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Evaluation of Anthhelmintic Potential of Pawpaw (*Carica papaya*) Seeds Administered In-Feed and In-Water for West African Dwarf (WAD) Goatss

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Abstract
Forty West African Dwarf (WAD) goats (average 10kg±0.25kg bodyweight) infected naturally with helminthes were used to evaluate the effectiveness of *Carica papaya* seeds in aqueous and crude extract forms against intestinal worms. The animals were randomly assigned into four treatment groups: GI, GII, GIII and GIV consisting of 10goats/group. GI animals were not treated while GII underwent Thiabendazole anthelmintic treatment. GIII and GIV were medically given the powdery and aqueous forms of *C. papaya* seed extract via feed and water respectively. Before anthelmintic treatments and after the 1st and 2nd two weeks of administering the anthelminth, faecal and blood samples were collected for parasitological and haematological analysis. Data collected were subjected to one-way ANOVA. Treatments of both aqueous and powdery forms of *C. papaya* seed extract resulted into a significant increase in lymphocyte counts, packed cell volume (PCV), red blood cell (RBC) and haemoglobin concentration. Conversely, there was significant decrease in the eosinophil counts. The reductions in the faecal egg counts of helminth when *C. papaya* seed extract were applied are comparable to those obtained for thiabendazole treatment. However, the effects of aqueous form of C. papaya seed extract were more conspicuous than the crude (powdery) extract administered via the feed. The results from this study suggests that extracts from *C. papaya* seeds could be of potential use as alternative anthelminthic to synthetic dewormers in therapeutic or sub-therapeutic use for production animals.
Keywords: Anthelmintic, haematology, helminthes, parasitology, thiabendazole

Introduction

Helminthosis caused by endoparasitic worms is one of the most crucial diseases accounting for high production losses in livestock production especially in ruminants. The disease is more prevalent in developing countries and this mainly due to the poor management systems adopted by farmers (Muthumani *et al.,* 2013). Helminth infestations in animals and human beings results in stunted growth coupled with extreme stress conditions which impede on the animals' comfort (Gibbs, 1986).

The key control strategies adopted to control worms throughout the world is the administration of chemical drugs against helminthes together with improved management farm practices (Geert and Dorny, 1995; Kumar et al., 2011). However, the present dependence on frequent exploitive anthelminthic drugs to control parasites is neither desirable nor sustainable. It is therefore becoming problematic with the recent wide-spread resistance to common anthelminthic drugs and this consequently leads to higher economic losses among farmers (Sangser, 1999; Mcleod, 1995). In order to maximize farm cost benefits and reduce loss due to worm infestation, trials on natural plants to prevent and cure helminthes are under investigation for alternative solutions.

Carica papaya is a tropical fruit available in abundance and its seeds as a case sample to fight helminthes and amoeba in human have proven positive from a study carried out by Okeniyi et al. (2007) in Nigeria. More so, a report from India by Goswami et al., (2013) revealed that, *C. papaya* seeds possess herbal medicinal property which is antiparasitic activity. Though, there are limited data on the ethno-veterinary property of *C. papaya* in ruminant animals, the trial on the efficacy of *C. papaya* as anthelmintic in WAD goats was investigated in this preliminary study.

Materials and methods

Seeds preparation

Seeds were freshly obtained from ripe pawpaw fruits and thoroughly washed with clean water to remove dirt and to prevent bacterial contamination. The washed seeds were then sun-dried and subjected to mechanical grounding machine to obtain a powdery form. To obtain a liquid form, 75g of the pawpaw seed powder was weighed and mixed with 150ml distilled water. The combination was centrifuged at 1500 rpm and the supernatant fluid was carefully filtered via sterile filter papers that were transferred into a conical flask for analysis. One ml of the filtrate would be expected to contain 500 mg of the active substance of the pawpaw seed powder.

Experimental animal and design

Forty WAD goats (average 10kg±0.25kg bodyweight; 12 months of age as observed using dentition method) with no history of prior de worming were used in the experiment. The goats were housed in individual pens which were cleaned and fumigated before the arrival of the animals. The animals were allowed to acclimatize for 2 weeks before commencing the collection of experimental data. The goats were then completely randomized into 4 experimental groups (G) (i.e. I, II, III and IV) consisting of 10 goats/treatment. All animals were adequately fed and water was provided *ad libitum* during the experimental period. GI animals received no de worming medication (i.e. control); GII were treated with thiabendazole (conventional anthelminth drug) at dosage rate of 50 mg/kg body weight peculiar to WAD goats (Hansen and Perry, 1990); GIII had the pawpaw seed extracts administered through their feeds at 300mg per day; while GIV animals where drenched with the aqueous crude extract at ratio 1:10 ml of water. Following the acclimatization period, both the *C. papaya* seeds extract and thiabendazole were administered for 3 consecutive days for the 1st two weeks. Similar procedure was repeated for the 2nd two weeks.

Parasitological analysis

Before anthelminth treatments and after the 1st and 2nd two weeks of administering the anthelminth treatments, the feacal samples of each goat in the experimental treatments were obtained and preserved in labeled sterile universal bottles for detection of the kind of helminthes eggs present by means of flotation techniques (Hansen and Perry, 1990). The floatation system, which involved the use of salted (NaCl) water, was used to determine the types of helminthes present in the faecal samples. The customized McMaster egg-counting technique was used for nematodes counts. In determining fluke count, the modified McMaster egg-counting technique as used for nematodes counts was engaged, except that saturated zinc sulphate solution was used for estimation of fluke egg counts.

Haematological analysis

Before anthelminth treatments and after the 1st and 2nd two weeks of administering the anthelminth treatments, haematological parameters were measured. Ten ml syringes and 25-gauge needles were used to collect blood samples from the jugular vein of each goat and transferred into suitably labeled EDTA bottles. Sahli's technique was used to determine the haemoglobin (Hb) concentration. Erythrocytes and leucocytes were counted manually by means of Neubau's method, i.e. the micohaematocrit method. White blood cell differential counts were also determined.

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using completely randomized design by SAS package (2000) and Duncan multiple range test was used to separate the significance of the treatment mean values (Field, 2000).

Results

Faecal Egg Counts

The research indicated that all animals were loaded with worms, starting with *Monienza spp*, *Haemonchus contortus*, *Faciola gangitica*, *Trichostrogylus spp* and *Strongyloides* (Bahrami et al., 2011). Chemical drug application together with *C. papaya* in powder and aqueous form resulted to a notable decrease of worms. Following the subsequent treatment, Group II and IV animals perfectly responded to treatment, but GIII animals were not successfully dewormed. Table 1 indicated significant differences at ($p<0.001$) by means of ANOVA tests.

Haematological analysis

As presented in Table 2, PCV mean values for GI, GII, GIII and GIV animals prior to treatments were 19.00±1.20, 22.00±1.50, 22.50±1.40 and 19.00±0.50 respectively. This parameter showed a significant increase by the treatment. The haemoglobin values before treatment were 6.50±0.50, 6.80±0.20, 6.80±0.40 and 6.30±0.60 for GI, GII, GIII and GIV animals in that order. There was also a notable increase from this parameter after administration. With regard to RBC, the mean values for GI, GII, GIII and GIV animals ahead of treatment were 11.40±0.40, 10.40±0.60, 11.20±0.70 and 11.40±0.50 accordingly. The RBC also indicated a significant increase after administration similar to first two parameters. Likewise, lymphocyte results indicated increase levels but eosinophil values diminished with drugs application. In Table 2, comparing the haematological parameters' values among groups with the aid of ANOVA test, the variations were significant at ($p<0.001$) (McDougall et al., 1991).

Discussion and conclusion

Remarkable diminution of helminthes was observed with powder and aqueous extract application in this study. The reduction of worms was more pronounced in GIV compare to GIII animals. Similar results were observed following the subsequent administration. This difference may probably be due to inability of animals to pick up sufficient active ingredients being administered in the feed during the first week. And the anthelmintic substance found in *C. papaya* seeds can be probably attributed to *papain*.

From the results obtained in this study, powder and aqueous extracts of *C. papaya* could be suggested as potent alternative for synthetic anthelminthic drugs. This is therefore a welcome innovation in organic health care for helminthes infestation. Since the seeds of *C. papaya* fruit are available mostly all year round with easy access for exploitation in tropical areas, therefore, the medication expenses on anthelmintic would be significantly reduced with subsequent increase in total farm profits for farmers. However, in addition to the high content of protein and good source of calcium, magnesium and phosphorus of *C. papaya* seeds, presence of high fat and some anti-nutritional factors such as phytic acid, tannins, trypsin inhibitors and oxalate have been attributed to the seeds (El-Safy et al., 2012; Elezuo et al., 2012). Because of these secondary metabolites and the high fat content related with *C. papaya* seeds, it is necessary to recommend that more studies should be carried out to evaluate possible harmful effect, normal dosage level and its cost effectiveness as anthelminthic in goats and other ruminant species prior to global recommendation for usage in livestock production.

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Table1: Helminths' faecal egg count in experimental goats treated with drug/pawpaw powder and aqueous extract

Helminth species **(epg)**	**Groups (G)**	**Before treatment**	***1st 14days**	****2nd 14days**
Monieza spp	I	2400±1.20	2550±0.80	2500±1.20
	II	2200±0.50	0	0
	III	2450±0.50	800±0.60	300±0.40
	IV	2250±1.00	0	0
Haemonchus contortus	I	1800±0.60	1750±0.50	1780±1.20
	II	1600±1.50	0	0
	III	1600±0.50	400±0.50	100±0.20
	IV	1500±0.60	0	0
Fasciola gangitica	I	800±0.50	1000±0.80	950±0.50
	II	900±0.60	0	0
	III	750±0.50	0	0
	IV	800±0.60	0	0
Trichostronglylus spp	I	1600±0.80	1700±0.50	1800±0.50
	II	1600±0.20	0	0
	III	1500±0.40	400±0.40	200±0.20
	IV	1700±0.40	0	0
Srongyloides spp	I	800±0.10	900±0.50	1200±0.40
	II	650±0.50	0	0
	III	950±0.60	300±0.40	0
	IV	950±1.20	0	0

epg: egg per gram of faeces;
Results are expressed as means ± SE (Standard error);
*1st 14days after administration of drug/pawpaw powder and aqueous extract;
**2nd 14days after 2nd administration of drug/pawpaw powder and aqueous extract

Table 2: Hematological parameters of experimental goats treated with drug/pawpaw powder and aqueous extract

Blood Parameters	**Groups (G)**	**Before treatment**	**1st 14days**	**2nd 14days**
Pack cell volume (%)	I	19.00±1.20	18.50±1.40	19.10±1.30
	II	22.00±1.50	33.00±1.09	32.00±1.20
	III	22.50±1.40	31.00±0.90	32.00±1.09
	IV	19.00±0.50	30.00±1.20	31.00±2.20
Haemoglobin (g/dl)	I	6.50±0.50	6.40±0.30	6.30±0.20
	II	6.80±0.20	10.50±0.40	9.60±0.30
	III	6.80±0.40	9.80±0.40	9.80±0.05
	IV	6.30±0.60	10.20±0.40	9.80±0.40
Red blood cell (x $10^6/mm^3$)	I	11.40±0.40	11.30±0.20	11.00±0.50
	II	10.40±0.60	15.33±0.45	15.60±0.50
	III	11.20±0.70	13.50±0.40	13.60±1.20
	IV	11.40±0.50	15.88±0.43	15.90±1.20
Lymphocytes (%)	I	60.00±0.60	57.00±1.00	54.00±0.50
	II	63.00±0.40	60.00±0.40	55.00±0.70
	III	62.00±0.30	60.00±0.30	60.00±0.40
	IV	60.00±0.40	62.00±0.40	58.00±0.40
Eosinophil (%)	I	2.50±1.50	2.30±1.60	2.40±1.60
	II	2.30±1.20	1.00±0.50	1.40±0.70
	III	2.50±1.25	0.90±0.09	1.00±0.09
	IV	2.40±1.30	1.10±0.09	0.90±1.2

Results are expressed as means ± SE (Standard error);
*1st 14days after administration of drug/pawpaw powder and aqueous extract;
**2nd 14days after 2nd administration of drug/pawpaw powder and aqueous extract

Conflict of interest declaration

The authors declare and confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome