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The effects of short-term feeding of fresh cassava (*Manihot esculenta*) foliage on gastrointestinal nematode parasite infections in goats in Cambodia

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Abstract. The antiparasitic effect of cassava (*Manihot esculenta* Crantz) was tested in goats artificially infected with gastrointestinal parasitic nematodes. Each experimental group consisted of 6 pen-fed goats kept on a slatted floor. The treatments compared were: 1) controls fed grass only, 2) cassava replaced grass for 3 weeks from the commencement of larval dosing period, and 3) cassava replaced grass for 3 weeks after the worm infection was patent. A total of 2000 mixed-species infective nematode larvae (L3) were administered to each goat in four doses of 500 L3/day, commencing 5 weeks after removal of previously acquired infections with ivermectin. The faecal egg counts (FEC) reduced in both cassava fed groups during the time of feeding, compared to the controls. Although, FEC increased differently with time ($P<0.05$), total adult worm burdens at slaughter (week 15) were not different between the treatment groups. No differences in live weight gain, or packed cell volume, between treatments were found. Whilst these results show limited evidence of an anthelmintic effect of cassava in the diet, they do suggest that feeding, or supplementation, of cassava over an extended period may prove beneficial.

INTRODUCTION

Gastro-intestinal nematode parasites are a major constraint of grazing ruminants in the humid tropics, including Southeast Asia (Sani & Gray, 2004). Singly the most important nematode pathogen is *Haemonchus contortus*, which results in enormous socio-economic losses to small-holder farmers throughout this region (McLeod, 2004), including Cambodia (Sorn & Muirden, 2002). Because of the widespread development of resistance of *H. contortus* to the anthelmintic drugs used to control nematode parasites, which is particularly severe in certain countries of the Asian tropics (Chandrawathani *et al.*, 2003; 2004), increasing interest is now being shown with regards to evaluating non-chemotherapeutic approaches to parasite control (Waller, 1997; 1999). Among these alternative approaches, the use of bioactive plants/forages has received recent research attention (Anathasiadou & Kyriazakis, 2004; Waller & Thamsborg, 2004).

Cassava (*Manihot esculenta* Crantz) is an annual crop in the tropics, usually grown for the production of tubers for human consumption. Additional benefits of this crop are obtained by feeding livestock with its foliage, which has been shown to enhance the growth rate and improve the milk yields of goats and dairy cattle (Wanapat, 2003; Preston & Rodrigez, 2004). Also, there is evidence of anthelmintic activity of cassava foliage on the basis of observed reductions in nematode faecal egg counts (FEC) of animals fed this diet (Granum *et al.*, 2003; Nguyen *et al.*, 2003; Seng & Preston, 2003). This has been attributed to phytochemicals, particularly the complex phenolics, which are generally referred as "tannins" (Nguyen, *et al.*, 2005). However, cassava foliage is also known to contain hydrocyanic acid (HCN) (Conn, 1994; Ravindran, 1995). Although this toxic compound renders cassava hazardous as a feed for monogastric livestock (Awoyinka *et al.*, 1995), it is usually neutralized through biodegradation by ruminants.

Although previous studies confirm an anthelmintic effect of tannins and related substances in temperate and tropical plants (Hoskin *et al.*, 2000; Kabasa *et al.*, 2000; Molan *et al.*, 2002) the direct effect of cassava foliage on nematode parasites of livestock remains unclear. Based on the studies of Seng & Rodriguez (2001) and Seng & Preston (2003) and using naturally infected goats, there is still insufficient evidence to state unequivocally that there is a beneficial anthelmintic effect against gastro-intestinal nematode infections of ruminant livestock. This study was designed to compare the direct effect of cassava foliage following a short-term feeding regimen to goats experimentally infected with mixed nematode infections. The aim was to validate the nematocidal effect of fresh cassava foliage on incoming larval infections and patent worm burdens.

MATERIALS AND METHODS

Location, animals, feeding and parasite infections

The study was carried out at the Center for Livestock and Agricultural Development farm (CelAgrid), located in the Kandal province approximately 26 km southwest of Phnom Penh, Cambodia. Eighteen male goats, 4 to 6 months of age and weighing approximately 13 kg live weight, were acquired from local breeders. They were injected subcutaneously with ivermectin (Ivomec® Merial, New Jersey, USA) according to the standard dose rate (200µg/kg) and housed individually in pens with raised slatted floors. They were sorted according to live weights and then randomized in blocks of three treatment groups, namely:

- Control [CO]: fed grass only
- Cassava / larvae [CaL]: cassava foliage substituted for grass for 3 weeks commencing at the time of larval administration
- Cassava / adult [CaA]: cassava foliage substituted for grass, 3 weeks after the larval dosing period, and fed for 3 weeks.

Larvae for the experimental inoculations were obtained from naturally infected goats from small-holder farms in the Kandal province. Fresh faeces were collected and larvae were cultured for 10 days at approximately 30°C, according to Hansen & Perry (1994). All goats then received doses of nematode infective larvae (L3), which were administered in four doses of 500 L3/day at 3-4 day intervals (each Monday and Friday) during two consecutive weeks (weeks 6 and 7). Thus, each goat received a total of 2000 L3 five weeks after the ivermectin treatment.

Both cassava and grass used in the trial were from the CelAgrid research farm.

Cassava was grown as a semi-perennial crop with repeated harvesting at 2 to 3 month intervals, cutting the stem at 50 to 70 cm above the ground. It was offered to the goats as fresh foliage form, containing main stem, petiole and leaf, after each daily harvesting. Cassava foliage used in the trial was the sweet variety which could be recognized by red petiole and pink root bark. This variety had been stated to contain less toxicity, particularly HCN content, than the bitter one which was distinguished by light green petiole and white root back (Chhay & Rodriguez 2001).

The grass sward consisted primarily of 3 species with the main component being paragrass (*Brachiaria mutica*). Neither of the forages was subjected to livestock grazing, to ensure worm-free status, and was harvested on a daily basis for feeding to the animals.

Measurements

The amount of grass and cassava offered and rejected by the animals were recorded daily and representative samples were taken fortnightly to determine dry matter (DM) (Undersander *et al.*, 1993), nitrogen and Hydro cyanide (HCN) contents (AOAC, 1990). Nematode faecal egg counts (FEC) were determined weekly for each animal according to Hansen & Perry (1994), and blood packed cell volume (PCV) was determined using the micro-hematocrit method. At the end of week 14 of the trial, all goats were slaughtered for worm recovery and enumeration (Hansen & Perry, 1994).

Data analysis

Data of the FECs were logarithmically transformed to normalize variances before analysis. FEC, PCVs and live weight were analysed using repeated measures analysis of variance (ANOVA) of the StatView® 5.0 (SAS Institute), to compare the dynamics of the treatment effect. Regression coefficients of FEC over time were calculated for each goat. Then the difference of FEC between treatment, average daily weight gain and total worm burden (TWB) were subjected to an analysis of variance (ANOVA) using the General Linear Model (GLM) of the MINITAB 14.1 software.

RESULTS

Faecal egg counts and packed cell volume

There was a significant difference of FEC between treatments over times ($P<0.05$). From week 9, the FEC steadily increased in both CO and CaA groups (Figure 1). However the FEC of the CaL goats remained relatively low until week 11, when they reached levels comparable with CaA at week 12. Subsequently, the trajectory of CaL FEC tracked the exponential increase of FEC in the CO group, but at a lower level (~2000 epg). For CaA, the egg counts declined during weeks 12 and 13 and only registered an increase

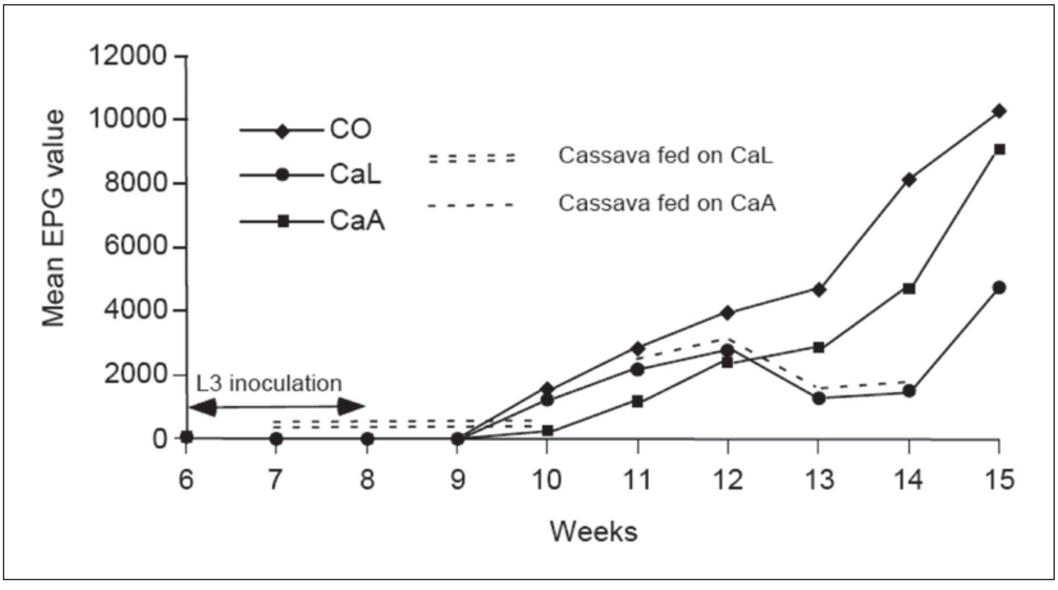

Figure 1. Mean faecal egg counts (FEC) expressed as eggs per gram faeces (EPG) in goats fed cassava foliage during L3 inoculation (CaL), feeding at 3 week after (CaA) and in control animals without cassava (CO).

at the final sampling occasion. The overall rate of increase of FEC for the three groups, from the time of infection until the termination of the trial, were significantly different between groups ($P<0.05$). The lowest value was found in CaA (546±127), followed by CaL (925±182) and CO (1221±200). There was no difference between groups in PCV values over the entire experimental period that ranged between 25- 30%. However, during the time that egg counts were rapidly increasing (week 11 onwards), there were correspondingly decreasing trajectories of PCV in all groups.

Total worm recovery

Haemonchus contortus was the most abundant species, accounting for >50% of the worm burdens in all groups (66%, 59% and 50% for CO, CaL and CaA respectively). Small numbers of *Teladorsagia circumcinta* and *Trichostrongylus axei* were also found in the abomasum of all animals. Additionally, *Trichostrongylus colubriformis* was identified in the small intestine of all animals (Table 1). There was no difference in establishment rates of all worm species, either in the abomasum or small intestines, between the treatment groups, although CO showed the highest values. However, differences in the mean total abomasal worm burdens just failed to reach significance.

Feed intake and weight gain

Dry mater (DM) and crude protein (CP) intake of both CaL and CaA groups during each cassava feeding period were higher than for the other two treatments, which received grass during this specific feeding time ($P<0.05$). Additionally it appeared that the CaA group consumed more cassava than the CaL group (Table 2). There was a significant interaction in live weights with time ($P<0.05$), although there were no overall differences in weight gain between treatments.

Tewe (1994) guaranteed the safety of cassava for livestock if it contains the cyanogens level less than 100 mg HCN equivalent kg^{-1}. In this present study, cassava foliage was found to contain HCN 580 mg/kg DM and goats were able to consume about 500 g of foliage in DM $head^{-1}$ day^{-1} which would ingest about 290 mg HCN per head equivalent to about 20 mg kg^{-1} of body weight day^{-1}. This amount was quite low than the report above that the animals could be assured from the risk of toxicity.

Table 1. Mean of worm burdens in the abomasums and small intestines of goats fed grass (CO), cassava foliage at L3 inoculation (CaL) and cassava foliage at adult worm establishment (CaA)

	CO	CaL	CaA	P. value
Abomasum				
H. contortus	513	379	304	0.121
T. circumcincta	29	13	4	0.247
Trich. axei	54	38	75	0.461
Total Abomasum	596	429	383	0.06
Small intestine				
Trich. colubriformis	183	208	221	0.755
Total number of worms	779	638	604	0.282

Table 2. Average dry matter intake (DM), crude protein intake (CP), average daily weight gain (ADG) and DM intake in % of body weight (BW) in each cassava feeding period of goats fed grass (CO), cassava foliage at L3 inoculation (CaL) and cassava foliage at adult worm establishment (CaA)

	DM (g/day)	CP (g/day)	ADG (g/day)	DM (% of BW)
Period 1: During the period that CaL received cassava foliage				
CO	260[b]	25.3[b]	5.47	2.04[b]
CaL	*401*[a]	*65.5*[a]	*10.47*	*3.04*[a]
CaA	274[b]	26.6[b]	-5.95	1.98[b]
Period 2: During the period that CaA received cassava foliage				
CO	301[b]	29.1[b]	2.85	2.36[b]
CaL	318[b]	30.8[b]	11.9	2.39[b]
CaA	*481*[a]	*68.7*[a]	*17.8*	*3.56*[a]

Letter in italic indicates the group and period that the goats were offered cassava foliage.
[a,b] Means within row with different superscripts are significantly different ($P<0.05$), and row means without superscript are not different ($P>0.05$).

DISCUSSION

The effect of cassava foliage and its suppression on FEC has been indicated in livestock previously. When young naturally infected goats were fed fresh foliage in a ration of either 50% or 100% cassava, there was a significant reduction in FEC after a 3-month feeding period compared to grass fed animals that had FEC approximately 5 times higher (Seng & Preston, 2003). On the other hand, in the present study the reduction in FEC in the groups CaL and CaA compared to the control group were only 35% and 50%, respectively. However, this reduction resulted from only 3 weeks of feeding cassava foliage.

Nguyen *et al.* (2003) also found a possible antiparasitic effect of cassava in naturally infected goats. When they offered foliage including cassava *ad libitum* for 5 months, FEC were lower than in goats fed grass in addition to rice bran and molasses urea block only. Furthermore, when Dung *et al.* (2005) replaced concentrate with cassava hay at rates from 250 to 1000 g/kg DM, in a diet that contained guinea grass and dried cassava tuber slices, FEC of naturally infected goats were gradually reduced with the increasing levels of cassava hay. A further study in two different geographical regions of Cambodia with grazing goats showed that dried cassava foliage had no influence on FEC in the highland area but were reduced by 35% in the lowland area, whereas ensiled cassava foliage reduced FEC by 25% and 70%, respectively (Ho & Preston, 2005b).

The antiparasitic effects of cassava hay have also been indicated in dairy cattle (Wanapat, 2003), as well as in grazing cattle and buffaloes (Netpana *et al.*, 2001; Granum *et al.*, 2003). Both according to Netpana *et al.* (2001) and Granum *et al.* (2003), cattle supplemented with 1kg / head/day of cassava hay resulted in a reduction of the FEC in comparison to control with approximately 30% and 50%, respectively. Interestingly, FEC were only reduced by 41% in cattle treatment with ivermectin according to Netpana *et al.* (2001).

In the study reported here there was an indication that the parasites were affected when goats were offered fresh cassava foliage, both at the time of larval intake and when adult worms were established. From our results, it is suggested that this effect was mainly directed towards abomasal nematodes. These findings lends support to *in vitro* studies with extracts from cassava leaves, which showed toxic effects on infective third stage *H. contortus* larvae attributed to condensed tannins found in these extracts (Lopez *et al.*, 2005).

Over the course of the present study, all goats showed little or no gain in live weight. This could be attributed to the fact that the level of daily DM intake was less than the normal intake required for growing goats, namely 3 - 5 % of body weight (Haenlein, 1995). During the first 2 weeks of feeding cassava foliage, goats in both CaL and CaA groups showed a reduction in live weight, possibly because they were previously fed on grass and had not been adapted to the cassava diet. Ho & Preston (2005a) conducted a digestibility trial with the same breed of goats used in this trial and they suggested that at least 10 days were required for the animals to gradually adapt to feeding on cassava foliage. Our study suggests that an even longer period of adaptation to cassava foliage is necessary – not only to restore expected weight gain in goats, but to also allow ample opportunity for any anthelmintic effects of cassava to be expressed.

This present study demonstrated only a limited effect of short term feeding of fresh cassava foliage in reducing nematode FEC and a clear cut direct anthelmintic effect of cassava on nematode worm populations could not be verified. Further studies are required on the effects of cassava foliage with respect to the amount and duration of feeding, particularly on the nematode parasite *H. contortus*, which is of overwhelming importance to owners of small ruminants in regions of South East Asia where cassava is grown as a staple crop for human consumption.

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