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Use of neem (*Azadirachta indica*) aqueous extract as a treatment for poultry coccidiosis in Borno State, Nigeria

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ABSTRACT: The anticoccidial efficacy of the aqueous neem leaf extract in comparison to amprolium showed that the aqueous extract dose of 800mg/kg compared favourably with 10mg/litre of amprolium in treating the disease, both showed 100% survival rates for infected and treated chickens with zero oocyst per gram at day 4 post treatment. Also, the mean weight (grams) of infected and treated chickens improved significantly ($P < 0.05$) at day 7-post treatment. Biochemical serum analysis of coccidial infected chickens showed a significant increase ($P < 0.05$) in alanine aminotransferase (ALT) levels (2.9-245.2) and a significant decrease in alkaline phosphate activity from 4643.9 to 2329.9.

Key Words: Neem; *Azadirachta indica*; Coccidiosis; Poultry; Borno State; Nigeria.

Introduction

The condition known as coccidiosis is one of the most important poultry diseases, most commonly occurring under intensive rearing conditions, where pathogenic populations of the causative agent easily build up (C.A.B.I., 2003), causing significant economic losses in the poultry industry by reducing weight gains and feed conversion efficiency (Raymond and Patricia, 2000). Also the disease coccidiosis is endemic in Nigeria, (Akpavie, 1998), particularly in Borno State (Biu and Dzugwahi, 2003; Biu, *et al.*, 2003^a, Biu, *et al.*, 2003^b, Biu, *et al.*, 2003^c) where intensive poultry operations are carried out (Akpavie, 1998). Much money is also spent in purchasing medication for treatment. However, the propensity for the development of drug resistance in the causative parasite has enabled the disease to retain its place as a rather tenacious enigma to the poultry industry.

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The neem (*Azadirachta indica* A. Juss) in Nigeria, forms about 90% of the trees in the forestry plantations (Donli and Buahin, 1998); and despite detailed studies carried out on the neem, it is apparent that information on its various uses is scanty in this semi-arid area of Borno State, particularly with respect to poultry diseases, hence the reason for this study to determine the efficacy of the neem leaf aqueous extract on chicken coccidiosis.

Materials and Methods

Fifty (50)mls of infective *Eimeria* oocyst suspension was obtained from the National Veterinary Research Institute (NVRI), and each 1ml of the oocyst suspension contained a total of 2000 mixed *Eimeria* oocysts, which was determined by the Mac Master technique (Kaufmann, 1996).

Twenty eight (28) 4 weeks old broiler chickens used for this experiment were all weighed before the commencement of the experiment using a triple beam balance (Florham Park NJ7932). They were then divided into seven (7) groups A to G) of four (4) chickens each and housed in wooden cages. Groups A – F were infected with an oral dose of 1ml of coccidian suspension each containing 2000 oocysts and observed for a period of four (4) days for clinical signs of coccidiosis, such as depression, ruffled feathers and bloody droppings. Group F served as positive control (infected and not treated) while Group G represented the negative control (not infected nor treated).

Following the infection, Groups A-D were given oral doses of 200mg, 400mg, 800mg and 1600mg of the aqueous neem leaf extract respectively to ascertain its efficacy against coccidiosis; while Group E was treated with Amprolium^R (an anticoccidial drug 250WSP KEPRO B.V. HOLLAND) at 10mg/litre of clean tap water.

Parameters evaluated

Clinical signs

The various groups were monitored daily following infection and during treatment for clinical symptoms of coccidiosis and their improvement.

Parasitology

Faecal droppings of each group was sampled daily and the oocyst per gram (OPG) of faeces evaluated for each group using the Modified Mac Master technique (Kaufmann, 1996).

Biochemical evaluation

Two mls of blood sample was collected at 2 days interval using the wing vein of chickens of each group (i.e. the infected and uninfected) into blood sample bottles without an anticoagulant. The coagulated blood samples were immediately centrifuged, the plasma separated and used for the serum metabolites (total proteins, albumin, uric acid, creatinine and urea), plasma transaminases (Alanine amino transaminase (ALT), Aspartate amino transaminase (AST) and Alkaline phosphatase (ALP) determinations (Reitman and Frankel, 1957; Seaton and Ali, 1984; Afonja, 1997). The tests for the plasma transaminases were based on pyruvate formation and its reaction with vanillin to give a yellow color (Reitman and Frankel, 1957) and the results were read in Karmen units (K.U>). Commercial kits (Randox Laboratories Ltd., UK) were used for the enzyme estimates (Reitman and Frankel, 1957; Rec, 1972).

Results were expressed as mean $\pm$ standard deviation (S.D.). Significance between control, treated and untreated groups were determined by students test. A probability level of ($P < 0.05$) was regarded as significant (Graph Pad InStat^R Dataset, 1998).

Results

Clinical signs

All chickens experimentally infected with coccidiosis showed clinical signs of depression, weakness, bloody diarrhea, anorexia, ruffled feathers, with death of few chickens. These clinical signs became less obvious following treatment with the neem leaf aqueous extract and amprolium^R.

Gross lesions

Gross lesions of infected and untreated chickens indicated congested lungs, liver with multiple grayish foci, bile imbibition, slight enlargement with multiple blunt edges, hydropericardium with congestion, the spleen showed focal congestion about 1/3 of its size, intestinal vessels were injected and had extensive mottled haemorrhages on the mucosa while the caeca contained bloody faeces, mucoid debris and haemorrhages on the mucosa.

Groups that died during treatment had congested lungs with froth; liver was enlarged and congested, intestinal and caecal mucosa with extensive haemorrhages.

Survival rate following treatment using doses of neem leaf aqueous extract and amprolium

Table 1 shows the survival rate of chickens following exposure to varying doses of neem leaf aqueous extract as treatment of coccidiosis. Treatment was best (100% survival rate) at the 800mg/kg dose, which compared favourably with amprolium^R at 10mg/litre; while the 200mg/kg and 400mg/kg doses had lower survival rates of 25.0% and 50.0% respectively. At 1600mg/kg dose, the neem leaf aqueous extract was initially effective but later was induced mortality, which could probably be due to toxicity. All members of Group F (positive control) died while those of Group G (negative control) all survived the experiment.

Oocysts per gram of faeces of coccidian- infected chickens before and during treatment

When the oocysts per gram (OPG) of the faeces of coccidian- infected chickens were counted before and during treatment with varying doses of neem leaf aqueous extract, the groups that received 200 and 400mg/kg doses only had their OPG drastically reduced from 57,000 and 81,100 to 5,800 and 3000 by the 4th day post treatment. While the groups that received doses of 800mg/kg and 1600mg/kg neem leaf aqueous extract, and 10mg/litre amprolium[®] had a total clearance of their oocysts by the 4th day post treatment (Table 2). Table 2 however shows that the positive control group had a total OPG of 140,400 by day 4-post infection and all the birds died.

Mean weights (g) of coccidian- infected chickens, before, during and after treatment

Table 3 shows the mean weights (g) of coccidian- infected chickens, before, during and after treatment with 200, 400, 800 and 1600mg/kg of neem leaf aqueous extract; and the results indicate that the low weights recorded before and during early treatment, increased significantly by day 7-post treatment.

The biochemical parameters of coccidian- infected and coccidian-free chickens

The biochemical parameters of coccidian- infected and coccidian- free chickens are shown in Table 4, which indicates that the ALT level increased significantly ($P<0.05$) from 2.9 μ mol/l in coccidian- free to 254.5 μ mol/l in infected chickens, ALP activity significantly decreased ($P<0.05$) from 4643.9 μ mol/l in coccidian-free to 2329.9 μ mol/l in infected chickens, but there were no significant changes ($P>0.05$) in the mean values of AST (μ mol/l) level, and urea (μ mol/l), albumin (g/dl), and uric acid (μ mol/l).

Table 1: Survival rate of coccidia-infected chickens treated with varying doses of neem leaf aqueous extract (percentage in parenthesis).

Group	No. of chickens	Dose (mg/kg) of extract	Period of treatment (Days)	No(%) chickens that survived.
A	4	200	4	1(25.0)
B	4	400	4	2(50.0)
C	4	800	4	4(100.0)
D	4	1600	4	2(50.0)
E	4	Amprolium (10mg/litre)	4	4(100.0)
F	4	⁺ ve control	-	All dead
G	4	⁻ ve control	-	4(100.0)

Inference: 1. 200mg and 400mg = Fairly doses for treatment with 25% and 50.0% survival rates.
 2. 800mg of aqueous neem leaf extract compared favorably with amprolium 10mg/litre and hence could be described as the correct therapeutic dose.
 3. 1600mg of aqueous neem leaf extract was initially good as testament, but induced mortality, could probably be due to toxicity.

Table 2: Oocysts per gram (OPG) counts, before and during treatment of coccidian-infected chickens with varying doses of neem leaf aqueous extract.

Treatment	No. of Birds	Dose(mg/kg) of extract	Day before treatment	Days of Treatment			
			0	1	2	3	4
A	4	200	57,000	47,400	31,300	24,500	5,800
B	4	400	81,100	60,800	32,100	22,800	3,000
C	4	800	78,100	66,300	16,000	1,300	0
D	4	1600	74,000	58,400	28,300	2,000	0
E	4	10mg/litre	84,700	71,110	14,600	2,100	0
F	4	-	83,700	98,500	120,900	140,400	All died
G	4	-	-	-	-	-	-

E = treated with amprolium^R

F = positive control (infected and untreated)

G = negative control (uninfected and untreated).

Table 3: Mean weight (g) of coccidian-infected chickens before, during and after treatment varying doses of neem leaf aqueous extract.

Groups	Dose(mg/kg)	No. of chickens	Mean range	±SD weight of	Chickens(grams)
			Before infection	During infection	After treatment
A	200	4	450±29.4 ^{a*} (420-480)	405±9.3 ^a (395-412)	665±63.6 ^b (620-710)
B	400	4	477.5±38.8 (430-520)	418±7.6 (410-425)	646±74.9 (585-730)
C	800	4	510±55.3 (48-565)	440±13.6 (410-480)	695±76.3 (600-785)
D	1600	4	448±35.7 (400-480)	405±13.2 (395-420)	687±53 (650-725)
E		4	523±61.3 (460-590)	450±43.6 (420-500)	778±113.6 (675-900)
F		4	442±81.4 (370-550)	373.3±41.6 (340-420)	613±80.9 (555-706)
G		4	487±8±23.5 (465-510)	523.7±33.0 (485-560)	845±135.4 (750-1000).

Table 4: Mean values of biochemical parameters for coccidian-infected and coccidian-free chickens (ranges in parenthesis).

Parameters	Mean ± SD biochemical	Values
	Coccidia-infected chickens	Coccidia free chickens
Creatinine	12.5±4.9 (4-27) ^{a*}	13.2±4.2 (6-21) ^a
Urea	1.0±0.3 (0.6-1.6) ^b	1.6±0.4 (1.1-2.4) ^c
AST	310.2±57.3 (225-422) ^a	279.2±42.3 (202-405) ^a
ALT	254.5±42.9 (201-309) ^b	2.9±1.9 (0.01-8.1) ^c
ALP	2329.9±2648 (116-11769) ^b	4643.9±2301.3 (1008-8153) ^c
Albumin	13.4±2.3 (6.3-17.5) ^a	13.9±1.8 (8.7-17.7) ^a
Uric acid	581.9±92.4 (415-756) ^a	526.7±122.6 (301-762) ^a

^{NB} Rows with different superscripts differ significantly.

Discussion

The anticoccidial efficacy study shows that the aqueous neem leaf extract had the best survival rate for the chickens at the dosage of 800mg/kg and compared favorably with amprolium^{Rx} (an orthodox treatment) 10mg/litre i.e. it suppressed the oocyst per gram.

The above findings agreed with the reports by National Research Council, (1992), that neem extracts contain the chemical *Azadirachtin* which has a significant efficiency on pests, deformatal effect on viruses, mites, fungal pathogens, plant parasitic nematodes, intestinal worms, bacteria, mollusks and protozoan parasites such as coccidian species. Also, the National Research Council, (1992) has it that aqueous neem leaf extract when taken orally produced an increase in red cells, white blood cells and lymphocyte counts thus enhancing the cellular immune response, increasing antibody production thus most pathogens can be eliminated before they cause the ill-feeling associated with disease.

In this study, coccidiosis was characterized by depression, weakness, bloody diarrhea, anorexia, ruffled feathers and death which became less obvious with treatment using aqueous neem leaf extract and amprolium respectively. Gross lesions of infected and untreated chickens congested lungs, liver with multiple foci, bile imbibition, slight enlargement with multiple blunt edges, hydropericardium with congestion, the spleen showed focal congestion about 1/3 of its size, intestinal vessels were injected and had extensive mottled haemorrhages on the mucosa while the caeca contained bloody faeces, mucoid debris and haemorrhages on the mucosa.

Groups that died during treatment had congested lungs with froth; liver was enlarged and congested, intestinal and caecal mucosa with extensive haemorrhages. These signs agree with the findings by Molta *et al.*, (1999); and C.A.B.I. (2003) which were completely eliminated at the end of 5 days treatment with amprolium at a dose rate of 30gm in 25 litres of tap water (Williams, 1996).

The disease coccidiosis also produced a significant increase in alanine amino transferase (ALT) levels which is indicative of hepatocellular damage, presenting large basophilic inclusions in the nuclei of hepatocytes as seen in inclusion body hepatitis of young broilers and replacement pullets, also an inflamed necrotic liver and crater like haemorrhagic edges suggestive of ulcerative enteritis (Akpavie, 1998). Alkaline phosphatase levels were significantly reduced in this study too. This is indicative of some damage to the bone marrow with severe growth depression as alkaline phosphatase activity is known to rise during active growth (Reddy *et al.*, 1988^a; Reddy *et al.*, 1988^b; Nagalakshmi, *et al.*, 1996).

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