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Comparative Anthelmintic Efficacy of Pineapple and Neem Leaves in Broiler Chickens Experimentally Infected with *Ascaridia galli*

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Abstract: A study was carried out in broiler chicken for comparative anthelmintic efficacy of neem and pineapple leave powder with piperazin hydrate for treatment of experimental *Ascaridia galli* infection. The affected birds had lower levels of Hb, PCV, Ca and P. The profile changed after treatment with neem and pineapple leave powder which was comparable to that observed after treatment with piperazine hydrate. Neem and pineapple leave powder treatment caused cent percent evacuation of the worms on 28th and 56th day post treatment respectively.

Key words: *Ascarida galli*, neem, pineapple, haemoglobin, calcium, phosphorus

INTRODUCTION

Ascaridiosis is still a cause of economic losses in modern poultry farming (Permin and Raving, 2001). Among the parasites next to the coccidium, *Ascaridia galli* infection in chicken is considered to be of great importance as it can cause extensive economic losses in different ways such as loss of weight, meat production, egg production and mortality of birds (Kamal, 1989). It is an intestinal worm and chickens under three months of age are mostly susceptible to this worm. Control of gastro-intestinal nematodes especially of *A. galli* is mainly based on regular anthelmintic treatment. Because of high cost of medicinal and unavailability of anthelmintics, the farmers cannot afford to purchase the anthelmintics. Further, frequent use of these anthelmintics increases the resistant population of nematodes (Walter *et al*., 1987). In this context, investigations on indigenous medicinal plants might contribute to develop effective and low cost herbal anthelmintics.

Some indigenous plants like neem, pineapple and tobacco have been reported to have anthelmintic properties. The present research work was conducted to evaluate the comparative efficacy of the leave of neem and pineapple with anthelmintic like piperazine hydrate and also to evaluate the haemato-biochemical changes of broiler chicken experimentally infected with *A. galli*.

MATERIALS AND METHODS

Sample collection and prevalence studies: In the present study intestine from 100 chickens were collected from different slaughterhouses in Aizawl to see the prevalence of *A. galli* infestation in poultry. The samples were brought to the laboratory immediately after collection. The mature parasites were recovered and collected after opening the individual intestine in normal saline solution following the procedure of Alcorn (2001). Further, the mature male and female worms were identified and separated based on their length.

Collection and authentication of plants: The plants *Azadirachta indica* (neem) and *Ananas comosus* (pineapple) were collected from different parts of Mizoram. The plants were submitted as a herbarium specimen for authentication to the Regional Office, Botanical Survey of India (BSI), Shillong had been identified respectively. Neem and pineapple leaves were collected and washed thoroughly with clean water for several times followed by double distilled water. Pineapple leaves were chopped into small pieces and then dried under shade at a well ventilated place. Both the leaves were ground to powder form and were stored at -4°C until use.

Culture and inoculation of infective eggs of *A. galli*: The collected female worms were macerated in a pestle and mortar with 5 ml PBS and washed 5-6 times in distilled water before placing them in clean petridishes for culturing in normal saline at 28-30°C for 21 days for embryonation in a B.O.D. incubator. Few drops of 5% formalin were added to the culture medium to prevent bacterial and fungal growth (Malik, 1981; Deka and

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Borah, 2008; Islam *et al*., 2008). When the infective stage was reached, the eggs were collected in a centrifuge tube containing normal saline. The pellet was collected after washing with normal saline for three times at 1000 rpm. The pellet was suspended in double distilled water to count the number of eggs as per the procedure of Choudhury (1989).

One hundred commercial (Vencobb 400) broiler chicks procured from M/s. Ranchaw Dawr, Durtlang, Aizawl, Mizoram, India were used in the present study. Each of the chicks was fed with 2 ml of the egg suspension containing 400 eggs of infective stage of *A. galli*. After the appearance *of A. galli* eggs in the droppings of the infected birds i.e. between 55-62 days post infection, the birds were then randomly divided into 4 groups consisting of 25 birds in each group. Group I was untreated while Group II, III and IV were treated with piperazine, neem and pineapple respectively.

Piperazine hydrate (Biprazine™, Brihans Laboratories) was administered at the rate of 200 mg/kg body weight orally as single dose while neem and pineapple powders were administered orally after mixing it with saturated sucrose solution at the rate of 100 mg/kg body weight and 1 g/kg body weight as a single dose respectively.

Collection and processing of blood: Blood samples for haematological and biochemical analysis were collected from wing vein on 0 day, 7th, 14th, 21st, 28th and 56th day post treatment. Five birds from each group were randomly selected and blood sample was collected for analysis. For haematological studies EDTA anticoagulated blood was used while serum was used for estimation of calcium and phosphorus. All the estimations had been carried out on the same day of collection of the samples.

Haematological estimation: Hemoglobin and PCV were estimated by following the procedure described by Jain (1986). The serum calcium and inorganic phosphorus in serum sample was estimated in a UV-Vis Spectrophotometer (Jenway- 6505) using commercially available diagnostic kits purchased from M/s.Crest Biosystems.

Evaluation of efficacy of treatment: The efficacy of treatment was determined based on worm count. Two birds from each group was sacrificed for determination of the presence of parasites in the intestine on 0, 7th, 14th, 21st, 28th and 56th day post treatment. Post mortem examinations were performed as described by Alcorn (2001). After decapitation, the entire gastrointestinal tract including the oesophagus was collected from each bird.

The gastrointestinal tract was opened in a longitudinal section and the contents carefully washed through a sieve for collection of mature parasite.

The efficacy of the drug on the basis of post mortem worm count was evaluated as per the method of Soulsby (1982):

$$AE\ (\%) = \frac{A - B \times 100}{A}$$

Where:

AE = Anthelmintic efficacy

A = Number of parasites in infected untreated (control) birds

B = Number of parasites in treated birds

Statistical analysis: The data generated were subjected to statistical analysis by employing ANOVA and Duncan post test for meaningful and accurate comparison and interpretation between control and treatment groups using SPSS Version 16.0.

RESULTS

Haemato-biochemical response: The haemoglobin levels in the group I declined gradually with advance stage of infection while in the treated groups Hb levels increased gradually. The change in the Hb levels in different groups is given in Table 1. There was significant differences in Hb content among the different treatment groups ($p<0.01$). Amongst all the treatment groups, neem treatment increased Hb level from 9.6±0.33 to 16±0.42 mg/dl by 21st day of post treatment. In the pineapple treatment there was no improvement in Hb level. The change in the Hb content of the birds in control and treated groups is shown in Fig. 1. PCV value declined to as low as 18.75±0.97% in untreated group while in other groups PCV increased due to treatments (Fig. 2). There were significant differences between the treatments ($p<0.01$). Neem and piperazine treatments had comparable results in PCV on 56th day of the treatment while the pineapple treatment has lower PCV compared to their counterparts. The analysis of biochemical profile of the infected birds showed lower Ca level. The decreased in the level of Ca however were controlled at relatively stable levels by treatments with piperazine, neem and pineapple leaves (Fig. 3). Pineapple treated group had high Ca levels on 21st and 28th day of post treatment (Fig. 4). The untreated birds had lower P levels than the treated groups. There were increasing trends in all the treated groups (Fig. 4). The pineapple treatment significantly increases the level of P from 3.2-9.4 mg/dl at 21st day of post treatment. This increase was accompanied by increase in Ca level however, it reflects some imbalances between Ca:P ratio in the pineapple treated group.

Table 1: Haemato-biochemical profile of the control and treated birds with piperazine and two herbal anthelmintics viz. neem and pineapple leaves on different stages of treatment

Parameter	Groups	0 day	7th day	14th day	21st day	28th day	56th day
Hb (g/dl)	Control	9.60±0.33	9.40±0.58	8.50±0.35	7.00±0.33	6.50±0.32	6.25±0.32
	Piperazine		10.00±0.35	10.50±0.32	12.50±0.34	15.00±0.33	14.00±0.31
	Neem		9.00±0.33	9.00±0.35	14.00±0.59	16.00±0.42	15.00±0.61
	Pineapple		9.50±0.50	10.00±0.35	9.00±0.57	9.50±1.25	9.00±0.35
PCV (%)	Control	28.50±1.06	25.50±0.71	21.00±1.06	21.00±2.16	20.00±2.25	18.75±0.97
	Piperazine		28.40±2.25	29.50±2.16	30.00±0.76	45.00±1.06	42.00±1.06
	Neem		28.00±1.06	35.00±0.76	40.00±1.45	48.00±0.57	45.00±0.42
	Pineapple		28.50±0.65	29.00±1.71	30.00±0.57	35.00±0.50	36.00±1.06
Ca (mg/dl)	Control	7.50±0.06	7.45±0.40	7.27±0.09	7.07±0.10	6.57±0.40	3.95±0.36
	Piperazine		7.47±0.21	8.14±0.45	8.85±0.24	9.30±0.24	9.95±0.23
	Neem		7.43±0.29	9.80±0.28	9.64±0.27	8.95±0.40	8.60±0.33
	Pineapple		7.60±0.22	9.70±0.65	10.80±0.41	10.50±0.28	9.19±0.23
P (mg/dl)	Control	3.20±0.45	3.04±0.42	3.40±0.45	3.22±0.30	2.97±0.13	2.27±0.25
	Piperazine		3.94±0.09	4.26±0.36	4.58±0.12	4.35±0.13	4.70±0.15
	Neem		3.67±0.22	4.70±0.20	4.58±0.21	3.97±0.36	3.79±0.21
	Pineapple		2.81±0.15	8.89±0.48	9.40±0.24	9.02±0.21	8.71±0.37

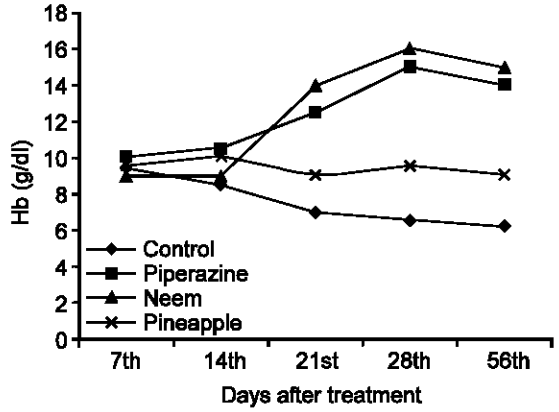

Fig. 1: Change in the Haemoglobin content of the birds in control and treated with piperazine, neem and pineapple leaves in different stages of treatment

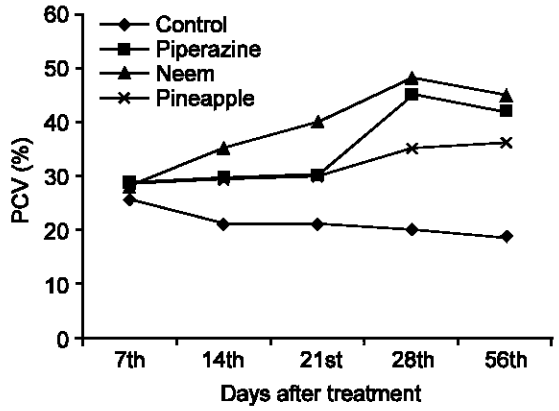

Fig. 2: Change in the PCV content of the birds in control and treated with piperazine, neem and pineapple leaves in different stages of treatment

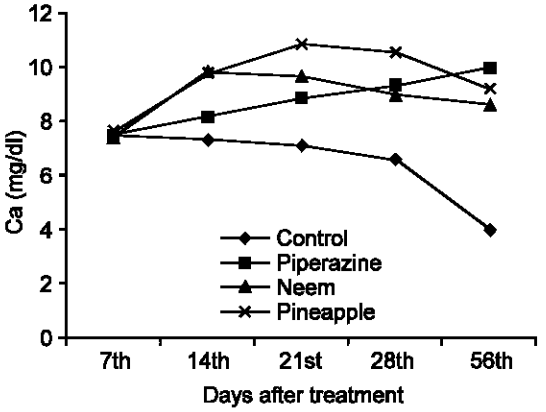

Fig. 3: Change in the Calcium content of the birds in control and treated with piperazine, neem and pineapple leaves in different stages of treatment

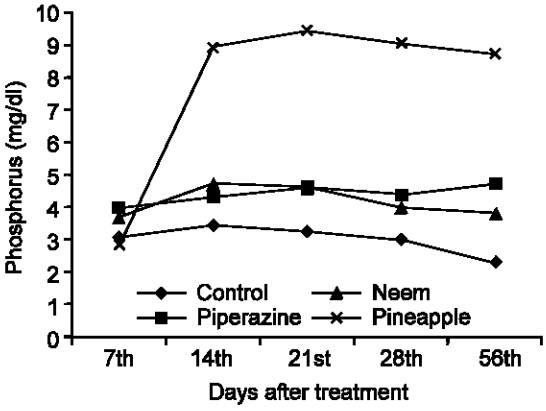

Fig. 4: Change in the phosphorus content of the birds in control and treated with piperazine, neem and pineapple leaves in different stages of treatment

Table 2: Comparative Anthelmintic Efficacy (AE) of piperazine, neem leave and pineapple leave on the broiler chickens experimentally infected with *A. galli*

		Number of parasites					
		Before	After treatment				
Groups	Anthelmintic used	treatment (0 day)	7th day	14th day	21st day	28th day	56th day
I	Control	42	43	45	40	45	38
II	Piperazine	44	16	00	00	00	00
III	Neem	43	26	16	12	07	00
IV	Pineapple	45	18	10	05	00	00
Anthelmintic Efficacy (AE) of piperazine, neem leave and pineapple leave							
	AE of Piperazine		62.79%	100%	100%	100%	100%
	AE of Neem		39.53%	64.44%	70%	84.44%	100%
	AE of Pineapple		58.14%	77.78%	87.5%	100%	100%

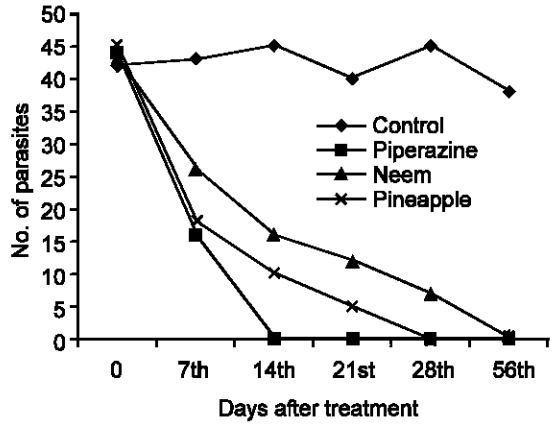

Fig. 5: Parasite load of the birds in control and treated with piperazine, neem and pineapple leaves in different stages of treatment

Efficacy of treatment: The worm burden prior to treatment (0 day) and after treatment on 7th, 14th, 21st, 28th and 56th with standard drug piperazine and two herbal anthelmintic viz. neem and pineapple leaves in the experimental birds is shown in Table 2 and Fig. 5. Piperazine citrate, pineapple and neem leave evacuated cent percent parasites on 14th, 28th and 56th day of post treatment respectively.

DISCUSSION

In the present study conducted during the month of January 2010 the prevalence rate of *A. galli* infection was found to be at 5%. The prevalence rate of *A. galli* infection is usually low during the winter season. Sandhu *et al.* (2009) reported lower prevalence rate of ascariasis in poultry of Punjab during winter months.

The decreased in Hb and PCV percentage in the untreated group in the present study compared to that of treated groups may be due to the destruction of the mucosa of the small intestine causing penetration and rupture of small blood vessels. This must have resulted to fall in number of RBC which consequently lowered Hb and PCV. Similar findings have been reported with *A. galli* infection in other parts of the country (Matta and Ahluwalia, 1982; Kumar *et al.*, 2003; Deka and Borah, 2008). Kumar and his co-workers (2003) and Deka and Borah (2008) opined that lower Hb concentration in infected birds can be correlated with the activities of early larval stage of *A. galli* in the process of penetration with resultant destruction of mucosa of small intestine and rupture of small blood vessels. Kumar *et al.* (2003) also reported that fall of Hb might be due to disturbance caused by worms rather than a direct blood lost.

The decrease level of calcium and phosphorus in the infective birds may be due to disturbances in the small intestine due to presence of worms. Anwar and Rahman (2002) reported that the decrease in Ca and phosphorus are indicative of pathological catabolic processes that have been contributed due to the effect of *A. galli* infection on the middle part of small intestine, where absorption of minerals occurred. The administration of the piperazine and herbal anthelmintic viz. neem and pineapple leaves in the birds caused removal of *A. galli* and as the treatment continued, the disturbances in the small intestine were lowered or removed which ultimately restored the capacity of the small intestinal absorption activities. This was clearly visible from the present finding that prior to treatment both the Ca and phosphorus were below the normal ranges. With piperazine citrate treatment cent percent evacuation of worms was recorded on 14th day of post treatment and it was correlated with the gradual rise in Ca and P level from 14th day onwards. With neem treatment cent percent evacuation of worms took 56 days which was correlated with the gradual rise in Ca and P level from 14th day of post treatment. Similarly with pineapple treatment cent percent evacuation of worms was recorded on 28th day of post treatment and it was correlated with the gradual rise in Ca and phosphorus level from 14th day onwards with the highest level Ca and phosphorus on 21st day of post treatment. The present findings indicates that treatment with both neem and pineapple leaves have comparable results in terms of haemato-biochemical profile though the treatment with herbal anthelmintic viz. neem and pineapple leaves took more days for cent percent evacuation of worms.

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