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Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*

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Abstract

Although the use of tanniferous plants or condensed tannins as an alternative to anthelmintics to control gastrointestinal nematodes has been largely documented in sheep, studies remain scarce in goats. The objective of this study was therefore to assess the possible impact of condensed tannins in goats infected with adult *Haemonchus contortus*. Two groups of cull goats were experimentally infected with 10,000 L3 of *H. contortus*. After 4 weeks, quebracho extracts, representing 5% of the diet DM, were administered for 8 days to one of the two groups. Goats of the second group remained as controls. One week after the end of quebracho administration, the goats were euthanised. Individual egg excretion and pathophysiological parameters were measured weekly during the study. At the end of the study, worm counts were assessed and histological samples from the abomasa were taken to count the numbers of mucosal mast cells, globule leukocytes and eosinophils. The administration of tannins was associated with a significant decrease in egg excretion, which persisted until the end of experiment. This reduction was not associated with any difference in worm number but with a significant decrease in female fecundity. No significant changes in the mucosal density of the three inflammatory cell types were detected between the two groups. These results indicate that the major consequence of tannin consumption in goats is a reduction in worm fecundity and egg output, which does not seem related to significant changes in the local mucosal response.

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1. Introduction

In the last decade, interest in alternative methods to control gastrointestinal trichostrongylosis of sheep and goats has increased in response to the development of anthelmintic resistance in populations of parasitic nematodes (Jackson and Coop, 2000). Among alternative methods, the possible use of tanniferous plants has been examined in several studies. The initial results obtained in New Zealand suggested that the consumption of tanniferous plants could affect the biology of different worm species and that condensed tannins could be responsible for these effects (Niezen et al., 1998a,b, 2002a,b). Further experimental *in vivo* studies using quebracho extracts (a highly rich source of condensed tannins) tended to confirm the initial results obtained with tanniferous plants (Athanasiadou et al., 2000a,b).

However, the vast majority of these studies, either in experimental or in grazing conditions, have been conducted in sheep. To our knowledge, until now, only one study has considered the possible effects of tanniferous plants in infected goats (Kabasa et al., 2000) despite the fact that this ruminant species presents several metabolic, physiological and immunological peculiarities (Silanikove, 2000; Hoste and Chartier, 1998), which could strongly modulate the interactions between tannins and nematode parasites compared to data acquired in sheep. Moreover, the most of the previous studies have concerned two main parasite species, i.e. *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. In contrast, information on the possible effects of tannins on one of the most pathogenic species, *Haemonchus contortus*, remains scarce (Molan et al., 2000; Athanasiadou et al., 2001a); although the species is highly pathogenic and widely distributed, particularly in tropical areas.

The current study was therefore designed in order to examine the possible consequences of consumption of condensed tannins on established populations of adult *H. contortus* in goats.

2. Materials and methods

2.1. Experimental design

Seventeen cull naïve Saanen goats were experimentally infected with 10,000 third stage larvae of *H. contortus*. They were reared indoors under conditions that excluded other nematode infection and offered a high quality pelleted food and gramineous hay. After 4 weeks of infection, the animals were divided into two groups, which were balanced according to the level of egg excretion and bodyweight. The tannin group contained nine goats which were given daily, for 8 days, 150 ml of an aqueous suspension of quebracho, a commercially available extract of the bark of a tropical tree *Schinopsis* sp. Overall the tannins represented 5% of the dietary DM. The remaining animals (eight goats) were used to provide an infected control group.

2.2. Parasitological techniques

Faecal samples were collected twice per week to measure nematode egg excretion. The egg counts were performed according to a modified McMaster technique (Raynaud, 1970).

At necropsy, the abomasa were collected to count the number of parasites both in the lumen and the mucosa based on a 10% aliquot method. The stage and sex of each worm was noted and the numbers of adult worms were used to calculate the male/female sex ratio (SR). In addition, the fecundity per capita was calculated by dividing the faecal egg counts (FECs) recorded on the day of slaughter by the total number of female worms recovered at necropsy.

2.3. Blood and histological analyses

Blood samples were collected weekly by venipuncture for haematocrits, circulating eosinophils and serum pepsinogen concentrations. Eosinophil counts were performed using Fast-Read slides[®] and the Carpentier's solution according to Dawkins et al. (1989) while pepsinogen concentrations were measured according to the method described by Kerboeuf (1975).

At necropsy, histological samples were collected from both fundus and pylorus to count mast cells, globule leukocytes and eosinophils in the abomasal mucosa according to the technique described by Larsen et al. (1994) and Huntley et al. (1995). The stained cells were enumerated at 400× magnification using a calibrated graticule encompassing an area of 0.25 mm². Mean cell densities for each tissue and each cell type were assessed from counts on 10 fields, which were randomly selected. The results have been expressed as the mean number of cells per mm² of mucosa.

2.4. Statistical analyses of the data

Worm burdens, fecundity per capita and histological counts were compared between the two groups using the non-parametric Mann and Whitney test. For the other measurements (faecal egg counts and blood pathophysiological parameters), the comparisons were performed using a repeated measures analysis of variance (SYSTAT 9.0 software) based on data collected on two separate periods. The first period corresponded to the time before the administration of quebracho extracts (D0–D28). The second period included both the period of tannin administration (D29–D36) and the subsequent period until necropsy on day 42.

Before analysis, data for egg counts, worm numbers and cell counts have been ($\log(x + 1)$) transformed to normalise their distribution.

Correlations between the different cell counts in the fundic and pyloric mucosae and the number of *H. contortus* were calculated using the Pearson's test.

3. Results

3.1. Faecal egg counts

Before the quebracho administration, no significant difference in egg excretion was observed between the two groups. In contrast, during the second period, corresponding to

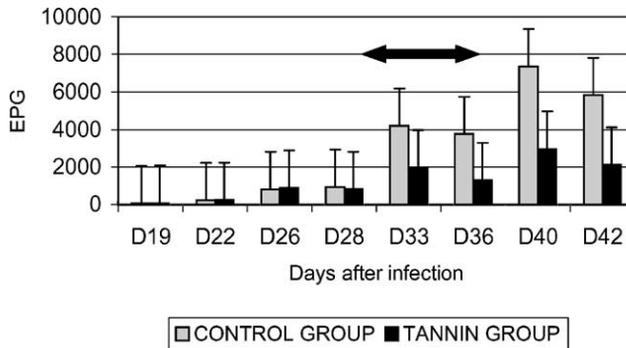


Fig. 1. Arithmetic mean faecal egg counts (eggs per gram) in the tannin and the control groups. Statistical difference ($P < 0.01$) was observed between both groups from D29 to D42. The arrow represents the 8-day period of distribution of quebracho extracts.

the tannin administration, faecal egg counts were significantly lower ($P < 0.01$) in goats receiving the quebracho extracts (Fig. 1). The reduction persisted after the quebracho administration was stopped (D37–D42). Overall, the reduction in egg excretion was 64% lower in the tannin group compared to the controls.

3.2. Worm burden and fecundity

There was no significant difference between the numbers of worms recovered from the tannin group animals and the controls. The mean number of worms recovered from the controls was 1342 compared to 1007 in the tannin group. The sex ratios were similar in the treated (SR = 0.73) and the control animals (SR = 0.79).

However, the fecundity per capita in the tannin group was significantly reduced ($P < 0.05$) by 57% compared to the controls. The fecundity per capita was 8.48 for the controls compared to 3.64 in the tannin treated group.

3.3. Pathophysiological measurements

Whatever the experimental period (before or after the administration of quebracho extracts), there was no significant difference between the two groups for any of the blood parameters, which were measured (Table 1).

3.4. Cellular response in the abomasal mucosae

For the three cell types and for the two anatomical sites, i.e. fundus or pylorus, there was no significant difference between the tannin and the control groups in cell counts. However, the number of inflammatory cells was usually higher in the fundic mucosae from the goats receiving tannins than in the control group (Fig. 2).

Table 1
Mean pathophysiological measurements in the control and the tannin groups throughout the study

	Dates						
	D0	D7	D14	D21	D28	D36	D42
Blood eosinophils (cells ($\times 1000$)/ml)							
Control group	23.4	32.6	36.5	34.5	41.4	49	45.6
Tannin group	33	32.1	32.2	36.5	41.9	44	43.2
Haematocrit (%)							
Control group	32	34	30	28	26	24	26
Tannin group	36	30	31	27	25	26	26
Pepsinogen concentrations (mUTyr)							
Control group	629.9	461.7	731.2	1049.5	1107.4	604	639
Tannin group	415.8	672.4	750.9	906.9	987.1	552	558

3.5. Correlations between mucosal inflammatory cells and *H. contortus* burdens

Negative and significant correlation coefficients were calculated between the number of globule leukocytes both in the fundus ($r = -0.668$) and in the pylorus ($r = -0.597$) and the number of *H. contortus* ($P < 0.01$).

In the fundus, the coefficient of correlations between the density of globule leukocytes and the number of mast cells or eosinophils were close to significance ($P < 0.06$). In the pylorus, similar relationships between the three cell types were significant ($P < 0.05$) (Table 2).

A significant relationship was also observed between the number of mast cells in the fundus and those in the pylorus ($P < 0.01$). A similar positive relationship was also observed for globule leukocytes at both sites ($P < 0.01$) as well as for eosinophils ($P < 0.06$) (Table 2).

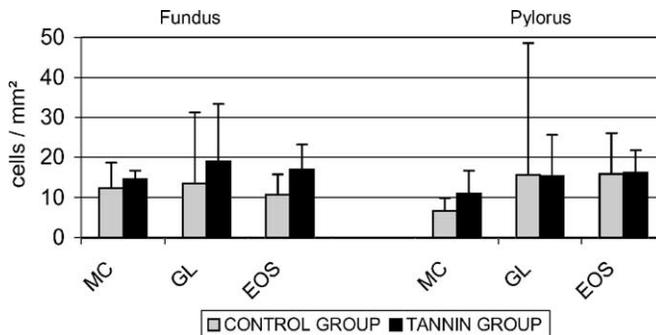


Fig. 2. Arithmetic mean density of inflammatory cells (mast cells: MC; globule leukocytes: GL; eosinophils: EOS) of fundus and pylorus in the two experimental groups.

Table 2

Values of correlation coefficients between the different inflammatory cell types in the two anatomical sites within the stomach

	Pylorus			Fundus		
	MC	GL	EOS	MC	GL	EOS
Pylorus						
MC	1					
GL	0.589	1				
EOS	0.016	0.476	1			
Fundus						
MC	0.594	0.416	0.253	1		
GL	0.289	0.585	0.091	<i>0.463</i>	1	
EOS	0.11	0.374	<i>0.459</i>	0.387	<i>0.456</i>	1

Significant values are indicated in bold ($P < 0.05$). Values close to significance are in italics ($P < 0.06$) (d.f. = 15).

4. Discussion

Despite the pathological importance of this nematode species, no information was available up to now on the in vivo effects of condensed tannins on *H. contortus* infections in goats. The consequences of tanniferous substances on this parasite were only observed in sheep in vitro and in vivo (Molan et al., 2000; Athanasiadou et al., 2001a).

The most significant result of the experiment was the decrease in faecal egg counts observed in the tannin group. The reduction in egg count occurred immediately after the administration of tannins and persisted after the cessation of tannin treatment. It represented an overall 64% reduction compared to the control group. Such a level could have major consequences on pasture contamination. Similar reductions in faecal egg count have also been observed in goats artificially infected with *T. colubriformis* and *T. circumcincta* (Paolini et al., in press). In addition, a rise in egg output has been reported in naturally infected goats given polyethylene glycol, an inhibitor of tannins (Kabasa et al., 2000). From these results, a depression on egg excretion is one of the main features associated with the consumption of tannins in goats infected with gastrointestinal nematodes.

In contrast to the data on egg excretion, no differences in worm populations were observed between the two experimental groups. This observation was confirmed by the lack of differences in the pathophysiological parameters (pepsinogen concentrations and haematocrit values), and suggested an absence of effect of condensed tannins, at the applied concentration, on established worm populations. This lack of effects on worm burden is similar to previous results in goats with *T. colubriformis* and *T. circumcincta* (Paolini et al., in press) and for some experiments in sheep with the same genera (Athanasiadou et al., 2001b; Bernes et al., 2000).

A significant effect upon *Haemonchus* per capita fecundity was evident in this study and similar findings have also been reported in tannin treated goats infected with *T. colubriformis* (Paolini et al., in press). The mechanism underpinning the reduced fecundity remains unclear although a direct effect of tannins on various organs of nematode, such as the female genitalium and/or the digestive tract could be envisaged.

An indirect effect of tannins by a stimulation of the local immune response, due to their protective effect for proteins against ruminal degradation (Mangan, 1988) has also been evoked as one option to explain their action on nematodes. In the study, an increased density of the three types of inflammatory cells was also noticed in fundus from the tannin group but the differences between the two groups remained non-significant.

Multiple studies on the local immunity against trichostrongyles of sheep have provided circumstantial evidence for an effector role for mast cells and epithelial globule leukocytes (Balic et al., 2000; Meeusen, 1999). However, relatively little information exists on the cellular components of local immunity in goats (Huntley et al., 1995; Patterson et al., 1996a,b). In addition, they were mostly acquired with the genus *Teladorsagia* or *Trichostrongylus*. The current study has provided unique information on the relationship between inflammatory cells and *H. contortus* in goats.

An increase in the number of mucosal mast cells and globule leukocytes has previously been mentioned in does showing an enhanced resistance against *T. circumcincta* and *Trichostrongylus vitrinus* (Patterson et al., 1996a). Etter et al. (2000) have observed a negative correlation between egg production from *T. colubriformis* and the number of intestinal globule leukocytes. In the current study, a negative, significant correlation between the number of globule leukocytes in the abomasal mucosae and the number of *H. contortus* was observed, confirming these previous results, and suggesting a major similar role for these cell types in does as in sheep.

The correlations between the three cell types in the two different anatomical sites suggest the occurrence of a general inflammatory response in the infected stomach. It is also noteworthy to underline the positive relationships found between the numbers of mast cells and globule leukocytes in each anatomical site. This is similar to what was observed by Etter et al. (2000) in the intestine. Both results tend to confirm that globule leukocytes derive from mast cells, in goats as in others species (Huntley et al., 1984). Overall, results from these studies suggest that globule leukocytes and, to a lesser extent, mast cells are important effectors of the local immunity against nematodes in goats as in sheep.

In conclusion, the results from the current study indicate that the main effects of condensed tannins from quebracho extracts on *H. contortus* in goats are a reduction in egg excretion and in the fecundity of the female worms, as previously described in sheep and in goats. The repercussions of the effects of tannins on the contamination of pasture and the subsequent epidemiology of trichostrongyle infection remain to be measured.

Differences in adaptation to tanniferous plants and digestive physiology and metabolism make goats a valuable model in which to examine the effects of tannins on nematode parasitism of the gastrointestinal tract. In particular, due to the physiological differences between sheep and goats, it can be postulated that comparisons of results acquired in the two host species would probably provide useful information and a better understanding of the mode of action of these botanical compounds on worm biology.

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