumed that the tannin-protein complex formed in the rumen dissociates when it reaches the acidic conditions of the abomasum, releasing the protein for post-ruminal absorption (Jones & Mangan 1977; Barry & Duncan 1984; Reed 1995). The released tannin however is presumably able to complex with other proteins in the lower gastrointestinal tract including the intestinal wall, digestive enzymes and dietary protein. This may result in reduced nutrient availability in the lower gastrointestinal tract. In the present study daily faecal dry matter output was significantly increased in the animals fed the high protein + QT diet (group 4)

as also reported by Niezen *et al*. (1995). Condensed tannins reaching the lower gastrointestinal tract have been shown to affect the functional state of the mucosa, resulting in increased intestinal mucus secretion (Sell *et al*. 1985) and enterocyte proliferation (Tebib *et al*. 1994). While such changes may carry a nutritional penalty they may enhance worm rejection (Douch 1990; McClure *et al*. 1992). The reduced worm establishment in lambs fed the low protein + QT diet (groups 2 and 5) supports this view.

Blood profiles

Although the plasma profiles of total protein, albumin and globulin concentrations differed between infected and uninfected groups, no dietary interactions were seen. The fact that plasma profiles differed only in relation to parasite infection suggests that dietary tannin does not significantly increase endogenous protein secretion from the gastrointestinal tract, unless increased endogenous secretions are compensated by increased protein availability due to protected passage through the rumen.

Infection with *Trichostrongylus colubriformis* was associated with a marked elevation in peripheral eosinophilia, an important indicator of helminth infection associated with the expulsion of the parasite from the gut (Dawkins *et al*. 1989). Eosinophilia were seen only when faecal egg counts declined and there was no significant correlation with dietary protein content, in agreement with Kyriazakis *et al*. (1996*b*). However, there was a tendency for animals fed the high protein diets (groups 3 and 4) to have an elevated eosinophilia whether or not QT was present. The inclusion of QT in the diet tended to elevate eosinophilia, this being more apparent than the effect of protein in the diet. The combination of the high protein diet + QT (group 4) had an additive effect, resulting in a statistically significantly higher eosinophilia compared to the uninfected controls (group 6) and infected lambs fed the low protein diet throughout (group 1) but this had no reflection on total faecal egg counts. Although, increased numbers of tissue and circulating eosinophils have been suggested as an index of protective immune response of animals to helminth parasitism (Datta *et al*. 1998), those lambs fed the low protein + QT diets (group 2 and 5) had only a slight elevation in circulating eosinophil concentrations but a 50% decrease in total daily nematode egg output compared to infected animals fed the low protein diet throughout (group 1). This could indicate that dietary QT may act locally on the mucosa to increase the numbers of tissue eosinophils before there is an elevation in peripheral eosinophil levels (Winter *et al*. 1997). Tissue eosinophilia, as blood eosinophilia, are associated with the responsiveness of sheep infected with *Trichostrongylus colubriformis* (Rothwell *et al*. 1993), and has been implicated in the rejection of worms from the gut in secondary infection (Stevenson *et al*. 1994). However, this is not supported by those lambs fed the high protein diet + QT (group 4) which had a significant elevation in eosinophil concentrations but no decline in daily nematode egg output compared to the low protein fed animals throughout (group 1).

The level of circulating antibodies to ovalbumin was not significantly influenced by infection, protein level or QT, although, in both high and low protein diets the inclusion of QT tended to decrease the responsiveness of the animal to ovalbumin. Thus, it would appear that the inclusion of dietary QT does not elevate general immune responsiveness of the animals, making it unlikely that it reduces parasite infection in lambs via a direct effect on the immune system.

Mechanism of action

The results presented here demonstrate that the inclusion of QT in the diet of low protein fed lambs reduces nematode infection as judged by faecal egg counts. The decline in infection was similar to that seen in infected animals fed a high protein diet. The mechanism through which QT may be reducing infection is unlikely to be due to an improved protein supply to the host as no increase in liveweight gain was observed. No elevation in the immune responsiveness of QT-fed animals was seen, indicating that there is little enhancement of the immune system due to dietary QT. An alternative is that QT may be directly toxic against the worm as suggested by Niezen *et al*. (1993, 1995). Condensed tannins are produced in plant tissue as a defence mechanism reducing herbivore consumption and reducing the plants' susceptibility to pest and fungal attack (Jansman 1993). Some plants have been observed to possess nematocidal properties reducing the number of plant parasitic nematodes (Taylor & Murant 1966; Chandel & Mehta 1990). Eucalyptus species have proved effective against the abomasal nematode *Haemonchus contortus* (Bennet-Jenkins & Bryant 1996) and the inhibitory effects of several plant extracts against *Trichostrongylus colubriformis* larval motility have been attributed to polyphenolics (Lorimer *et al*. 1996). The fact that the inclusion of QT in the high protein diet showed no effect on reducing total daily faecal egg counts suggests that any beneficial effects of dietary tannin are reduced by excess protein in the diet, presumably due to the tannin being bound by excess protein. If the insoluble complex between tannin and protein at neutral pH is unable to dissociate in the acidic conditions of the abomasum and/or reforms as the pH returns toward neutral in the small intestine, there may be insufficient free tannin to exert toxicity against the parasite. Furthermore, if QT reduces infection through increased mucus production and epithelial damage,

complexing with protein may reduce these adverse effects. Studies of the direct effect of QT on the infective and adult larvae are in progress.

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