



EFFICACY OF QUEBRACHO TANNIN INCORPORATED FEED ON *HELIGMOSOMOIDES BAKERI* INFECTION IN MICE

¹Ngongeh, L.A. & ²Fakae, B. B.

¹Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike

²Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

ABSTRACT

The anthelmintic potential of quebracho extract due to its condensed tannin content has been reported. The present study investigated the efficacy of quebracho tannin (QT) on *Heligmosomoides bakeri* infection in outbred albino mice. Thirty male mice, 7-8 weeks old, weighing 20-23g each were used in the study. They were randomly placed into five groups of six mice each. Mice in four groups were infected with 150 *H. bakeri* infective larvae (L3) of *H. bakeri* while the last group remained as uninfected controls. Infection was monitored by packed cell volume (PCV), body weight changes (BW), faecal egg counts (FEC) and worm burdens (WB). Mice fed feed containing 6 and 8% quebracho extract had their FEC reduced compared to those of mice fed feed containing 4 and 0% quebracho extract. There was no statistical significant difference between the animals on QT feed and the non-treated group at the termination of the study on D22 ($F = 0.343$, $P = 0.795$). Mice kept on 6 and 8% quebracho tannin feed had their worm burden much reduced though not significantly ($F = 0.343$, $P = 0.094$) when compared to the untreated control. There was no significant difference between the 4 and 8% feed-inclusion groups ($P > 0.05$), although the 8% group had the lowest worm burden. Quebracho tannin therefore demonstrates a dose dependent anthelmintic potential against *H. bakeri* infection in mice.

KEYWORDS: Quebracho tannin extract; feed inclusion; anthelmintic potential, mice; *Heligmosomoides bakeri*

INTRODUCTION

As the quest for more sustainable methods of control of animal gastrointestinal (GI) nematodosis continues, inclusion of quebracho tannin (QT) extract into feed is being tried (Paolini *et al.*, 2003) as a means controlling GI nematode parasites of domesticated animals. Mice infected with *Heligmosomoides bakeri* were found to have their faecal egg counts (FEC) and worm burden (WB) reduced following drenching with quebracho extract (Ngongeh and Fakae, 2005). A more practical approach to the use of quebracho tannin would be the incorporation of determined quantities of quebracho extract as part of the diet. This study was therefore designed to investigate the efficacy of quebracho tannin incorporated feed on *H. bakeri* experimental infection in outbred albino mice. The objective of this trial was to obtain data, which may validate the dietary inclusion of condensed tannin in animal feed as a sustainable parasite control in animals in intensive and extensive management systems.

MATERIALS AND METHODS

Plant extracts

Commercial soluble quebracho tannin extract in powder form kindly donated by Professor Peter Buttery of University of Nottingham was used. It was obtained from the bark of a dicotyledon *Schinopsis* species that grows in the tropics.

Experimental feeds

Four experimental feeds were formulated. The four classes of feed were comprised of a standard commercial feed, chicken's growers mash (Guinea Feed, Bendel Feed and Flour Mill Ltd.) containing 0,4,6 and 8% quebracho tannin extract (powder) weight by weight (w/w). The feed

was properly mixed, moistened, pelleted and dried in an oven at 150°C.

Experimental Animals

The mice were obtained from Experimental Parasitology Unit, Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka (UNN). Thirty male mice, 7-8 weeks old and weighing 20-23g each were used in the study. They were randomly placed into 5 groups of 6 mice each. They were allowed to acclimatize in the groupings two weeks before the start of the experiment.

Preparation of infective larvae and infection of mice

Faecal cultures were prepared according to the method of Fakae *et al.* (1994). Infection of mice was done according to the method of Fakae *et al.* (2001). Mice in four groups were infected with 150 *Heligmosomoides bakeri* infective larvae (L3) per mouse while one was reserved as the uninfected control.

Feed incorporation trial

The specially formulated feeds were introduced to the different groups of animals from 12 DAI throughout the experiment and fed *ad libitum*. The 0% quebracho-included feed was fed to the uninfected control and the infected control mice, corresponding to groups 1 and 2 respectively. Quebracho-incorporated feed (4,6 and 8% QT) was then fed to the mice of groups 3, 4 and 5 respectively.

Faecal egg counts

1g of faeces collected as described above from each mouse was dispersed in 30ml of saturated sodium chloride solution of specific gravity 1.28. The suspension was passed through a coffee strainer and made up to 45ml with additional salt solution. Well-mixed aliquots were

counted in a standard McMaster counter slide (Hawksley, England) in a standard McMaster counter technique, and expressed as eggs per gram (epg) of faeces (MAFF, 1997).

Post-mortem Worm Counts

This was done as described by Ngongeh (2008; 2011). Briefly, each mouse was sacrificed with diethyl-ether (May and Baker, Ltd, England) and the gastrointestinal tract was removed immediately. The entire length of the small intestine was opened, by cutting along its longitudinal axis with a pair of fine scissors. The adult worms from the intestine were recovered by suspending each intestine on a fine thread and dipping into Hanks balanced salt solution (HBSS), and incubated at 37°C in an incubator. Within 2-3 hours all worms had migrated into the saline. The intestine was then discarded and more of the fresh saline was added. After removal of the intestine the incubation continued overnight (20 hours) to ensure complete disentanglement of the worms. At the end of the incubation saturated sodium chloride was added to the Hanks saline containing the worms to make up a 30% v/v solution. The worms then died within 30 minutes of this treatment, relaxing the tight spiral coils characteristic of the living worms, thus making counting easier.

Weight Measurements

Mice were weighed weekly from the first day of the study using desktop balance (Sartorius-GMBH-Gottinge, Germany)

Haematology

Blood was collected from the tail, by tail snip and gradual milking into disposable heparinised capillary tubes (Camlamb Ltd, Cambridge). The tubes were filled up to two-third full and sealed with cristaseal (Hawksley, England). The percentage of packed cell volume (PCV) of individual mice was determined by a microhaematocrit centrifuge (Haemofuge A, Heraeus sepatech) and centrifuged for 5 minutes at 17000g. The PCV was determined using a micro-haematocrit reader (Heraeus Reader).

Statistical analysis

Results were analysed using standard statistical procedures, ANOVA and Student's t-test and difference with probability level, $P < 0.05$ was accepted as significant.

RESULTS

Changes in faecal egg counts

Faecal egg counts (FEC) in mice fed with 6 and 8% quebracho feed increased markedly following commencement of feeding (Fig 1). The FEC however dropped 4 days after to levels comparable to those fed 0 and 4% QT feed. There was no statistical difference between the animals on QT feed and the non-treated group at the termination of the study at D22 ($F = 0.343$, $P = 0.795$).

Weight changes

There was a marked loss in weight in all the infected groups of mice from D14 compared to the uninfected group. However, while the infected but not treated group gained weight from D21 to D24, the quebracho treated groups kept losing weight (Fig 2).

PCV changes

The changes in PCV are shown in Fig 3. All infected mice suffered negative PCV changes at 14 DAI. The 6% and

control groups showed the greatest and least negative PCV changes respectively at 14DAI. Following treatment from 14DAI, the PCV changes for 4,6 and 8% groups started appreciating attaining a maximum at 21DAI with the 8% and 4% groups attaining the greatest PCV changes comparable to the PCV changes of the naïve control. However, the negative changes for the infected control continued their fluctuating negative changes from 14 DAI until end of the study. There was no significant change in PCV ($F = 1.219$, $P = 0.328$).

Post Mortem Worm Counts

Worm burden of untreated group (0% QT feed) and those fed with 4% QT feed did not differ (Fig 4). However, mice kept on 6 and 8% QT feed had their worm burden much reduced though not significantly ($F = 0.343$, $P = 0.094$) when compared to the untreated control. There was no significant difference between the 6 and 8% QT feed-inclusion groups ($P > 0.05$), although the 8% group had the lowest worm burden.

DISCUSSION

The obtained results indicate that the feed-inclusion of extracts of quebracho tannin in *H. bakeri* infected mice was beneficial because there was a reduction in both FEC and worm burden especially at 6 and 8% levels. Proanthocyanidins have been reported to have the potential of reducing nematodes in the gastrointestinal tract (Khan and Diaz-Hernandez, 2000). Quebracho feed inclusion at similar level fed for seven days to sheep infected with *T. colubriformis* also had both FEC and fecundity and worm burden reduced (Athanasidou *et al.*, 2000). Feeding a supplement of cassava hay containing moderate levels of condense tannins have also been shown to reduce nematode egg counts in both cattle and buffaloes (Netpana *et al.*, 2002). The present study being equally a short-term trial, is comparable to both studies. Some studies have shown that quebracho tannin inhibits parasite Gluthathione S-Transferase *in vitro* (Fakae *et al.*, 2000). The inhibition of such protective enzyme could affect the worms adversely with possible slowing down of some physiological activities that may lead to reduced worm fecundity or subsequent expulsion.

Weights of mice on higher doses of quebracho tannin preparations were affected when compared to controls. This may have been due to reduced digestibility of the feed (Athanasidou *et al.*, 2000). It is also possible that the quebracho bound the proteins both digested and non-digested leaving nothing to be absorbed into the system. The duration of the study may have been too short, as the mice might have needed some time to adjust to their new diets. Weight changes may also be attributable to reduced feed intake. According to the theory of co-evolution of plants and herbivores, herbivores develop mechanisms to inactivate such substances the plants may produce to defend them against being devoured by animals. This has been shown by insects' and larvae's adaptation to tannins by developing an alkaline milieu or by means of surfactant substances (Berenbaum, 1980).

A number of experiments also suggest the existence of adaptation processes. Thus feed containing tannins was not accepted by sheep until after a conditioning phase (Burns *et al.*, 1972). Another adaptation example was the fact that the live weight gain of lambs, which had received

tannin-containing bird's-foot trefoil (8-10% tannin in DM) for longer periods of time, was higher as compared to animals that had been less conditioned to this (68 as compared to 45g / day). (Barry and Reid, 1984).

Changes in PCV of infected mice were similar to those in the earlier studies. Fakae *et al.* (1994) had reported that the pathology of *H. bakeri* infection in respect of mouse haematology is mainly lowering of the PCV at 14 DAI. It was interesting to observe that following introduction of the quebracho tannin-rich feed, the changes in PCV started

appreciating, peaking at 21 days after infection (DAI). The appreciation in PCV are likely as a consequence of reduced worm burden, and particularly the 8% group with the least worm burden attended the greatest PCV changes amongst the treated groups indicating that the QT feed was beneficial to the animals. This feed inclusion trial does suggest that there is a good prospect of control of certain gastrointestinal nematode infections by inclusion of condensed tannins in feeds.

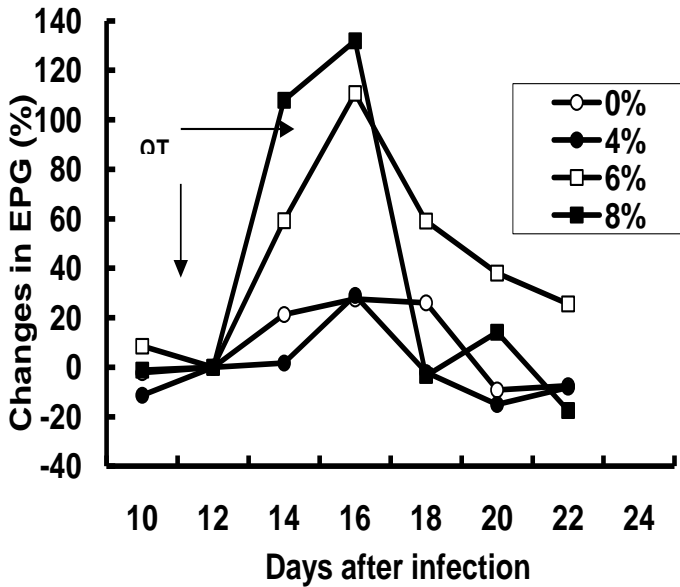


Figure 1. EPG in feed inclusion

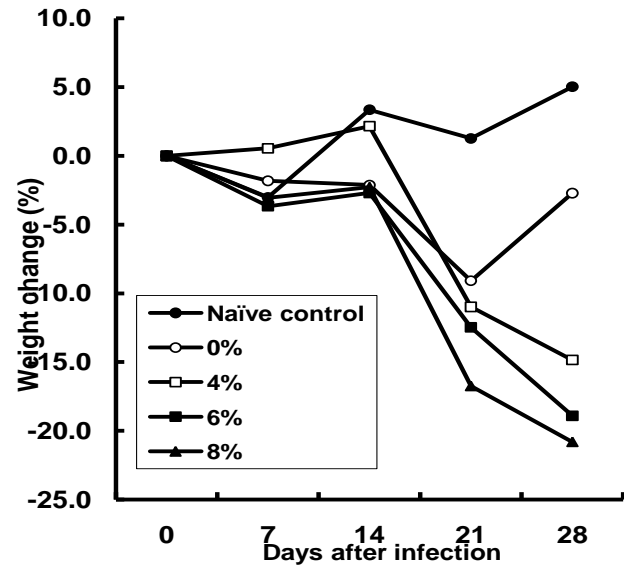


Figure 2. Changes in body weight in feed inclusion

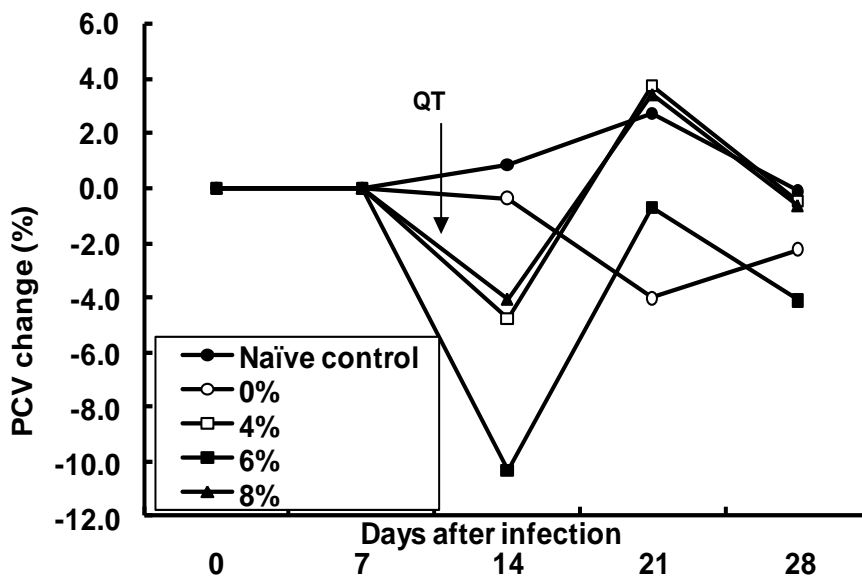


Figure 3. PCV changes

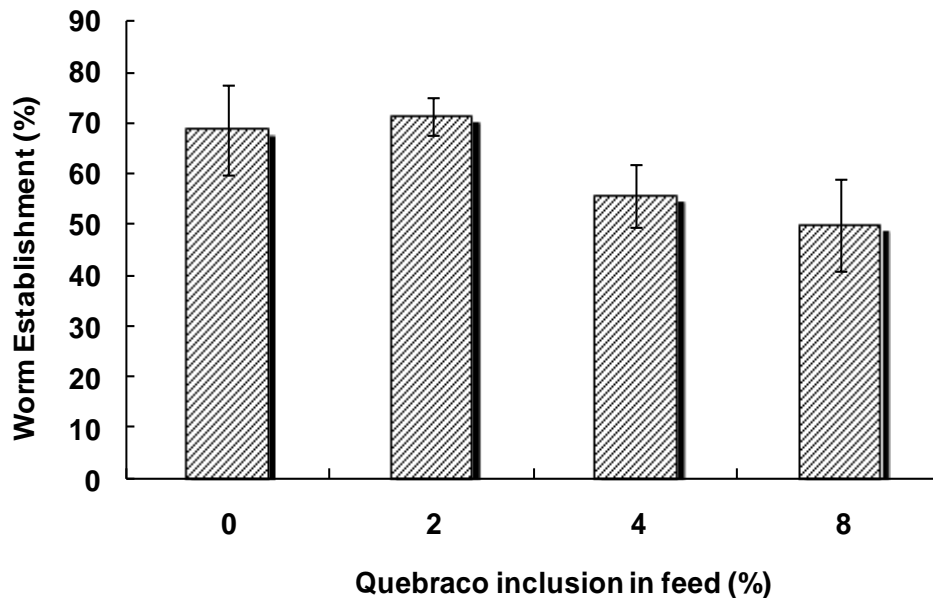


Figure 4. Worm counts in feed inclusion

REFERENCES

- Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R. L. (2000) Effects of short term exposure to condensed tannins on adult *Trichostrongylus colubriformis*. *Veterinary Record*. **146**: 728-732.
- Berenbaum, M. (1980) *American Naturalist*. **115**: 138-146.
- Barry, T.N. Reid, C.S.W. (1984) Nutritional effects attributable to condensed tannins, cyanogenic glycosides and oestrogenic compounds in New Zealand forages. In: *Forage legumes for energy-efficient animal production. Proc. of a trilateral workshop held in Palmerston North, N.Z., April 30 – May 4, 1984* (R.F. Barnes, P.R. Ball, R.W. Brougham, G.C. Marten, D.J. Minson eds.), 251-259. Printed by USDA Agric. Res. Serv., Washington D.C.
- Burns, J.C. Mochrie, R.D., Cope, W.A. (1972) *Agron*, **J. 64**: 193-195
- Chiejina, S.N. (1986) The epizootiology and control of parasitic gastroenteritis of domesticated ruminants in Nigeria. *Helminthological Abstracts*. **55**: 314-429.
- Fakae, B.B., Harrison, L.T.S., Ross, C.A., Sewell, M.H. (1994) *Heligmosomoides polygyrus* and *Trypanosoma congolense* infections in mice. A laboratory model for concurrent gastrointestinal nematode and trypanosome infection. *Parasitology*. **108**: 61-68.
- Fakae, B. B, Cambell, A. M., Barret, J., Scott, I. M., Teesdale, P.H., Lieban, E., and Brophy, P. M. (2000) Inhibition of glutathione S-transferase (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. *Phytotherapy Research*, **14**: 630-634.
- Fakae, B.B., Brophy, P.M. (2001) Primary inhibition of parasitic nematodes and mammalian glutathione S-transferase (GSTs) by extracts from *Piliostigma thinnigii*. *Nigerian Journal of Biochemistry and Molecular Biology*. **6** (3)
- Khan, L.P., Diaz-Hernandez, A. (2000) Tannins with anthelmintic properties. In: *Proc. International Workshop on Tannins in livestock and human nutrition*. (Editor J.D. Brooker), ACIAR Proceedings No. **92**: 171.
- MAFF (1977) *Manual of Veterinary Parasitological laboratory techniques*. Technical bulletin No. 18, HMSO, London, 36-37.
- Netpana, N., Wanapat, M.W., Pongchompu O., and Toburan W. Effects of condensed tannins in cassava hay on fecal parasitic egg counts in swamp buffaloes and cattle International Workshop. Current Research and Development on the Use of Cassava as Animal Feed. Merkan International Workshop. (Personal communication).
- Ngongeh, L. A., Fakae, B.B. (2005) Effect of quebracho tannin on *Heligmosomoides polygyrus* infection in tropical outbred albino mice. *30th Annual conference of the Nigerian Society for Animal Production*, 20-24 March 2005, Nsukka.
- Ngongeh, L.A. (2008) Comparison of mean faecal egg counts and worm burdens of strongest and weakest responder male mice infected with *Heligmosomoides bakeri* with those of their F1 offspring. *The Proceedings of the 45th Annual Congress of the Nigerian Veterinary Medical Association (NVMA)*. 20th-24th October. Owerri, Imo State, Nigeria. Pp 246-248.