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Toxicity and nutritional assessment of aqueous *Azadirachta indica* (neem) leaf extract in broiler chicks

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Abstract

A study was carried out to evaluate the toxicity and effects of aqueous *Azadirachta indica* leaf extract (ALE) on the performance, haematology and serum biochemistry parameters of broiler chicks. One hundred and forty four, seven-day old broiler chicks were randomly assigned to four treatments which contained 0, 20, 40 and 60ml ALE per litre of water representing T_1, T_2, T_3 and T_4 respectively in a Completely Randomized Design experiment. Each treatment was replicated three times with five birds in each replicate. Feed and water were provided *ad libitum*. There was no significant (P>0.05) difference in weight gain, feed and water intakes, feed conversion and protein efficiency ratios among the treatment groups. Also no significant (P>0.05) effect was observed in the survival of birds treated with ALE and the control. ALE did exert any significant influence (P>0.05) on the haematological and serum indices of the birds. In conclusion, results of this study indicate that aqueous ALE could therefore, be used in broiler chicks production without any deleterious effect on the performance and blood indices of the birds.

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Introduction

Adequate nutrition is very important for maximized poultry production. Stiff competition between man and livestock for the major conventional feed ingredients such as cereal grains, oil seeds, roots and stem tubers has resulted in scarcity and exorbitant costs of poultry and aqua feeds in Nigeria (Aniebo *et al,* 2008b; Anyaehie and Okorie, 2008). The reduction of feed cost using cheaper and unconventional feed resources (Muriu *et al*., 2002) is an important aspect of commercial livestock production. The need therefore, to develop balanced and economical feeds to compliment and possibly replace the scarce supplies from the expensive conventional sources, led to the search for alternative and readily available cheaper feedstuffs not consumed by man (Aniebo *et al*, 2008a; Onu and Aniebo, 2011). The search has led to the discovery that many tropical browse plants and legumes are not only good sources of protein, minerals and vitamins for poultry, but they also contain nutritionally important pigments (Esonu *et al*., 2006).

Blood profiles are important indices of the physiological state of animals (Khan and Zafar, 2005). Blood constituents provide valuable media for clinical investigations and nutritional evaluations of an animal (Aderemi, 2004). The ingestion of numerous dietary materials has been reported by Church *et al.* (1984) to have measurable effects on blood constituents. Thus, blood provides proximate measures for long term nutritional status of animals (Kerr *et al*., 1982). However, blood picture varies with certain conditions such as stress, infections and toxicity (Khan and Zafar, 2005).

Neem (*Azadirachta* indica) is an indigenous tropical plant, which predominates in Nigeria. It is known by names such as "Ogwu-iba" in Igbo and "Dogonyaro" in Hausa. Neem is medicinal as it has anticoccidial effect in broilers and is used as pesticide (Tipu *et al*., 2002; Esonu *et al*., 2006). Neem leaves like most tropical tree leaves contain bioactive compounds (Kausik *et al*., 2008; Akpan *et al*., 2008) which may affect nutrient utilization. These bioactive compounds may also alter the hematological and serum biochemical parameters of animals.

Unfortunately, the high fibre content in Neem leaf meal poses serious intake and digestibility problems in poultry diets (Udedibie and Opara, 1998). Therefore, the use of leaf extracts could overcome this barrier towards tapping the good nutritional characteristics of Neem leaf meal. The leaves extract contain nimbin, nimbinene, 6-desacetylnimbiene, nimbandiol, nimbolide and quercetin (Mitra *et al*., 2000). An aqueous extract (10%) of tender leaves is reported to possess anti-viral properties against, fowl pox, IBD and New Castle disease virus (NDV) and significantly enhances the antibodies production against the IBD and NDV (Sadekar *et al*., 1998). Information on the use of Neem leaf extracts in poultry production is very limited. This study is therefore intended to determine the effects of Neem leaf extracts on the performance, haematology and serum biochemistry of broiler chicks.

Materials and methods

In order to investigate the toxicity and effects of aqueous *Azadirachta indica* leaf extract (ALE) on the performance, haematology and serum biochemistry parameters of broiler chicks, a study was conducted at the Poultry Unit of the Teaching and Research Farm, Department of Animal Science, Ebonyi State University, Abakaliki, Nigeria.

Neem (*A. indica*) leaves were harvested fresh and subjected to manual extraction. Exposure to sunlight was avoided to prevent the loss of active components. The leaf extract was obtained by cutting one kilogram of fresh Neem leaves, the leaves were separated from the stalk, washed, drained, chopped and pound in a mortar. After which, it was further squeezed with hand to get the deep green extract which was filtered with filter paper to obtain a homogenous extract. Extract sample was oven-dried and subjected to proximate analysis as described by AOAC (1995). The proximate composition of the test ingredient is shown in Table 1.

A total of one hundred and forty four (160) broiler chicks of the Marshal breeds were procured from a reputable source. The birds were brooded together for one week in a deep litter pen, kerosene stoves and lanterns were used to provide the necessary heat required to maintain the optimum temperature range at this stage. At the 8th day, 144 birds were randomly allotted to four treatments in a Completely Randomized Design (CRD), and each treatment was replicated three times with 12 birds per replicate. The birds were raised on conventional deep litter system, with open sided house. All the pens were located in one house to have identical environment. Feed and water were provided *ad libitum* throughout the experimental period of 35 days. Strict sanitation practices were maintained in the house before and during the course of the experiment. Other routine poultry vaccination and management practices were maintained.

Commercial broiler starter diet was procured and used in the experiment. *Azadirachta indica* leaf extract was added to the chicks drinking water at four different levels representing T_1, T_2, T_3, and T_4, having 0, 20, 40 and 60ml ALE per liter of water respectively as their level of inclusion and served within the interval of three days. T_1 (0ml ALE/liter of water) served as the control.

The birds were weighed at the beginning of the trial and thereafter, weekly. Daily feed intake per group was determined by weighing the feed offered and the left-over the following morning. Water intake was determined by adding the amount evaporated and the left over and the value obtained are subtracted from the total amount served previously. Feed conversion ratio was calculated by dividing the average daily feed intake by the average daily weight gain. Protein efficiency ratio was determined by first determining the daily protein intakes. PER was calculated by using the values obtained from daily protein intake to divide the weight gain of the birds. Birds in all the treatment groups were monitored for fatality or casualty on daily basis throughout the duration of the experiment. Few dead birds were subjected to *post mortem* examination to determine the cause of their death.

At the end of the feeding trial, blood samples were collected from three (3) birds of similar weights from each replicate for the determination of the haematological and serum biochemical indices. Samples were collected from the wing vein of the birds by venipunture using disposable needle (21 – gauge needle) and syringes. The birds were fasted overnight (12hrs) and bled in the morning (7.00 – 8.00am) to avoid excessive bleeding and temporary elevation of many blood metabolites by feeding. The collection site was cleaned with alcohol and zylene applied to dilate the veins. Sterile cotton was used to cover the punctured vein after collection. The blood were collected in sample bottles containing ethylene diamine-tetra acetic acid (EDTA) which served as anticoagulant for haematology while the bottles for serum biochemical indices were free of EDTA

Hematological indices were estimated using standard procedures (Jain, 1986) for its hemoglobin, red blood cells (RBC), packed cell volume (PCV) and white blood cells (WBC) contents as described by Makinde *et al*. (1991), Mafuvadze and Erlwanger (2007) and Tripathi *et al*. (2008). Others such as mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were obtained by calculation according to standard formulae (Schalm *et al*., 1975 and Jain, 1986) as shown below:

$$MCV = \frac{PCV \times 10}{RBC\ count\ (m\ 10^6/mm^3)}$$

$$MCH = \frac{Hb\ (g/dl) \times 10}{RBC\ (m10^6/mm^3)}$$

$$MCHC = \frac{Hb\ (g/dl) \times 10}{PCV\%}$$

Statistical analyses

All the data obtained were subjected to one way analysis of variance for completely randomized design (Steel and Torrie, 1980) using computerized statistical analysis of SAS (2000). Where statistical significance was observed, the means were compared

using the Duncan's New Multiple Range Test (Obi, 2002).

Results and discussion

The results of the effects of aqueous *Azadirachta indica* leaf extract on the growth performance and mortality rate of starter broilers are presented in Table 3. There was no significant ($P > 0.05$) difference in all the parameters measured. The importance of nutrition as the most influential factor in animal growth and development cannot be overemphasized. The comparable weight gain of the birds fed Neem leaf extract and the control is an indication that quantity of toxic factors such as terpenes and limonoids (Ogbuewu *et al.*, 2011; Kabeh and Jalingo, 2007) was minimal to have depressed the growth of the birds served the treated water. This corroborates Verma *et al.* (1998) who had earlier reported a non-significant difference in weight gain of chicks fed Neem leaves. The weight gain recorded in this study has further manifested the potentials in leaf extracts as source of nutrients needed by animals. The results of present study disagree with the findings of Chakeravarty and Prasad (1991) and Durrani *et al.* (2008), who reported that boilers fed on diet containing Neem (*A. indica*) leaves and Neem leaf infusion respectively, had higher body weight gain.

Table 1. Performance of broiler chicks served aqueous *A. indica* leaf Extract supplement.

Parameters	T_1	T_2	T_3	T_4	± SEM
Initial body weight (g)	127.07	130.54	133.38	133.49	8.26
Final body weight (g)	813.57	803.51	816.81	815.25	49.57
Body weight gain (g)	686.49	672.97	683.43	681.76	46.47
Daily weight gain (g)	24.51	24.02	24.40	24.34	1.65
Total feed intake(g)	1523.48	1515.49	1532.71	1534.27	14.69
Daily feed intake (g)	54.41	54.12	54.74	54.70	0.53
Total water intake(ml)	4482.85	4435.52	4448.20	4461.53	31.77
Daily water intake (ml)	160.11	158.42	158.87	159.35	1.14
Feed water intake ratio	1:2.94	1:2.93	1:2.90	1:2.9	0.31
Feed conversion ratio	2.24	2.28	2.24	2.27	0.14
Daily protein intake	11.43	11.37	11.50	11.51	0.14
Protein efficiency ratio	2.15	2.12	2.13	2.12	0.14
Mortality	2	1	1	0	

Table 2. Haematological parameters of starter broiler chicks served *A. indica* leaf Extract supplement.

Parameters	T_1	T_2	T_3	T_4	± SEM
Packed cell volume (%)	27.17	27.90	28.18	28.17	0 .05
Haemoglobin (g/dl)	8.00	9.00	8.83	8.52	1.81
Red Blood Cell ($x10^6/mm^3$)	2.83	3.00	2.98	3.01	7.78
White Blood Cell ($x10^6/mm^3$)	17.60	15.13	16.93	17.00	5.70
MCV(fl)	96.01	93.00	94.56	92.06	4.45
MCH(pg)	28.27	30.00	27.28	27.64	1.52
MCHC (%)	29.44	32.26	28.85	29.53	6.61

There was no significant (P> 0.05) difference in feed intakes of treated birds and the untreated birds. The lack of variations in feed consumption among the treatment groups is an indication that the concentration of toxicants in the extract was within the tolerable limits of the birds as against the use of whole Neem leaf. This is most likely as terpenes and limonoids (Ogbuewu *et al*., 2011; Kabeh and Jalingo, 2007) as well as Azadirachtin (Ogbuewu *et al*., 2010b) have been implicated in the unpalatability and poor acceptability of Neem leaves. However, earlier workers, Kabeh and Jalingo (2007) had reported no marked variations in feed intakes of birds fed Neem leaves, while use of some other whole leaf such as fluted pumpkin was reported to increase feed consumption in broilers (Nworgu, 2007).

Table 3: Serum Biochemical characteristics of broiler chicks served aqueous *A. indica* leaf Extract supplement.

Parameters	T_1	T_2	T_3	T_4	± SEM
Total protein (g/dl)	6.57	6.80	6.68	5.77	0 .05
Albumin (g/dl)	2.30	2.62	2.33	2.60	1.81
Globulin (g/dl)	3.03	3.10	3.33	3.08	7.78
Urea (mg/dl)	27.60	29.63	32.91	34.66	5.70
Creatinine (g/dl)	1.33	1.03	1.30	1.66	4.45
Glucose (mg/dl)	105.33^{c}	104.93^{c}	95.60^{b}	86.06^{a}	1.52
Cholesterol(mg/dl)	88.06^{c}	82.53^{c}	72.66^{b}	65.33^{a}	6.61

There was no marked (P > 0.05) variation in the water intakes of the broilers among the treatments. Daily water intakes in this study varied from 158.42 - 160.11 ml per bird. The daily water intake is not in harmony with the reports of Nworgu *et al.* (2007) (77.66-79.97 ml/bird/day). However, it compared favourably with the daily water intake recorded for broiler starters (Aniecheonwu, 2010). Variations could be attributed to the type of bird, season, text ingredient and environment in which the experiment was carried out. The non significant difference in water consumption observed in this study disagrees with the report of Durrani *et al.* (2008) who reported a significant difference in the water intakes of the treated groups and the control.

The result of feed: water intake ratio agreed with the reports of Aduku (2004) that poultry consumes 2-3 times amount of water per unit of feed consumed. This is however in contrast with Nworgu *et al.* (2007). Significant (P>0.05) differences did not exist in feed conversion and protein efficiency ratios among the treatment groups. This is an indication that the Neem leaf extract did not impair nutrient availability, digestion, absorption and utilization. This is evident in the comparable weight gain of the birds. However, this observation is at variance with the findings of Chakeravarty and Prasad (1991) and Durrani *et al.* (2008) who reported better feed conversion ratio of broilers fed commercial ration and water containing Neem (*A. indica*) infusion than others.

The result of mortality records showed that the death of two birds in diet 1 and one bird each in diets 2 and 3 respectively was due to coccidiosis infection. The non association of mortality to toxicity of ALE contrasts the report of Kabeh and Jalingo (2007) that Neem leaves caused severe growth depression and 50% mortality in broiler chicks. In addition, the non-significant effect of ALE extract on the performance and survival of the broiler chicks may be due to the hygienic situation of experiment. In this study birds were kept in clean disinfected environment following all hygiene regulations.

The haematological indices of the starter broilers fed Neem leaf extract are presented in Table 4. There was no significant (P>0.05) variation in the haematological indices of the birds among the treatments. The values of the PCV, Hb, WBC and RBC

fall within the normal range for healthy broiler chickens as reported by Mitruka and Rawsley (1977), Anon (1980) and IACUC (1998). Hacbath *et al.* (1983) reported that there is a strong influence of diets on haematological traits with PCV and Hb being very strong indicators of the nutritional status of animals. The results of this study showed that Neem leaf extract slightly increased the hemoglobin concentration and packed cell volume of the birds. This indicates that these animals were not stressed by the leaf extract. The values of these blood parameters obtained from birds fed NLE may indicate better nutrient availability and utilization by the birds. This suggests that the birds were properly nourished and were able to obtain essential amino acids and minerals necessary for the normal functioning of the haematopoetic tissues. The results of present study disagree with the findings of Sadre *et al.* (1984), Gowda *et al.* (1998) and Biu *et al.* (2009) that Neem preparations fed to laying hens significantly reduce the content of haemoglobin, erythrocyte count and packed cell volume. The variations observed by the authors on the haematological effect of Neem extracts may however, depend on the mode of application, concentration and exposure time.

The effect of the treatments on the serum biochemistry is as shown in Table 4. This revealed that the treatments did not significantly ($P>0.05$) influence the measured serum biochemical parameters of the broiler starters but for the glucose and cholesterol where significant ($p< 0.05$) reductions were observed as ALE inclusion increased. The non significant ($P >0.05$) values for total protein, albumin and globulin obtained in this study suggests nutritional adequacy of the dietary proteins for starter broilers. The marked decrease in serum glucose value in the present study could be attributed to the presence of bioactive compounds contained in neem leaves which have the ability to block the energy metabolic pathway (Chattopadhyay, 1996). The result is in line with the findings of Halim (2003) who reported that aqueous neem root and leaves reduced blood glucose level in rats exhibiting antidiabetic activity. The progressive decrease in serum cholesterol observed in this study is in agreement with the reports of Chattopadhyay, et al. (2000) and Ogbuewu *et al.,* 2010a,b) that administration of neem leaf extract decreased serum cholesterol significantly without changing serum protein, blood urea and uric acid levels in rats nprobably suggests a general decrease in lipid mobilization. It is possible that ALE has indirect inhibitory effects exerted at the levels of HMG-CoA reductase, a key enzyme in cholesterol biosynthesis (Ogbuewu *et al.,* 2009)

Conclusion

Azadirachta indica is readily available in Nigeria and is a rich source of protein in poultry. Therefore, the result of this experiment shows that oral supplement of ALE supported growth of broiler birds served treated water. The comparable growth parameters of birds fed ALE with those fed the control are an indication of tolerable toxic factor quantity. Hence, ALE can be used in broiler chicks production to improve growth especially where conventional nutrient sources either scarce or expensive without adversely affecting the haematological and serum traits of the animals.

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