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Short communication

Exposure of *Melia azedarach* fruits to *Eimeria* lowers oocyst output in yearling Tswana goats

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Abstract

This study assessed the effects of *Melia azedarach* fruits on oocyst output of goats naturally infected with *Eimeria* species. The nineteen 12-month-old male Tswana goats weighing 21.5 kg were allocated to either a grass hay basal diets (control = 10) or to grass hay basal diet + *M. azedarach* fruits (treatment = 9). The animals were individually penned, given feed and clean water for 21 days. There was a significant ($P < 0.001$) difference in oocyst per gram (OPG) between the two groups in weeks 2 and 3. *M. azedarach* maintained an oocyst count of 6400 OPG throughout the study. In contrast, OPG of the control animals increased in the second week, reaching a peak of 33,000 OPG by week 3. There was no difference ($P > 0.05$) in body weight between the groups by week 2. The use of novel plants to control parasites in livestock opens opportunities for sustainable and less frequent use of anthelmintics.

Keywords: *Melia azedarach*; *Eimeria* species; Goats; Condensed tannins; Parasites

1. Introduction

The plant *Melia azedarach* Linn (Meliaceae), also known as Chinaberry or Persian lilac tree, is a deciduous tree that is native to northwest India, Persia. Khan et al. (2001) reported that *M. azedarach* is used against intestinal worms and other stomach disorders. In China, extracts of its bark have anthelmintic properties (Oelrichs et al., 1983). *M. azedarach* has been tested for its anti-fungal properties (Carpinella et al., 2003), for *in vitro* efficacy against the tick *Boophilus microplus* (Borges et al., 2003) and anti-microbial properties (Khan et al., 2001).

Some small stock farmers in Botswana have observed that animals consume ripe fruits of *M. azedarach* especially during the dry season. Based on a paradigm shift from the continued use of drugs to more sustainable and biological strategy, in the control of parasites, the objective of the present study was to evaluate the effects of exposure of fruits of *M. azedarach* for 3 weeks on *Eimeria* oocyst excretion in naturally infected goats.

2. Materials and methods

2.1. Site of study

The trial was carried out at Botswana College of Agriculture (BCA) small stock experimental unit in 2004, about 10 km from Gaborone city. The vegetation at BCA is a mixture of

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Acacia savanna with broad leaved middle layer trees. The soil type of the area has been classified by De Wit and Nachtergaele (1990). The average annual rainfall of the area is 500 mm, while monthly mean minimum and maximum temperatures are 12.8 and 28.6 °C, respectively.

2.2. Study protocol

Ripe fruits of *M. azedarach* were collected on campus and around Gaborone city; sun dried and kept in bags pending the feeding trial. Nineteen male goats with an average age of 12 months were used in the study. The animals were sourced from BCA farm, where animals graze and browse with little or no feed supplementation. External and internal parasite control is only carried out when infestation is heavy enough to warrant intervention. The animals were brought into the feeding pens for a week and fed the control diet to accustom them to the experimental pens. The animals were cared for according to International Guidelines for Biomedical Research Involving Animals (CIOMS, 1985). The animals were blocked, according to weight and initial oocyst count, into two groups and randomly allocated into two treatments; control ($n = 10$; 21.1 ± 0.9 kg; 5440 ± 0.1 oocyst per gram (OPG) and *M. azedarach* treatment ($n = 9$; 21.9 ± 0.9 kg; 7138 ± 0.1 OPG). The animals were housed and fed individually in pens (with concrete floors) for 3 weeks. Each animal had a trough for feed and a bucket for clean water, which was filled daily. The control and *M. azedarach* treatment animals were given a diet comprising the following ingredients; ground maize 19%, grass hay 60%, soya bean oil cake 10%, molasses 8.5%, urea 1.75%, salt 0.5%, dicalcium phosphate 0.5% and formulated to have 12.7% crude protein. The animals were fed 800 g/day at 8.00 am to allow for refusal of 10% above the estimated intake of 3.5% of live weight. In addition to this diet, *M. azedarach* treatment animals were given *M. azedarach* fruits at a rate of 200 g/day.

2.3. Faecal oocyst count and weighing

Faecal samples were taken weekly and oocysts counted using a modified McMaster method where one egg was taken to represent 50 oocysts/g fresh faeces (Niezen et al., 1998). Animals were weighed initially, then weekly for 2 weeks.

2.4. Chemical analyses

A sample (100 g) of *M. azedarach* was taken each time a new bag was opened and bulked. At the end of the trial a subsample was taken for crude protein analysis. Soluble condensed tannins were extracted in duplicate from finely ground pulp, with 10 ml of aqueous acetone (70:30, v/v). Condensed tannins were estimated according to Makkar (1995). The amount of condensed tannins (g/kg DM) as leucocyanidin equivalents is presented. Crude protein (Kjeldahl method) was determined following the method of the Association of Official Analytical Chemists (AOAC, 1996).

2.5. Statistical analyses

Data for oocysts was analysed using the General Linear Model (GLM) of SAS (1999) for repeated measures according to Wolfinger and Chang (1998), to find the effect of the inclusion of *M. azedarach* fruits on OPG. GLM was used to test effect of diet on live weight. Oocysts were transformed using $\log_{10}(\text{OPG} + 1)$ to normalise the data before analysis. Pairwise comparisons of means were computed with lsmeans statement with *pdiff* option. The results are reported as least means ± SED and the significance level was $P < 0.05$.

3. Results

3.1. Oocyst count and live weight

There was no difference ($P > 0.05$) in oocyst count between the treatment groups after 1 week of treatment but at weeks 2 and 3 there was a significant difference ($P < 0.001$; Fig. 1). The oocyst count of *M. azedarach* treated animals remained relatively stable at 6400 OPG throughout the trial, while counts of the control animals increased significantly from week 2 onwards, reaching a peak of 33,000 OPG by week 3 (Fig. 1). No difference ($P > 0.5$) between the live weights of the control diet and those supplemented with fruits of *M. azedarach* was observed. Weights of both groups declined to 16.5 ± 0.9 and 17.9 ± 0.8 in week 1 and to 17.9 ± 0.9 and 19.1 ± 0.9 in week 2, respectively. Overall, weight loss after 3 weeks was 2.8 and 3.2 kg for the *M. azedarach* treatment and control, respectively.

3.2. Chemical composition

The animals were observed eating the pulp of the fruits and spitting out the seeds. The fruit pulp of *M. azedarach* contained 120 g/kg DM crude protein and 40 g/kg DM condensed tannins. The basal diet contained 114 g/kg DM crude protein.

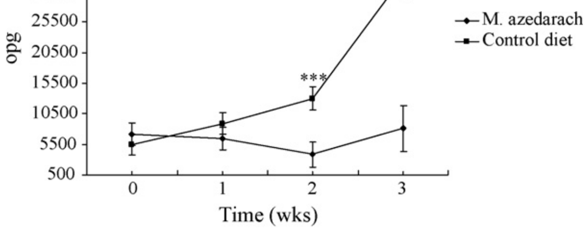

Fig. 1. *Eimeria* oocyst output of male Tswana goats fed a diet with M. azedarach fruits and the control diet.

4. Discussion

This study showed that feeding *M. azedarach* fruits pulp to goats prevented increases in *Eimeria* oocyst counts. On average, *M. azedarach* maintained the *Eimeria* oocyst count at 6400 OPG, which is comparable to a value observed by Faizal et al. (1999) when goats were dosed with toltrazuril, an anticoccidial or albendazole, an anthelmintic, or a combination of both. In contrast, control animals in the present study had their oocysts increase exponentially. Since *M. azedarach* did not reduce increment but rather suppress oocyst and the fact that often symptoms of clinical coccidiosis occurs after the damage has taken place, it would be sensible to use *M. azedarach* as a preventative measure possibly before weaning. The potency of *M. azedarach* has been observed elsewhere. For instance, extracts from leaves, unripe fruits and stems of *M. azedarach* have been reported to be insecticidal (Bohnenstengel et al., 1999), fungicidal (Carpinella et al., 2003), and acaricidial (Borges et al., 2003). Therefore, these results are consistent with the general remedial effects of *M. azedarach*. This makes *M. azedarach* a candidate for natural control of coccidosis.

It was estimated that at a daily feeding rate of 200 g/kg fruits, the pulp of *M. azedarach* supplied 7.3 g/kg/day condensed tannins. Even though this quantity of tannins is lower than the 80 g/kg solution of quebracho tannins used by Athanasiadou et al. (2000) to dose sheep against internal parasites, *M. azedarach* resulted in a 75.4% reduction in oocyst count at the end of the study. This is encouraging since, according to Houdijk et al. (2005) and Paolini et al. (2003), a high reduction in faecal egg output would impact on the epidemiology of gastrointestinal parasite infections and keep pasture contamination at lower levels. One or more chemical constituents, separately, or in combination, may be responsible for anthelmintic activity observed in *Calotropis procera* (Iqbal et al., 2005). Fruits of *M. azedarach* are known to contain alkaloid azaridine, a resin, tannin and meliatannic, and benzoic acids (Carrotola 1939; cited by Oelrichs et al., 1983). Any of these compounds, singly or in combination, may be responsible for the effects observed in the present study.

This experiment was designed to see the effects of *M. azedarach* within the 3-week feeding period but the difference in oocyst output was observed in approximately 2.5 days, however, the difference at that time was not significant. The action of condensed tannins extract (quebracho) against *Trichostrongylus colubriformis* in sheep was observed within 2 days (Athanasiadou et al., 2000). According to these authors (Athanasiadou et al., 2000) the faecal egg count decline was similar to that observed when animals were treated with anthelmintic drugs, which may suggest that tannins may had a direct effect on the parasites. Min et al. (2004) found that suppression of faecal egg count was more rapid (within 5 days) than would be expected from an immune response for goats fed condensed tannins from *Sericea lespedeza* forage. Rapid effects were observed when *M. azedarach* extracts were test against the tick *B. microplus in vitro* (Borges et al., 2003) and after dosing sheep with extracts of *Spondias mombin* (Ademola et al., 2005). *Eimeria* species are intracellular parasites, parasitizing intestinal epithelial cells of goats (Abo-Shehada and Abo-Farieha, 2003) and the consequent ingestion of condensed tannins, which bind to the intestinal mucosa, may cause autolysis or unavailability of free protein to the parasites (Ademola et al., 2005). Hounzangbe-Adote et al. (2005) observed a reduction in excretion of *H. contortus* eggs by sheep fed *Zanthoxylum zanthxyloides* (Fagara leaves) without major changes in worm populations. This was attributed to the effects of *Z. zanthxyloides* on female nematodes' fertility rather than on the worms themselves. These reports on helminths, in comparison to results from the present study, suggest that there may be different mechanisms employed by different plant compounds on internal parasites, i.e. direct effects, or suppression of fertility.

Live weights of the two groups were similar. Although the *M. azedarach* fed-group received about 112.9 g/day of protein, compared to 91.3 g/day of the control, they lost weight after 3 weeks just like the control animals (2.8 vs. 3.2 kg; *M. azedarach* treatment and control, respectively). These losses in the second week may have occurred because animals were still unfamiliar with the diets since they began to gain weight the following week. The lack of difference in live weight when animals are fed tannin-containing diets has previously been reported and may indicate that protein availability had not been increased (Butter et al., 2001).

It is concluded that exposure of *M. azedarach* fruits to *Eimeria* reduced oocyst counts in goats. The effects occurred within a week, which discounts the possibility of an immune response. Condensed tannins may be responsible, but since *M. azedarach* contain an array of other chemicals reported to have pesticidal effects, any of these chemicals, singly or in combination, may be responsible for the observed oocyst reduction. The use of novel plants compounds to help control parasites in livestock production opens opportunities for sustainable and less frequent use of anthelmintics.

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