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The effects of condensed tannins supplementation of foods with different protein content on parasitism, food intake and performance of sheep infected with *Trichostrongylus colubriformis*

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The aims of the present study were to investigate (1), the potential anthelmintic properties and (2), the nutritional consequences of commercially available condensed tannins on parasitised sheep fed, *ad libitum*, either a high- or a low-protein food. For this purpose, forty-eight previously parasite-naïve sheep (*n* 12) were infected with 2000 *Trichostrongylus colubriformis* larvae/d for a 67-d experimental period. Two experimental foods were made: a low (L), formulated to be inadequate in meeting the requirements of growing sheep for metabolisable protein (MP), and based on wheat, citrus pulp, and oatfeed; a high (H), expected to be above the requirements of growing sheep for MP, based on similar ingredients but supplemented with protected soyabean meal. Two additional foods were made by adding 60 g *Quebracho* (a condensed tannins (CT) extract)/kg fresh matter to foods L and H (foods LQ and HQ respectively). This level of *Quebracho* supplementation has been previously shown to reduce the level of parasitism in restrictedly fed, parasitised sheep. The experiment was divided into two periods: period 1 (P_1, day 1–38) and period 2 (P_2, day 39–67), each one associated with different phases of an intestinal parasitic infection. Six sheep from each group were slaughtered at the end of P_1, and the remaining sheep were slaughtered at the end of P_2 (day 67). Although faecal egg counts (FEC; number of parasite eggs/g faeces) and total egg output were reduced in sheep offered the supplemented foods during P_1 ($P<0{\cdot}05$), worm burdens on day 38 were unaltered. Neither *Quebracho* supplementation nor food protein content during P_2 affected FEC and worm burdens. Food intake and performance were higher in sheep offered food HQ compared with sheep offered food H ($P<0{\cdot}05$); no differences were observed in sheep offered foods LQ and L throughout the experiment. The previously shown anthelmintic properties of CT were not observed following *ad libitum* intake of either low- or high-protein foods supplemented with *Quebracho* extract. Higher levels of CT supplementation may be required to reduce parasitism and consequently improve the performance of parasitised sheep, when fed *ad libitum*. Supplementation with CT conferred advantages on the performance of parasitised sheep on a high- but not on a low-protein food.

Food intake: Nematodes: Protein: *Quebracho*: Sheep: Tannins

The control of helminth infections in both ruminant and non-ruminant farm animals has traditionally been anthelmintic drug-dependent. However, the development of anthelmintic-resistant strains of parasites (Jackson & Coop, 2000), which is particularly prevalent for small-ruminant nematodes, the increasing concern for drug residues in animal products and the growth of organic farming systems have increased the need for alternative sustainable approaches to control parasitism. Among the alternatives under consideration, the use of plants with anthelmintic properties appears to be a promising approach for controlling gastrointestinal parasitism in both temperate and tropical climates (Niezen *et al.* 1996). Although many of the active plant metabolites involved have not yet been identified, there is evidence that the consumption of forages high in condensed tannins can reduce parasitism in grazing

Abbreviations: FEC, faecal egg count; FM, fresh matter; H, high-protein food; HQ, high-protein food+*Quebracho* extract; L, low-protein food; LQ, low-protein food+*Quebracho* extract; L_3, infective larvae; MP, metabolisable protein; P_1, period 1; P_2, period 2; VH, very-high-protein food.

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and browsing ruminants (Niezen *et al.* 1998; Hoskin *et al.* 1999). Recent *in vitro* and *in vivo* studies suggest that condensed tannins extracts can affect directly different nematode parasites of sheep and red deer (Molan *et al.* 2000; Athanasiadou *et al.* 2000*a*, 2001).

Condensed tannins are the most widespread fraction of tannins, which are phenolic secondary plant metabolites. The presence of condensed tannins, mainly in the woody and to a lesser extent in the leafy parts of the plants, has been thought to act as a defence against insect and mammalian herbivores (McArthur *et al.* 1991). Nevertheless, the biological significance of condensed tannins has not been clearly defined, since the consumption of condensed tannins can lead to both detrimental and beneficial consequences in ruminants. High concentrations of condensed tannins in forages have been considered responsible for reducing food digestibility and intake (Barry & Duncan, 1984) of grazing ruminants; on the other hand, moderate concentrations appear to increase wool growth (Wang *et al.* 1996) without any effect upon food intake of grazing sheep. Regarding the anthelmintic properties of condensed tannins, current research is focused in reducing the level of parasitism and consequently improving performance in parasitised hosts.

In a recent study, a condensed tannins extract (*Quebracho* extract) was incorporated into a high-protein food and fed to parasitised sheep for a period of 10 weeks (Athanasiadou *et al.* 2000*b*). Although parasitic worm burden and faecal egg counts (FEC) were lower in sheep offered the tannin-supplemented foods than in sheep offered the unsupplemented foods, live-weight gain of sheep on both foods was similar throughout the experiment. This was contrary to the expectation that live-weight gain of parasitised sheep offered the supplemented food should have increased, since sheep performance is directly related to the level of parasitism (Coop *et al.* 1982). These findings were consistent with data from a previous study, where *Quebracho* extract was added to both low- and high-protein foods of sheep parasitised with the same nematode (Butter *et al.* 2000). In this case, condensed tannins supplementation was expected to improve the performance of the host, not only by reducing the level of parasitism, but also by increasing protein availability in the small intestine of the hosts offered the low-protein foods. The latter was expected because condensed tannins have the ability to protect dietary protein from rumen degradation (Mangan, 1988). The unexpected lack of improved performance of sheep in both the above studies was assumed to be attributable to the adverse effects of condensed tannins, especially upon DM digestibility (Butter *et al.* 2000). Although sheep in both studies were offered high-energy foods, they were not able to overcome this adverse effect of condensed tannins by modifying their intake (Kyriazakis & Emmans, 1995), because they were offered restricted amounts of the foods.

The aim of the present study was to investigate whether *ad libitum* food intake of low- and high-protein foods supplemented with *Quebracho* extract would reduce the level of parasitism in sheep, during the initial worm establishment and when an established parasitic infection was present. To the best of our knowledge, this is the first study where a condensed tannins extract has been offered to parasitised sheep fed *ad libitum*. As a consequence of the reduced level of parasitism, the performance of parasitised sheep was expected to be improved. The benefit on the performance of sheep was expected to be larger in sheep offered the low-protein-supplemented food than in those offered the high-protein-supplemented food, as the former could also benefit from the extra protein released post-ruminally. We hypothesised that when tannin-supplemented foods were offered *ad libitum*, sheep would have the opportunity to eat to the extent required to overcome any adverse consequences of condensed tannins upon digestibility.

Materials and methods

Animals

Fifty-four Texel × Greyface female sheep were used. They were reared indoors under conditions that minimised the likelihood of nematode infections. After weaning at 8 weeks of age (mean live-weight 29·5 (SD 4·60) kg), they were transferred to individual pens. The experimental unit was a concrete-floored animal shed and contained two rooms of forty-eight and sixteen pens respectively. Sheep were randomly allocated to forty and fourteen pens of each room respectively. All pens contained a food trough and either a water bowl or an automatic drinker, which gave free and continuous access to water. Sheep also received a minimum of 16 h artificial light/d. The experiment took place from July to September 1999 at latitude 51° N.

Once penned, sheep were injected with a coccidiostat (Bimalong 25 %, 2 ml subcutaneously; Cross Vetpharm Group Ltd, Dublin) and a Clostridia/*Pasteurella* vaccine (Heptavac, 2 ml subcutaneously; Hoechst Roussel Vet Ltd, Milton Keynes, Bucks.). During a 2-week acclimatisation period they were offered a high-quality pre-experimental food *ad libitum*, with 876 g DM and a calculated yield of 102 g metabolisable protein (MP) and 11·7 MJ metabolisable energy/kg DM. One week before the start of the experiment, sheep were drenched with benzamidazole (Panacur 10 %; Hoechst Roussel Vet Ltd, Milton Keynes, Bucks.) at 0·2 ml/kg live weight.

Condensed tannins

Cold-soluble *Quebracho* extract was used as the source of condensed tannins (*Quebracho* ATO; Roy Wilson Dickson Ltd, Chester, Ches.). This type of *Quebracho* extract contains 793 g condensed tannins/kg DM and a small amount of simple phenolics. It is a fine powder, which in the present experiment was added in the foods of sheep at 60 g/kg fresh matter (FM), before pelleting. The above concentration was comparable with concentrations of condensed tannins found in plants, and has been considered responsible for reducing the parasitic burden of infected sheep when included in their foods (Athanasiadou *et al.* 2000*b*). As certain types of condensed tannins are sensitive to oxidation at temperatures around 100°C (Larrauri *et al.* 1997), the pelleting of the foods was performed at the lowest possible temperature (approximately 80°C), similar to temperatures used in previous studies (Athanasiadou *et al.*

2000*b*; Butter *et al.* 2000), where the anthelmintic properties of *Quebracho* have been observed.

Experimental foods

Three basal foods were formulated (Table 1). Foods L, H and VH were calculated to yield 49, 115 and 148 g MP/kg DM respectively and were formulated to be isoenergetic. Food L was formulated to be inadequate in meeting the MP requirements of growing sheep, when offered *ad libitum*, but also inadequate for an optimum microbial growth in the rumen (Agricultural Research Council, 1980). Foods H and VH were formulated to be above the MP requirements in growing sheep. Food VH was included to ensure that food H was indeed not limiting for growth, as parasitism imposes nutrient (protein) demands on the host. If these increased requirements are not met, then not only the growth of the animal would be penalised, but also its ability to maintain an effective immune response would be compromised (Coop & Kyriazakis, 1999) In addition, foods L and H were supplemented with 60 g *Quebracho* extract/kg FM to form foods LQ and HQ respectively.

Before pelleting the foods, a quantity from each food was supplemented with 19·6 g acid insoluble ash (Fine; BDH, Lutterworth, Leics.)/kg FM, as a marker to estimate DM digestibility of the foods (see p. 700).

Infective larvae

Sheep were trickle-dosed with infective larvae (L_3) of *Trichostrongylus colubriformis*, a common intestinal nematode of sheep in temperate climates. L_3 were harvested using a standard Baerman procedure after a 10-d incubation period (20–21°C) of faeces collected from monospecifically infected donor sheep. The strain used to infect the donor sheep was the Moredun ovine anthelmintic susceptible strain. Following the harvesting of L_3, larvae were maintained in tap water (1000 L_3/ml) at 4°C, until oral administration to sheep.

Experimental design

One day before the start of the experiment, sheep were allocated into one of five groups, taking into account their live weight (mean live weight 32·2 (SD 5·23) kg). From day 1 of the experiment, sheep from each group were offered one of the five experimental foods *ad libitum* (*n* 12 for L, LQ, H, and HQ groups; *n* 6 for VH group). Sheep were infected with 2000 L_3 *T. colubriformis*/d, 5 d/week, throughout a 67-d experimental period. This intake of larvae is within the range that young sheep may ingest through grazing moderately contaminated pasture, and is capable of establishing a subclinical infection in growing sheep (Coop *et al.* 1979; Kyriazakis *et al.* 1994).

The experiment was divided into two periods: P_1 (days 1–38) and P_2 (days 39–67). In *T. colubriformis* infections the first period has been associated with high worm establishment (acquisition of immunity) in previously parasite-naïve sheep (Dobson *et al.* 1990*a*). At the end of this period (day 38) six sheep from groups L, LQ, H and HQ were slaughtered and their intestines removed for worm recovery (see pp. 702–703). This was expected to provide evidence for a reduced level of parasitism attributable to a direct anthelmintic effect of condensed tannins. During P_2, the acquired immunity of the host is expected to be expressed (Dobson *et al.* 1990*b*); hosts reduce the numbers of incoming larvae and the fecundity of adult worms, and eventually reject the adult worm population. On the last day of the experiment (day 67) all remaining sheep were slaughtered and their small intestines removed for worm

Table 1. Ingredients and composition of low- (L), high- (H) and very high- (VH) protein foods offered *ad libitum* to 10-week-old sheep trickle-infected with 2000 *Trichostrongylus colubriformis* infective larvae/d

Foods …	L	H	VH
Ingredients (g/kg fresh matter)			
Wheat	350	276	170
Oatfeed	194	207	207
Citrus pulp	347	210	160
Soypass (protected soyabean meal)	0	100	200
Hi-pro soya	0	98	155
50% fat premix	25	25	25
Molasses (CMS 20)*	50	50	50
NaCl	10	10	10
Dicalcium phosphate	9	4·2	1·1
Limestone flour	13	18	20
Minerals and vitamins mix†	2·5	2·5	2·5
Composition (g/kg DM)			
DM	874	877	879
Crude protein	80	188	228
Metabolisable energy (MJ/kg DM)	11·4	11·4	11·4
Fermentable metabolisable energy (MJ/kg DM)	10·3	10·3	10·3
Effective rumen degradable protein	59	104	133
Digestible undegraded protein	11	49	82

* CMS 20 soluble condensed molasses supplied by Internal, Cobham, Surrey, UK.
† Minerals and vitamins mix supplied by Scotmin Ltd, Ayr, Scotland, UK.

recovery. Information expected to be obtained during the second experimental period was related to possible further reduction in level of parasitism and as a consequence improved performance of parasitised sheep.

In order to estimate the DM digestibility of the foods, sheep were introduced to the foods that contained the marker on days 15 and 45 of the experiment. The first week was considered a period of acclimatisation and faecal samples were collected during 5-d periods of P_1 (days 22–27) and P_2 (days 52–57) of the experiment (see p. 700).

Measurements

Food intake was recorded daily. Food refusals were weighed early in the morning and then the food troughs were refilled with fresh food. Sheep were weighed on the first day of each week before the morning feeding; last live-weight recording was conducted on day 64 of the experiment. Faecal samples were collected on the third day of each week directly from the rectum; they were then either processed immediately or stored at 4°C and processed within 2 d. Nematode eggs/g fresh faeces were estimated using a modified flotation technique (Christie & Jackson, 1982); polyallomer centrifuge tubes (Beckman Coulter UK Ltd, High Wycombe, Bucks, UK) were used to separate the meniscus containing the eggs.

For determination of DM digestibility, faecal samples of 10–15 g were collected daily from each sheep, during days 22–27 and 52–57. At the end of the collection periods, faeces were stored at −20°C until analysis for acid-insoluble ash. DM of the faeces was determined for each sheep for every day of the collection periods to determine total egg output (see p. 704). Samples from each collection period were mixed thoroughly for each sheep and subsamples were used for analysis. Food digestibility was estimated by calculating the amount of acid-insoluble ash contained in the foods and faeces during the two faecal collection periods of the experiment. The ash residue of the samples was estimated by incineration at 500°C. Acid-insoluble ash was determined after washing the ash residue of the foods or faeces by HCl (25 ml HCl/100 ml water) (Helrich, 1990).

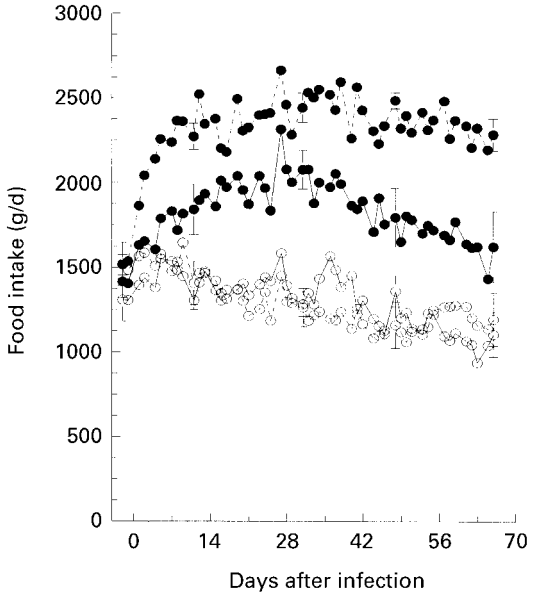

Fig. 1. Daily voluntary food intake of sheep parasitised with 2000 *Trichostrongylus colubriformis* infective larvae/d, 5 d/week, and offered either a low- (○) or a high- (●) protein food supplemented with either 0 (—) or 60 g (---) *Quebracho* extract/kg fresh matter. The vertical bars indicate standard errors.

Following the estimates of DM digestibility, total faecal output and total egg output were calculated. Total faecal output was expressed in g dry faeces/d. Total egg output represented the total amount of *T. colubriformis* eggs excreted daily (eggs in DM faeces/d). This is considered a more reliable indicator for monitoring the parasitic infection than FEC, which is a concentration number.

At slaughter, the small intestines of sheep were ligated, removed and the worms were recovered from digesta and the intestinal mucosa following incubation in physiological saline (8·5 g NaCl/l) at 37°C for 4 h. The total worm burden and sex ratios were estimated from 2 % samples of digesta and mucosal digest from each sample. *Per capita* fecundity was calculated by dividing the FEC recorded the day before slaughter by the number of female worms recovered on the slaughter day; this is widely used as an indicator of the fecundity of female worms.

Statistical analysis

All data collected were analysed by Genstat 5, release 3.2 (Lawes Agricultural Trust, 1993). Before any further analysis, comparisons were made between sheep offered foods H and VH over the 67-d experimental period (n 6) to ensure food H was indeed not limiting for the growth of parasitised sheep. Following this, statistical analysis focused on data from L, LQ, H and HQ groups.

Live-weight gain (g/d) was determined by linear regression for each sheep. During days 15–27 and 45–57, when sheep were offered the foods that contained the marker, daily food intake was corrected for the consumption of acid-insoluble ash. Live-weight gain, mean food intake and food conversion efficiency for groups L, LQ, H and HQ were analysed (1), for P_1 and P_2 with six sheep per treatment (sheep that remained in the experiment throughout), by a two-way ANOVA within a repeated measurement analysis (split plot), using protein level and *Quebracho* extract as factors, and (2), for P_1 only, with twelve sheep per treatment by a two-way ANOVA using the protein level and *Quebracho* extract as factors. The latter analysis was performed to benefit from increased statistical power during this period (n 12). Digestibility and total faecal and egg output were analysed as above: for the two faecal collection periods (n 6, days 22–27 and 52–57 of the experiment) by a repeated measurement structure, or for the first collection period only (n 12, days 22–27).

FEC were analysed separately for P_1 (n 12) and for the whole experimental period (P_1 and P_2, n 6 for sheep that remained in the experiment throughout), by an antedependence model for repeated measurements (Kenward, 1987) with protein level and *Quebracho* extract in the foods as factors. Worm burden and *per capita* fecundity were analysed by two-way ANOVA with protein level and

Quebracho as factors for each slaughter point separately. FEC, worm burden, *per capita* fecundity and total faecal and egg output were transformed before analysis (log x + 1) to stabilise the variance. For transformed data, back-transformed means with 95 % CI (lower and upper limit) are reported.

Results

In most cases, the results obtained by the two-way ANOVA for P_1 (*n* 12) were not different from those obtained by the repeated measurement analysis (P_1-P_2). Unless otherwise stated, results from P_1 will not be reported separately.

Food intake

Daily food intake of sheep offered L, LQ, H and HQ over the whole experimental period (*n* 6) is presented in Fig. 1. There were no differences observed between sheep offered foods H and VH (mean over the whole experimental period, 1813 *v.* 1937 g/d respectively; SED 245 g/d). Sheep offered the H foods (H and HQ foods) had higher mean food intake compared with sheep offered the L foods (L and LQ foods) over the whole experiment (Table 2). Average effect of *Quebracho* supplementation was statistically significant. However, a significant protein × *Quebracho* interaction indicated that sheep offered food HQ had significantly higher intake compared with sheep offered food H, whereas the intakes of sheep offered foods LQ and L were very similar. Mean food intake of all sheep was significantly decreased during P_2 compared with P_1. Interactions with period were not significant.

Live-weight gain and food conversion efficiency

Sheep offered foods H and VH had similar live-weight gain throughout the experimental period (mean live-weight gain, 199 *v.* 207 g/d respectively; SED 52 g/d). Although sheep (*n* 12) offered the *Quebracho*-free foods showed significantly higher live-weight gain than sheep offered the *Quebracho*-containing foods (218 *v.* 174 g/d respectively, SED 17 g/d, $P<0{\cdot}05$) during P_1, this effect was not significant over the whole experimental period (Table 2, where *n* 6). Sheep on the H foods showed higher live-weight gain compared with sheep on the L foods over P_1 and P_2. A significant protein × *Quebracho* interaction indicated that sheep offered food HQ had significantly higher live-weight gain compared with sheep offered food H, whereas live weights of sheep offered foods LQ and L were similar. Live-weight gain during P_2 was significantly reduced in all sheep compared with live-weight gain during P_1. However, the reduction in live-weight gain of sheep offered the H foods was significantly more pronounced compared with the reduction in sheep offered the L foods. In addition, the reduction observed in live-weight gain of sheep during P_2 was more severe in sheep offered *Quebracho*-free foods than in sheep offered *Quebracho*-containing foods.

Food conversion efficiencies of sheep offered L, LQ, H and HQ foods throughout the experiment are also reported in Table 2. Although during P_1 (*n* 12) sheep on *Quebracho*-free foods converted food significantly more efficiently than sheep on *Quebracho*-containing foods (0·122 *v.* 0·083 g gain/g food respectively; SED 0·009 g gain/g food, $P<0{\cdot}001$), this effect only tended to be significant during P_1 and P_2 ($P=0{\cdot}093$, where *n* 6). Sheep offered the H foods converted food significantly more efficiently compared with sheep offered the L foods over P_1 and P_2. However, sheep offered food LQ tended to convert

Table 2. Mean voluntary food intake (VFI), live-weight gain (LWG) and food conversion efficiency (FCE) of sheep infected with 2000 *Trichostrongylus colubriformis* infective larvae/d and offered foods low (L) or high (H) in protein supplemented (+) or not (−) with 60 g *Quebracho* extract per kg FM, throughout a 67-d experimental period*

Time period†	Protein	*Quebracho*	VFI (g/d)	LWG (g/d)	FCE (g gain/g food)
P_1 (*n* 6)	L	−	1410	132	0·094
		+	1353	51	0·037
	H	−	1936	297	0·152
		+	2400	323	0·134
P_2 (*n* 6)	L	−	1170	44	0·031
		+	1181	44	0·032
	H	−	1734	100	0·051
		+	2373	168	0·071
SED			164	32·3	0·015
Statistical significance (*P*):					
Quebracho			<0·05	>0·1	<0·1, >0·05
Protein			<0·001	<0·001	<0·001
Time period			<0·001	<0·001	<0·001
Quebracho × protein			<0·05	<0·05	<0·1, >0·05
Quebracho × time period			>0·1	<0·05	<0·01
Protein × time period			>0·1	<0·001	<0·01
Three-way interaction			>0·1	>0·1	>0·1

* For details of diets and procedures, see Table 1 and p. 698.
† P_1 has been associated with high worm establishment (days 1–38) and P_2 has been associated with the expression of host immunity towards parasites (days 39–67).

food less efficiently than sheep on food L, whereas sheep on H and HQ foods had similar food conversion efficiencies (P=0·074). Although sheep from all feeding treatments converted food less efficiently during P_2 compared with P_1, the reduction in food conversion efficiency during P_2 was significantly more pronounced in sheep offered the H foods compared with those offered the L foods. In addition, the reduction observed in food conversion efficiency of sheep during P_2 was significantly more severe in those offered the *Quebracho*-free foods compared with sheep offered the *Quebracho*-containing foods.

Digestibility and total faecal output

DM digestibility and backtransformed data of mean faecal output are presented in Table 3. DM digestibility and total faecal output of sheep offered foods H and VH were similar over the whole experimental period (digestibility 0·70 *v.* 0·76; SED 0·05 for sheep offered H and VH foods respectively). The addition of *Quebracho* extract did not alter DM digestibility. There was a significant increase in digestibility of all foods on days 52–57, compared with digestibility observed during days 22–27. Period × *Quebracho* interaction did not affect DM digestibility.

Mean faecal output was significantly higher in sheep offered the H foods compared with sheep offered L foods. A significant reduction in total faecal output was observed during days 52–57 compared with days 22–27 of the experiment. This reduction was significantly more pronounced in sheep offered the L foods (days 22–27, 476 (95 % CI 428, 528) *v.* days 52–57, 213 (95 % CI 191, 236) g dry faeces/d) compared with sheep offered the H foods (days 22–27, 676 (95 % CI 594, 770) *v.* days 52–57, 508 (95 % CI 445, 578) g dry faeces/d). The addition of *Quebracho* extract in the foods did not affect the total faecal output of sheep throughout the experiment.

Faecal egg counts and total egg output

FEC of sheep offered foods H and VH were similar during P_1 and P_2 (e.g. day 59 of the experiment, 503 (95 % CI 320, 788) *v.* 940 (95 % CI 600, 1470) eggs/g faeces respectively). Backtransformed FEC of sheep offered L, LQ, H and HQ foods for P_1 (n 12), and P_1 and P_2 (n 6) are presented in Fig. 2 (a and b). During P_1, sheep offered the *Quebracho*-containing foods had significantly lower FEC compared with sheep offered the *Quebracho*-free foods (Fig. 2(a)). There were no differences in FEC of sheep offered L or H foods, and protein × *Quebracho* interaction was not significant during P_1. There were no differences observed in FEC of sheep offered foods that differed in protein level or *Quebracho* content, over P_1 and P_2 (Fig. 2(b)).

Backtransformed total egg outputs of sheep are presented in Table 3. Total egg outputs of sheep offered foods H and VH were similar (for days 52–57, 7·2 (95 % CI 5·2, 10·0) *v.* 10·5 (95 % CI 7·6, 14·6) × 10^4 eggs/d, respectively). Sheep offered the *Quebracho*-containing foods tended to have lower total egg output compared with sheep offered the *Quebracho*-free foods. A significant protein × period interaction indicated that sheep on the H foods increased their total egg output during days 52–57 (6·3 (95 % CI 5·0, 8·1) × 10^4 eggs/d), compared with days 22–27 (3·0 (95 % CI 2·4, 3·6) × 10^4 eggs/d); sheep offered the L foods had similar egg output during both periods (3·0 (95 % CI 2·4, 3·8) *v.* 4·2 (95 % CI 3·4, 5·1) × 10^4 eggs/d respectively).

Table 3. Mean dry matter digestibility, total faecal output (TFO, backtransformed data) and total egg output (TEO, backtransformed data), calculated by the addition of acid-insoluble ash in the foods of sheep infected with 2000 *Trichostrongylus colubriformis* infective larvae/d and offered foods low (L) or high (H) in protein supplemented (+) or not (−) with 60 g *Quebracho* extract per kg FM, throughout a 67-d experimental period*

Time period	Protein	*Quebracho*	Digestibility (kg digested DM/kg food DM)	TFO† (g dry faeces/d)	TEO (×10^4)† (eggs excreted/d)
Days 22–27	L	−	0·59	578	5·4
		+	0·58	539	3·6
	H	−	0·63	713	5·6
		+	0·67	765	2·9
Days 52–57	L	−	0·78	239	3·4
		+	0·81	214	3·3
	H	−	0·77	376	7·3
		+	0·72	619	10·6
SED			0·06	−	−
Statistical significance (P):					
Quebracho			>0·1	>0·1	<0·1, >0·05
Protein			>0·1	<0·001	>0·1
Time period			<0·001	<0·001	>0·1
Quebracho × protein			>0·1	>0·1	>0·1
Quebracho × time period			>0·1	>0·1	>0·1
Protein × time period			<0·1, >0·05	<0·01	<0·05
Three-way interaction			>0·1	>0·1	>0·1

* For details of diets and procedures, see Table 1 and p. 698.
† For the 95 % CI, see p. 702.

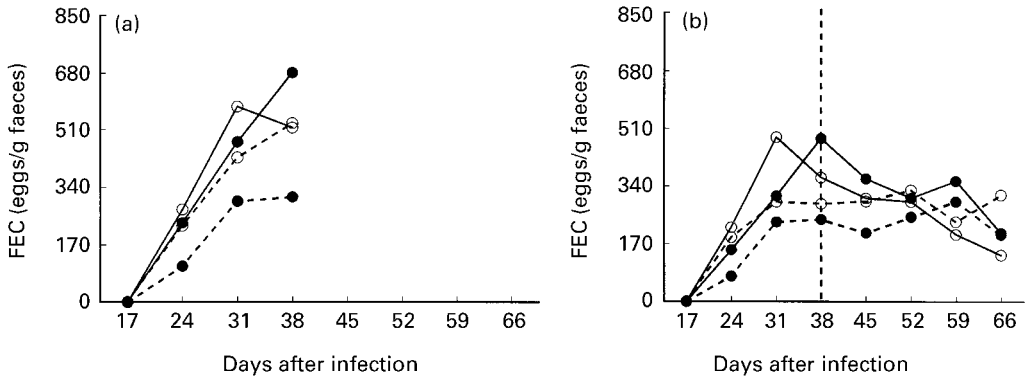

Fig. 2. Backtransformed means of faecal egg counts (FEC) of sheep infected with 2000 *Trichostrongylus colubriformis* infective larvae/d, 5 d/week, and offered either a low- (○) or a high- (●) protein food, supplemented with either 0 (—) or 60 g (---) *Quebracho* extract/kg fresh matter during (a) period 1 (days 1–38, *n* 12) and (b) period 2 (days 39–67, *n* 6). For indication of the variation within each treatment (95 % CI), see p. 702.

Worm burdens and per capita fecundity

At the first slaughter point (day 38) worm burdens, sex ratio and immature stages of parasites between sheep on different feeding treatments were very similar (Fig. 3(a)). At the end of the experiment (day 67) worm burdens between sheep offered foods H and VH were similar (total worm burden, 13 945 (95 % CI 12 502, 15 555) *v.* 12 639 (95 % CI 12 331, 14 098) worms respectively). Worm numbers recovered from sheep offered the L foods were significantly lower compared with worm numbers recovered from sheep offered the H foods (11 550 (95 % CI 10 492, 14 696) *v.* 15 533 (95 % CI 14 110, 17 100) worms respectively, $P<0{\cdot}05$). Sheep that consumed *Quebracho*-containing foods had higher worm burdens than sheep on *Quebracho*-free foods (16 030 (95 % CI 14 637, 17 553) *v.* 11 133 (95 % CI 10 166, 13 998) worms respectively, $P<0{\cdot}01$). The protein × *Quebracho* interaction was not significant.

There was no difference observed in the fecundity of worms between sheep offered foods H and VH. *Per capita* fecundity of female worms, at the end of P_1, was lower in sheep offered the H foods, compared with sheep offered the L foods (0·09 (95 % CI 0·07, 0·10) *v.* 0·12 (95 % CI 0·11, 0·14) eggs/female respectively, $P<0{\cdot}05$). However, a significant protein × *Quebracho* interaction was present indicating that fecundity of female worms was lower in sheep offered food HQ than in sheep offered food H (0·05 (95 % CI 0·03, 0·06) *v.* 0·12 (95 % CI 0·11, 0·14) eggs/female respectively, $P<0{\cdot}001$); *per capita* fecundity of female worms was similar in sheep offered foods L and LQ. Fecundity of female worms at the end of the experiment was not affected by protein or *Quebracho* extract in the foods of sheep.

Discussion

To the best of our knowledge, this is the first experiment where the consequences of *ad libitum* intake of foods supplemented with a condensed tannins extract on parasitism of sheep were investigated. The *ad libitum* intake of parasitised sheep on either high- or low-protein foods supplemented with *Quebracho* extract did not cause

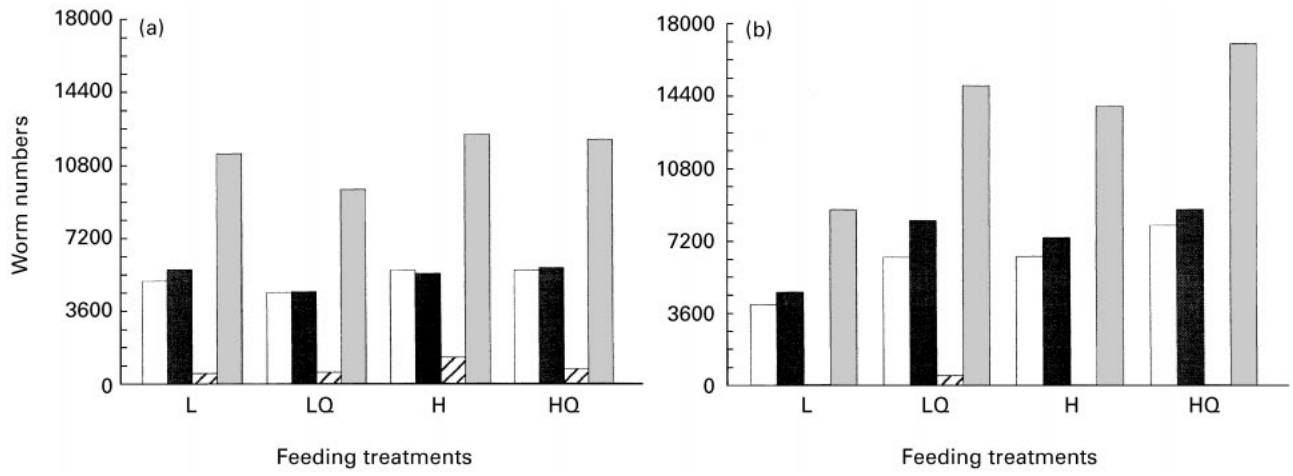

Fig. 3. Backtransformed means of adult male (□), adult female (■), immature (▨) and total worm burdens (■) of sheep infected with 2000 *Trichostrongylus colubriformis* infective larvae/d, 5 d/week, and offered either a low- (L) or a high- (H) protein food, or the L and H foods supplemented with 60 g *Quebracho* extract/kg fresh matter (LQ and HQ respectively) at the end of (a) period 1 (day 38, *n* 6) and (b) the end of period 2 (day 67, *n* 6). For indication of the variation within each treatment (95 % CI), see pp. 702–703.

the expected reduction in the level of intestinal parasitism in sheep. Such a reduction has been previously seen on parasitised sheep offered *Quebracho*-supplemented foods, on a food-restricted basis. As a result, the performance of sheep offered the supplemented and unsupplemented foods was similar. Although the performance of sheep offered the low-protein food supplemented with *Quebracho* extract was expected to be further improved due to the extra protein flow in their small intestine, the results did not support this hypothesis either.

Faecal egg counts and worm burden

An 'anthelmintic' effect of *Quebracho* extract towards *T. colubriformis* was only evident on FEC of sheep offered the *Quebracho*-containing foods, during the first experimental period (days 1–38). The reduction in FEC could be attributed to a dilution of the faeces, due to the addition of the 'inert' *Quebracho* extract in foods LQ and HQ. However, since total egg output was also reduced during P_1, the reduction in FEC observed during the same period should be mainly ascribed to the effects of condensed tannins. Since this reduction was observed within the first 5 weeks of the infection, it is likely that it could be attributed to a direct effect of *Quebracho* towards the parasites *per se* and was not a consequence of an enhanced immune response, that could result from an increased protein flow (van Houtert *et al.* 1995). In addition, it appears that only the fecundity or the egg-laying ability of female worms was affected by *Quebracho* extract, which is in agreement with previous observations (Athanasiadou *et al.* 2000*b*), since worm burdens recovered at day 36 of the experiment were not reduced by the consumption of the supplemented foods.

The lack of a direct anthelmintic effect of *Quebracho* extract on worm burden at the end of P_1 and P_2, and on FEC during P_2 contradicts previous evidence for a direct effect of *Quebracho* towards a *T. colubriformis* population over a 10-week experimental period (Athanasiadou *et al.* 2000*b*). In the previous study, FEC and worm burdens were reduced in sheep offered foods that contained similar amounts of *Quebracho* extract to the present study (60 g/kg). However, sheep were offered restricted amounts of supplemented foods (about 80 % of their expected *ad libitum* food intake), whereas in the present study they were offered the supplemented foods *ad libitum.* It has been reported that animals can consume a high proportion of their intake (80–90 % of the expected *ad libitum* intake) within a short period of time when they are fed restrictedly, whereas they spread their food consumption throughout the day when they are fed *ad libitum* (Bornett *et al.* 2000). It is possible that condensed tannins need to reach a certain concentration in the gastrointestinal tract of sheep to be effective towards parasites and this might not be easily achieved via *ad libitum* feeding. In addition, *ad libitum* intake can lead to increased flow rates of digesta (Grovum & Hecker, 1973), which would reduce the retention time of condensed tannins in the digestive tract, and thus render them less effective against parasites. Increased food intake has also been considered responsible for reduced efficacy of anthelmintic drugs in sheep due to increased digesta passage rate (Ali & Hennessy, 1995).

The surprising consequences of low protein nutrition on the parasitic burden of sheep during P_2 also merits discussion. The second experimental period (day 39–67) has been associated with the expression of immunity in previously naïve sheep (van Houtert *et al.* 1995). During this period, high protein nutrition is expected to enhance immunity and contribute to a reduction in the level of parasitism (Coop & Kyriazakis, 1999). In the present study worm burdens recovered at the end of the experiment, were lower in sheep offered food L rather than H. This result was probably due to the very low protein intake of sheep offered food L; they ingested 2 g MP/kg live weight during P_1 and only 1·2 g MP/kg live weight during P_2. To the best of our knowledge, the effects of such low protein intake have not been previously addressed in parasitised sheep. Such very low protein intakes could have led to changes in the natural environment of the parasites. Since parasites have very specific nutritional demands (Matthews, 1998), which are independent of those of the host, failure to meet them could cause reduction in their survival and/or maturation (Bundy & Golden, 1987). In addition, low protein nutrition may alter the biochemistry and/or the physiology of the gut and create an unsuitable environment for the establishment and/or the survival of parasites. It is evident from the above that the negative relationship between host protein nutrition and parasitism might not be linear, as has been previously suggested (Datta *et al.* 1998).

Food intake, DM digestibility and performance

The addition of *Quebracho* extract at 60 g/kg FM in the foods did not reduce DM digestibility in the present study. Evidence in the literature is inconclusive about the relationship between condensed tannins and digestibility. Previous studies have reported a reduced DM digestibility (Dawson *et al.* 1999) and an increased total faecal output (Butter *et al.* 2000) in rats and sheep offered *Quebracho*-supplemented foods, which is contrary with the findings of the present experiment. The differences observed between the latter and the present studies could be attributed to the different markers used to estimate the digestibility (Thonney *et al.* 1985), or to possible adaptation of the sheep digestive tract to tannins. However, the latter does not seem possible, as relevant experimental evidence (Butter *et al.* 2000) has indicated that such an adaptation had not occurred in parasitised sheep, following 5 weeks of *Quebracho* intake. Interestingly, although digestibility and intakes of sheep offered foods LQ and L were similar, sheep offered food LQ had lower live-weight gain than sheep on food L. This could be due to possible loss of endogenous protein, as will be discussed later.

Voluntary food intake and performance were identical in sheep offered foods H and VH; this confirmed that protein intake was not limiting the performance of parasitised sheep offered food H. However, food intake of sheep offered HQ was approximately 20 % higher compared with food intake of sheep offered H and VH. This percentage was higher than the dilution caused to food H by the addition of *Quebracho* (6 % w/w). It is suggested that the increased intake of sheep offered food HQ, compared with sheep offered food H, was possibly a mechanism to compensate for dietary or

endogenous protein loss caused by condensed tannins. Although condensed tannins have the ability to increase protein availability in the small intestine of the host (Barry & McNabb, 1999*a*), recent studies have suggested that the absorption of non-NH_4-N in the small intestine of sheep may be reduced following consumption of *Quebracho* extract (Komolong *et al.* 2001). This suggests that some of the dietary protein ingested by sheep may not be absorbed and could be excreted, whilst bound to condensed tannins. The 20 % increase in food intake of sheep would have undoubtedly led to an increased gut fill, which could have been greatly responsible for the increased live-weight gain seen on this treatment.

If we accept that sheep on food HQ increased their intake to overcome possible losses of endogenous protein, the question arises as to why sheep offered food LQ did not also increase their intake. The expectation was that sheep on the low-protein-supplemented food would increase their intake to at least account for the *Quebracho* dilution, as their food was highly digestible. It is possible that sheep on food LQ could not achieve this, due to the adverse consequences of condensed tannins on rumen microflora (McAllister *et al.* 1994). Such effects would arise from either the accentuation of N deficiency for the growth of rumen microbial population or directly from condensed tannins excess. Although the daily amount of *Quebracho* extract consumed by sheep offered food LQ was lower (70 g/d) compared with that of sheep on food HQ (140 g/d), most of the condensed tannins in the rumen of sheep on food HQ would be expected to be present in complexes with dietary protein, due to dietary protein excess (Barry & McNabb, 1999*b*). On the other hand, most condensed tannins present in the rumen of sheep on food LQ would be expected to be free to bind to microbial protein, due to the lack of dietary protein. High concentrations of free condensed tannins have been considered responsible for inhibiting and/or reducing the growth of rumen micro-organisms (Jones *et al.* 1994; Titus *et al.* 2000).

Previous studies have provided evidence for the anthelmintic properties of condensed tannins when consumed by parasitised sheep, either under restricted feeding conditions (Athanasiadou *et al.* 2000*b*; Butter *et al.* 2000), or under grazing conditions (Niezen *et al.* 1998). The present experiment was performed to investigate whether *ad libitum* food intake of foods supplemented with condensed tannins from *Quebracho* extract can reduce the level of parasitism and consequently improve the performance of sheep infected with *T. colubriformis*. The results indicate that the level of gastrointestinal parasitism in sheep may not be affected following *ad libitum* intake of foods supplemented with *Quebracho* extract, at the previously suggested level of supplementation (Athanasiadou *et al.* 2000*b*). It is concluded that another factor which could influence the anthelmintic properties of condensed tannins, besides their type and the amount offered to parasitised animals (Niezen *et al.* 1998; Athanasiadou *et al.* 2001), is the feeding regimen of parasitised sheep. An investigation into the anthelmintic properties of different condensed tannins, including *Quebracho*, under different feeding strategies seems to be an area where effort could be usefully diverted.

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