

Final Technical report

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Project title: **DO DIETARY TANNINS (POLYPHENOLICS) AFFECT THE SUSCEPTIBILITY OF RUMINANTS TO PARASITIC INFECTION?**

RNRRS Programme: **Livestock production science**

Production System: **Semi-arid production systems and high potential systems**

Project Leader/Institution: **Prof Peter Buttery, University of Nottingham**

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EXECUTIVE SUMMARY

Endoparasitic control is still heavily reliant on the use of anthelmintic drugs, however, frequent use and mis-use of anthelmintics is leading to the development of multiple resistance. In the tropics and subtropics where marginal levels of nutrition lead to greater consequences to infection, animal death due to nematode infection remains widely apparent. Here, anthelmintics are either unaffordable, of inferior quality or 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 parasitic infection solely, or in conjunction, with limited drug use. In tropical and subtropical regions many plants contain condensed tannins due to stress induced by environmental conditions. The aim of the studies reported was to determine whether the inclusion of a model condensed tannin, quebracho tannin, could reduce the establishment and persistence of small intestinal nematode infections and whether this was influenced by dietary protein concentration.

Initial work using the *Nippostrongylus brasiliensis*-rat model demonstrated that the inclusion of 40 g quebracho tannin/kg in both high (250 g/kg) and low (100g/kg) protein diets significantly ($p < 0.05$) reduced the number of *N. brasiliensis* worms establishing in the small intestine. Total daily faecal egg counts were also significantly ($p < 0.05$) reduced by dietary quebracho tannin, and high dietary protein concentration. Data obtained from using the *Trichostrongylus colubriformis*-sheep model also demonstrated that faecal egg counts were significantly ($p < 0.05$) reduced when 50 g quebracho tannin/kg was included in a low protein diet (97g/kg). Increasing the dietary protein concentrations (to 222 g/kg) also reduced faecal egg counts to similar levels. The inclusion of quebracho tannin in a high protein diet did not significantly ($p > 0.2$) reduce total daily faecal egg counts. Haematological and serological parameters did not show any significant ($p > 0.2$) differences between dietary treatments. Subsequent investigations indicated that dietary quebracho tannin was not reducing worm establishment and persistence by elevating the host immune response. Further studies suggested that quebracho tannin was acting through a direct toxic effect against the nematodes. Evidence for this came from two sources. (1) Studies in rats showed that while quebracho tannin reduced the infection of *N. brasiliensis*, a lumen dwelling nematode, the mucosal inhabiting nematode, *Trichinella spiralis*, was unaffected by the presence of dietary quebracho tannin. (2) *In vitro* data demonstrated that *N. brasiliensis* survival was compromised by incubating worms in quebracho tannin-containing media. Concentrations as low as 0.01% (w/v) quebracho tannin proved effective at accelerating worm death.

Thus, dietary quebracho tannin may be an alternative to increasing dietary protein concentration, which increases the hosts' capacity to mount an immune response and expel the worm burden from the small intestine. These data suggest that feeding plants high in condensed tannins may be a suitable alternative to anthelmintics, especially in areas of the tropics and subtropics.

The project has resulted in a collaboration being established with the University of Sokoine in Tanzania which is currently enabling these observations to be tested in target regions.

BACKGROUND TO PROJECT

Animals infected with gastro-intestinal parasites show a reduction in productivity, with parasitism world-wide having enormous economic significance being one of the major contributors to loss of productivity in target regions of the project (semi-arid/high potential production systems of Africa and India). Clinical and subclinical infections reduce animal survival, depress growth rates, wool and milk production and reproductive performance (as reviewed by Parkin and Holmes, 1989). The nutritional status of the animal influences the pathogenesis of the infection (Gibson, 1963; Lunn, Northrop and Wainwright, 1988). Animals subjected to marginal levels of nutrition are more susceptible to infection leading to a greater decrease in productivity (Niezen, Charleston, Hodgson, Mackay and Leathwick, 1996). It has been well documented that animals receiving a high plane of nutrition, namely the supplementation of dietary protein, are able to withstand some of the debilitating effects associated with parasitism (Poppi, MacRae, Brewer and Coop, 1986; Abbot, Parkins and Holmes, 1988; Bown, Poppi and Sykes, 1991; Kyriazakis, Oldham, Coop and Jackson, 1994; van Houtert, Barger, Steele, Windon and Emery, 1995; van Houtert, Barger and Steele, 1996). Poppi, Sykes and Dynes (1990) reported approximately 40 % reduction in liveweight gain, depressed feed intake, wool and milk production as some examples of the loss of productivity in the ovine host when exposed to sub-clinical infections. In addition to inducing anorexia in the host, the presence of parasites in the intestinal tract also causes an increase in the secretion of endogenous proteins through plasma and whole blood loss, sloughed epithelial cells and increased mucus production (Coop, Sykes and Angus 1982; Poppi *et al.* 1986; Sykes, Poppi and Elliott, 1988) further accentuating the demand for dietary protein to overcome these losses. An adequate protein supply also helps the animal to mount an immune response to the parasite.

Endoparasitic control is still heavily reliant on the use of anthelmintic drugs, although the frequent administration and mis-use 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.

Published data which indicates that polyphenolic feedstuffs could act as potential natural anthelmintics include papers reporting effective control of a number plant parasitic nematodes by wild sage (*Lantana camara*) (Chandel and Mehta, 1990). Eucalyptus species containing Mannich bases (containing an aromatic ring side chain) fed to goats have proved to be effective anthelmintics against *Haemonchus contortus* but not *Ostertagia* (Bennet-Jenkins and Bryant 1996), and the use of a nematode larval motility inhibition assay has shown certain plants to have anthelmintic properties against *Trichostrongylus colubriformis* (Lorimer, Perry, Foster and Burgess, 1996). Further evidence that forages containing polyphenolics have direct beneficial effects at reducing parasitic infection in ruminants comes from New Zealand where parasite-infected lambs and sheep have been shown to have improved liveweight gains and reduced faecal egg counts when grazing sulla (a condensed tannin containing forage) compared with those grazing lucerne (condensed tannin-free) (Niezen, Waghorn, Waghorn and Charleston, 1995; Robertson, Niezen, Waghorn, Charleston and Jinlong (1995)). The mechanism through which these tannins can reduce infection is still unknown but speculation indicates that the beneficial properties could be due to tannins complexing with dietary soluble protein, preventing rumen degradation and thereby increasing the duodenal protein supply once the complex dissociates in the acidic conditions of the abomasum (Martin and Martin 1983; Martin *et al.* 1985) hence increasing the protein supply to the host. Additional protein supply in parasitised animals has been shown to help the host to expel the worm burden from the GI tract more rapidly, as judged by the presence of nematode eggs in the faeces, by elevating the capacity to mount an immune response. Bown *et al.* (1991) infused casein into the abomasum of infected animals and observed a 55% decline in mean faecal egg counts with a 2-fold drop in worm burdens recovered at slaughter after 12 weeks of infection. van Houtert *et al.* (1995) recorded similar

decreases in lambs supplemented with 100g fishmeal/day. A further speculation is that the presence of condensed tannins in the New Zealand forages mentioned above could be acting directly against the worm as an anthelmintic (Niezen, Waghorn, Waghorn and Charleston 1993).

PROJECT PURPOSE

To provide information which can be used to increase the productivity/production potential of livestock, including draft animals, in particular ruminants in areas where intestinal parasites are currently a major problem. The specific aim was to test the hypothesis that dietary inclusion of polyphenolics (tannins) could reduce the intestinal parasitic burden in sheep. Many plants in the tropics and subtropics contain high concentrations of tannins. To develop strategies to test any significant positive findings in target regions.

RESEARCH ACTIVITIES AND OUTPUTS

(a) Experimental programme and results.

These are described in detail in the Ph.D thesis of N.Butter which is attached. An outline is given below.

The research activities of the project were to obtain data on the influence of dietary tannin on parasitic burden in sheep, the possible mechanism of action and its interaction with the plane of nutrition which may be used to design follow-up studies with indigenous feedstuffs. To obtain these data several rodent trials and two sheep trials were carried out. As a model system quebracho tannin was used. This condensed tannin is readily available, being imported into the UK for the leather industry. It is difficult to get large quantities of plant materials high in tannins in the UK. The rodent trials involved the gastro-intestinal nematode *Nippostrongylus brasiliensis* for preliminary work prior to the large animal studies. This nematode is often used as a model for the sheep nematode *Trichostrongylus colubriformis* infection. Sheep trial 1 investigated the effect of dietary quebracho tannin (50g/kg diet) on *Trichostrongylus colubriformis* infection in sheep fed with a restricted intake

calculated to give a growth rate of 100g/d. The second sheep trial, again using *Trichostrongylus colubriformis*, involved a more elaborate study investigating the benefits of dietary quebracho tannin inclusion in a low protein diet prior to and during worm infection compared with the effects of dietary protein supplementation during the infection period alone or in combination with QT. Diet combinations and changes are outlined in Table 1. In conjunction with dietary manipulation several blood parameters were analysed to investigate possible influences of infection, dietary quebracho tannin and increased protein concentration on the physiology and immune status of the animal. Immune status was further investigated (1) through a challenge infection of *Trichostrongylus colubriformis* at the conclusion of the trial and following a drench of an anthelmintic (Parafend, Ofendazole 2.265%) and (2) by measuring the plasma IgG response to a subcutaneous injection of ovalbumin, 50 and 60 days post the original infection with *Trichostrongylus colubriformis*. Possible mechanisms of action through which quebracho tannin could be reducing nematode infection were investigated by immune suppressing tannin- and control-fed infected rats and also by comparing the effect of tannin on lumen-dwelling nematodes (*Nippostrongylus brasiliensis*) or mucosal inhabiting nematodes (*Trichinella spiralis*). Mucosal inhabiting worms are only exposed to gut contents for a brief period of time prior to establishing in the gut mucosa, while lumen-dwelling worms are continuously exposed to gut contents. Finally some *in vitro* studies were conducted to evaluate the direct effects of QT on nematode viability.

The major results from the research activities reported above are presented below. Initial rat trials demonstrated that the inclusion of 40 g/kg quebracho tannin into both high (250g/kg) and low protein (100g/kg) diets reduced the number of *Nippostrongylus brasiliensis* worms establishing in the small intestine and reduced number of eggs *in utero* in female worms (Figure 1). Subsequent rat trials demonstrated that the inclusion of quebracho tannin in the low protein diet reduced faecal egg counts to levels seen in high protein-fed controls. The addition of quebracho tannin in the high protein diet did not have any additional beneficial effect (Figure 2). In both trials two types of quebracho tannin were used, both being effective at reducing parasitic infection. The extracted tannin was the commercially available tannin chemically extracted with metabisulphite, oxalic acid and EDTA to improve colour and solubility for use in the leather industry (QT). Consequently the

compound may contain residues from the extraction process. A small quantity of chemically untreated or 'pure' quebracho tannin was also available for use (uQT). The purpose of using both tannin types was to ensure that the reductions in parasitic infection were due to the presence of condensed tannins in these compounds and not due to chemical residues from the extraction process. Both sheep trials conducted involved the commercially available extracted quebracho tannin. Sheep trial 1 showed a reduction in faecal egg counts from tannin-fed lambs, particularly male lambs, when fed a restricted diet (calculated to give a growth rate of 100g/d) containing 50 g quebracho tannin/kg diet (Figure 3). Sheep trial 2 in which animals were fed just below *ad libitum* (4% of body weight/day) resulted in similar results being obtained. Quebracho tannin was able to reduce faecal egg counts when included in a low protein diet (97g/kg) (Figure 4). The inclusion of dietary tannin into the low protein diet after *Trichostrongylus colubriformis* infection had established (as judged by the presence of nematode eggs in the faeces) (diet group 5) also reduced faecal egg counts as did increasing the protein concentration of the diet (to 222g/kg group 3). Dietary tannin has been observed to increase faecal output. When faecal egg counts observed in one gram faeces were multiplied by daily faecal output (measured by chromium dilution in the faeces) the inclusion of dietary tannin in the high protein diet (group 4) did not have any effect on mean total daily faecal egg counts when compared to low protein fed controls (Table 2). The immune status of the animal, measured through the IgG response of the host against the injection of a foreign protein, ovalbumin, showed no effect of diet or infection being observed (Table3). The trial was concluded with a challenge infection of *Trichostrongylus colubriformis* to all animals. Only the lambs which remained uninfected during the trickle infection (group 6) were susceptible to worm infection, hence all of the lambs exposed to the trickle infection had mounted an immune response to prevent any further infective *Trichostrongylus colubriformis* larvae from establishing in the small intestine, irrespective of diet.

A further rat trial was carried out comparing the effect of dietary quebracho tannin on the establishment of lumen-dwelling nematodes (*Nippostrongylus brasiliensis*) and mucosal inhabiting nematodes (*Trichinella spiralis*). Mucosal inhabiting worms are only exposed to gut contents for a brief period of time prior to establishing in the gut mucosa, while lumen-dwelling worms are continuously exposed to gut contents. Table 4 shows that lumen-dwelling worms were reduced by the inclusion of dietary

tannin but mucosal inhabiting worms remained unaffected, suggesting that quebracho tannin is only effective when in direct contact with the worms. Subsequent *in vitro* studies with *Nippostrongylus brasiliensis* showed that as little as 0.1% QT was effective at accelerating worm death.

In summary, dietary quebracho tannin inclusion in a low protein diet reduces lumen-dwelling small intestinal nematode infection in both the monogastric and ruminant to similar levels as observed when high protein diets are fed. The inclusion of tannin to high protein diets is not additive. Indications are that quebracho tannin is exerting its beneficial effect of reducing infection in the low protein diet by direct toxicity against the worm. The lack of response in infected animals fed high protein tannin diets may be due to the complexation of quebracho tannin with excess dietary protein forming an insoluble and inactive complex.

(b) Publications

In addition to thesis the following publications were produced.

BUTTER, N.L., DAWSON, J.M., WAKELIN, D. AND BUTTERY, P.J. (1998) Effect of dietary tannin and protein level on the susceptibility of sheep to parasitic infection. *Proceedings of the Society of Animal Science* **97** (Abstract)

BUTTER, N.L., DAWSON, J.M., WAKELIN, D. AND BUTTERY, P.J. (1999) Dietary tannins acting as anthelmintic agents? *Proceedings of the IX International Symposium of Ruminant Physiology* (Abstract)

BUTTER, N.L., DAWSON, J.M. AND BUTTERY, P.J. (1999) Effects of dietary tannins. In: *Secondary plant products – considerations for animal feeds* Nottingham University Press, UK (eds J.C. Caygill and I Muelier-Harvey) pp 51-71.

BUTTER, N.L., DAWSON, J.M., WAKELIN, D. AND BUTTERY, P.J. (2000) Effect of tannin and protein concentration on nematode infection (*Trichostrongylus colubriformis*) in lambs. *Journal of Agricultural Science, Cambridge*, *in press*.

A further full length publication is being prepared.

(c) Other outputs

(i) Dissemination of results:

Seminar given to a MAFF sponsored meeting on Secondary Plant Products entitled 'Tannins: Implications for Ruminants'.

Poster presented at British Society of Animal Science, 1998. Awarded the Presidents prize for the best poster.

Research results distributed to the staff of the Department of Animal Production, University of Sokoine, Tanzania.

Detailed discussions with Prof E Owen and colleagues to ensure that our results are available to the group working in Zimbabwe on Acacia (R7351)

(ii) Exploitation of findings

The project activity of catalysing work to be conducted in the tropics where large quantities of forages containing tannins other than quebracho tannin, the model tannin used in the above studies was accomplished by forming a collaboration with the University of Sokoine in Tanzania and the starting of a further research collaboration funded by DIFD R7424/ZC0113. 'Can feeding locally-available plant material rich in tannins reduce parasitic burden in ruminants and hence improve their productivity?'

All anticipated outputs were achieved.

CONTRIBUTION OF OUTPUTS

The output from the above studies has potentially identified a method to improve performance of livestock (including draught animals) in various crop/livestock and livestock production systems. In particular they have enabled us to develop potential strategies for the use of locally available resources (plants high in tannin) to combat parasitic infections in ruminants and thereby optimise livestock production and improve their contribution to the crop/livestock farming system. Further studies in the target regions are required and these are currently underway.

Table 1. Dietary groupings of lambs fed quebracho tannin with high or low protein (sheep trial 2)

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 p.i.)	Low	Low + QT	High	High + QT	Low + QT	Low

Low = Low protein - 97 g/kg

High = High protein – 222g/kg

+ QT, quebracho tannin added at 50 g/kg (QT)

Table 2. Effect of dietary treatment (high and low protein concentration and/or dietary quebracho tannin) on mean faecal output and number of *T. colubriformis* eggs passed post diet change of sheep, (sheep trial 2).

Group	1 (L-L)	2 (LQT-LQT)	3 (L-H)	4 (L-HQT)	5 (L-LQT)	s.e.d. (25 df.)
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 (x10 ⁴ DM)	212.3 ^a	104.8 ^b	112.2 ^b	204.0 ^a	83.3 ^b	39.52

For key see table 1. a,b,c means with different superscripts within a row are significantly different (p<0.05). Values represent the average of 17 time points taken from day 23-71 p.i. for 6 animals in each infected group (1-5).

Table 3. Mean IgG response to ovalbumin 23-71 post infection of sheep with *T. colubriformis* (sheep trial 2).

Group	1 (L-L)	2 (LQT-LQT)	3 (L-H)	4 (L-HQT)	5 (L-LQT)	6 (uninfected)	s.e.d. (30 df)
OD ¹ (410 nm)	3.49 ^a	3.33 ^a	2.66 ^{a,b}	1.84 ^b	2.93 ^{a,b}	2.59 ^{a,b}	0.607

^{a,b} means with different superscripts within a row are significantly different (p<0.05)
 OD¹ Optical density scaled to a positive control having an optical density of 1.00 IgG was measured using an ELISA system. For key to diet groupings see table 1.

Table 4 *Effect of dietary QT on the number of T. spiralis and N. brasiliensis worms recovered from the small intestine of rats.*

	L	L + QT	s.e.d. (d.f.)
<i>T. spiralis</i> , d 2 p.i.	935	930	84.9 (14)
<i>T. spiralis</i> , d 5 p.i.	675	614	90.9 (14)
<i>N. brasiliensis</i> , d 5 p.i.	1442 ^a	1090 ^b	82.8 (11)

^{a,b} means with different superscripts within a row are significantly different p<0.05.
 L=low protein (100g/kg casein). n= 8 rats/group.

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