

Air-dried neem leaf extract: effects on the growth performance and blood profile of broiler chicken

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Abstract

This study was conducted to evaluate the efficacy of aqueous extract of air-dried neem leaves on growth performance and blood profile of broiler chickens. One hundred and sixty (160) broiler chicks were used for the experiment and were intensively managed. The chicks were randomly assigned into four treatment groups administered, 30, 40 and 50mLs of 4% aqueous neem leaf extracts and defined as T1, T2, T3 and T4, respectively. Growth parameters (daily weight gain, feed intake, water intake and feed conversion ratio) and both haematological and serum indices were measured. Data were subjected to One-way Analysis of Variance and significant means were separated by Duncan Multiple Range Test at 95% probability. Result showed a significant ($p < 0.05$) influence of aqueous neem leaf extract on feed conversion ratio only at starter phase. The birds on 50 mLs aqueous neem leaf extract had poorest (1.58) feed conversion ratio while best (1.48) values were observed from those in the control and 40 mLs. It also showed that neem leaf extracts had no significant ($P > 0.05$) effect on all the haemataological parameters and serum indices at both starter and finisher phases. It was concluded that the use of air-dried neem leaf extract can be adopted as a replacement to antibiotics in broiler chicken production without any adverse effect on their growth performance and blood status.

Keywords: neem leaf extract, performance, blood status, broiler chicken

Introduction

The importance of poultry industry to the socio-economic development of any country cannot be overemphasized as a result of its ability to provide animal protein at a relatively shorter duration at reasonable cost to the consumer. Many synthetic drugs and growth promoters are supplemented to the diets of broiler chickens to effect rapid growth, but their use have shown many disadvantages like high cost, adverse side effect on health of birds and long residual properties (Nayaka *et al.*, 2012). Exploring new antibiotics from the medicinal plants is a priority resistance to increase the production of broilers. In Nigeria, a good number of different herbs and plants parts ranging from leaves meal and extracts,

seeds, fruits and tree barks have been used in researches as alternatives to the conventional feeds, feedstuff, growth promoters and antibiotics. In some recent researches, *Aspilia africana*, *flamboyant seeds*, bitter kola, *Amarathus cruentus*, *Mucuna utilis*, *Azadirachta indica*, *Curcuma longa* and *Cinnamomum zylenicum* and host of others have been successfully used as supplement to enhance the health and performance of livestock particularly monogastric animals including poultry (Rahman *et al.*, 2014).

Neem tree is a widely researched tree that has attracted world-wide recognition due to its vast range of medicinal potentials like antibacterial, antiviral, antifungal, Antiprotozoal, Hepatoprotective and anti-

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coccidial effect in broiler chickens and used as pesticide (Akpan *et al.*, 2008). Various other properties have been documented in respect of its many bioactive components (Akpan *et al.*, 2008) that may also influence haematological and serum biochemical parameters in animals. Growth performances and blood profiles are important indices of physiological state of animals (Khan and Zafer, 2005). Serum biochemistry and haematological features have attracted many researchers in order to make clinical predictions of the health status in animals, birds and even humans. The blood picture varies with certain conditions such as stress, infections and toxicity and blood constituents provide valuable media for clinical investigations and nutritional evaluation (Khan and Zafer, 2005). Therefore, the present study is designed with the main objective of determining the effect of neem leaf extract on growth performance, haematology and serum biochemical indices of broiler chickens.

Materials and methods

Experiment site

The experiment was carried out at the poultry unit of the Directorate of University Farm of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The area lies on latitude 7° 10' N and longitude 3° 2'E, it received a mean precipitation of 1037mm per annual an average temperature of 34.7°C and an average relative humidity of 82% throughout the year (Google Earth, 2017).

Aqueous extraction of neem leaf

Fresh neem leaves were harvested and air dried until it became very dry. One litre of boiled water was added to 40g of air-dried neem leaf for 5 hours which was later sieved to get the neem leaf extract. The prepared extract was further diluted at the rates 0, 30, 40 and 50ml in one litre of water.

Experimental animals and their management

One hundred and sixty day-old broiler chicks were purchased and were allocated into four treatment groups of forty birds each. Each treatment was sub-divided into four replicates with ten birds per replicate. The birds were fed *ad-libitum* throughout the experimental period. Diet composition is presented in Table 1. Only the birds in control group were given antibiotics.

Data collection

The birds were weighed upon arrival to get their initial weight while they were also weighed on weekly basis and recorded appropriately so as to ascertain their weight gain. Their feed intake and water intake were measured and the feed conversion ratio was calculated. At 28th and 49th day, two birds were selected and marked from each replicate for blood collection. With a 4ml syringe fitted with a 24-gauge sterile hypodermic needle, 5 mLs of blood was carefully drawn from the jugular vein in the neck region. 2 mLs of the blood was put into a sterilized bottle containing Ethylene diamine tetra acetic acid (EDTA) as anticoagulant, and mixed gently to prevent coagulation, while the rest of the blood was put into a plain bottle which had no anticoagulants in it for serum biochemical analysis. All blood samples were taken to the laboratory for analysis. The haemoglobin (Hb) content was determined with a digital photo colorimeter (Model 312E by Digital Photo Instruments, Germany) at a wavelength of 625nm and expressed in gramme (g) units. Packed cell volume (PCV) was determined through the Winthrosemicrohaematocrit technique, and expressed in percentages (%). Red blood cell (RBC) counts was obtained with a Coulter Electronic counter (Model Z by Coulter Electronic Ltd. London), and the values were expressed in millions per microlitre (μL) of blood ($\times 10^6/\mu\text{L}$). The

total leucocytes or white blood cells (WBC) were counted with an improved Neubauer haemocytometer and expressed in thousands per microlitre (μL) as described by Peters *et al.* (1982). Other haematological parameters that were computed include mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from total RBC, PCV and Hb (Ritchie *et al.*, 1994), respectively. Total protein was determined by the Biuret method and serum cholesterol

by the enzymatic colorimetric technique (Odoh and Bratte, 2015). Serum albumin was analyzed colorimetrically according to the method of Douma and Briggs (1972) and Peters *et al.* (1982). Globulin was estimated as the difference between total protein and albumin.

Data analysis

All data were subjected to one-way Analysis of Variance ANOVA as contained in SAS (2012). Means that were significantly ($p < 0.05$) different were separated by Duncan Multiple range test of the statistical software.

Table 1: Percentage composition of experimental diets (%)

Ingredients	Starter	Finisher
Maize	47.00	53.50
Soyabean Meal	18.50	16.50
Fish Meal	2.00	0.40
Groundnut Cake	17.50	13.80
Wheat Offal	10.00	10.80
Bone Meal	3.00	3.00
Oyster Shell	1.00	1.00
Vitamin Premix	0.25	0.25
Methionine	0.25	0.25
Lysine	0.25	0.25
Salt	0.25	0.25
Total	100	100
Calculated analysis		
Crude protein (%)	23.00	20.10
Crude fibre (%)	3.61	3.51
Ether extracts (%)	4.04	3.88
Metabolisable energy (MJ/Kg)	11.47	11.62

Results

The result of effect of neem leaf aqueous extract on the growth performance of broiler chicks is shown in Table 2. At starter phase, there was no significant ($p > 0.05$) difference in all the parameters measured except feed conversion ratio (FCR). Poorest feed conversion ratio (1.59 ± 0.04) was observed in birds that took water containing 50 mLs neem leaf extract while

the best (1.48 ± 0.02) was observed in the birds on 0 and 40 mLs of neem leaf extract. At finisher phase, there was no significant ($p > 0.05$) difference in all parameters measured. The live weights ranged from 2067.63 (30 mLs/l) to 2175.59g. The daily feed and water intake ranged from 121.11 to 124.39g and 346.47 to 362.40 mLs, respectively.

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Table 2: Effect of air-dried neem leaf extract on growth performance of broiler chickens

Parameters	Inclusion level of neem leaf extract (ml)			
	0	30	40	50
Starter Phase				
Initial weight (g)	38.30±0.51	35.05±0.58	38.48±0.99	38.33±1.37
Final weight (g)	1046.25±10.87	1036.81±14.25	1046.25±22.49	1011.5±9.18
Weight gain (g)	1007.95±10.764	998.76±13.92	1007.78±21.89	973.20±9.58
Daily weight gain (g)	35.99±0.38	35.67±0.49	35.99±0.78	34.76±0.34
Total feed intake (g)	1491.95±11.58	1545.60±15.59	1495.78±33.50	1547.64±25.03
Daily feed intake (g)	53.28±0.42	54.13±0.56	53.42±1.19	55.27±0.89
Total water intake (mLs)	3394.43±17.00	3388.21±21.08	3290.14±62.36	3397.87±31.68
Daily water intake (mLs)	121.23±0.61	121.01±0.75	117.51±2.23	121.35±1.13
Feed Conversion Ratio	1.48±0.02 ^b	1.52±0.01 ^{ab}	1.48±0.02 ^b	1.59±0.04 ^a
Finisher phase				
Final weight (g)	2175.59±10.77	2067.63±83.61	2174.44±42.08	2144.86±21.73
Weight gain (g)	1129.34±15.91	1030.83±85.60	1128.19±57.99	1133.33±27.85
Daily weight gain (g)	53.78±0.76	49.09±4.08	53.72±2.76	53.97±1.33
Total feed intake (g)	2389.01±25.63	2612.19±119.38	2545.17±25.49	2543.29±53.66
Daily feed intake (g)	113.76±1.22	124.39±5.68	121.19±1.21	121.11±2.56
Total water intake (mLs)	7275.93±104.88	7444.27±366.09	7610.44±329.69	7337.14±292.69
Daily water intake (mLs)	346.47±4.99	354.49±17.43	362.40±15.69	349.39±13.94
Feed Conversion Ratio	2.16±0.01	2.57±0.19	2.27±0.12	2.25±0.03

^{a,b} Means on the same row with different superscripts are significantly (P<0.05) different.

The effect of neem leaf extracts on haematological parameters of broiler chickens at starter and finisher phases are presented in Table 3. The haematological parameters were not significantly (p<0.05) influenced by the trial diets. At the starter phase, Packed Cell Volume (PCV) range was between 32 and 34.67% while White Blood Cell Count (WBC) ranged from 10.73 (30 mLs NLE) to 13.10 x10⁹ /l (control). For the finisher phase, Red Blood Cell Count (RBC) ranged from 1.93 (0ml NLE) to 2.27 x10⁹ /l (50 ml NLE) while the white blood cell ranged from 9.90 to 11.17 x 10⁹ /l.

The effect of neem leaf extracts on serum

indices of broiler chickens at starter and finisher phases are presented in Table 4. The results on the biochemical composition of the blood of the birds indicated that there was no significant (P>0.05) difference among the treatment groups in all the serum biochemical indices at both starter and finisher phase. Aspartate aminotransferase ranged from 68.67 (50ml NLE) and 81.00 U/l (30ml NLE) while alanine transaminase ranged from 27.67 (30ml NLE) and 32.33 U/l (50ml NLE). The values of globulin ranged from 1.20 (50ml NLE) to 1.83 g/dl (30ml NLE) while alanine transaminase ranged from 25.00 (0ml NLE) to 34.00 U/l (30ml NLE).

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Table 3: Effect of air-dried neem leaf extract on haematological parameters of broiler chickens

Parameters	Inclusion level of neem leaf extract (ml)			
	0	30	40	50
Starter phase				
Pack Cell Volume (%)	33.00±1.16	32.33±2.60	32.00±1.15	34.67±0.67
Haemoglobin (g/dl)	9.73±0.37	9.97±0.16	9.97±0.32	10.70±0.32
Red Blood Cell (x10 ¹² /l)	2.73±0.03	2.53±0.35	2.43±0.30	2.77±0.09
White Blood Cell (x10 ⁹ /l)	13.10±1.73	10.73±0.94	11.6±0.70	11.2±0.45
Heterophil (%)	32.00±4.35	37.33±4.18	27.67±5.37	30.67±5.81
Lymphocyte (%)	69.33±2.40	61.67±4.67	69.67±6.07	68.67±5.93
Eosinophil (%)	0.00±0.00	0.00±0.00	1.00±0.58	0.00±0.00
Basophil (%)	0.33±0.33	0.00±0.00	0.67±0.33	0.00±0.00
Monocyte (%)	1.67±0.33	1.00±0.58	1.00±0.58	0.67±0.67
MCV (µm ³)	120.85±5.50	129.93±8.78	134.59±13.63	125.45±2.76
MCH (pg)	35.65±1.76	39.63±1.25	41.96±4.36	38.74±1.58
MCHC (%)	29.50±0.71	30.65±0.12	31.16±0.14	30.86±0.61
Finisher phase				
Pack Cell Volume (%)	33.00±1.15	33.67±1.67	30.67±3.17	33.00±2.08
Haemoglobin (g/dl)	10.33±0.80	10.83±0.49	9.67±1.01	10.43±0.35
Red Blood Cell (x10 ¹² /l)	2.03±0.37	2.43±0.33	1.93±0.43	2.27±0.12
White Blood Cell (x10 ⁹ /l)	10.53±0.37	9.90±0.71	10.63±1.01	11.17±0.76
Heterophil (%)	25.33±8.88	26.00±3.00	29.33±7.22	22.33±6.36
Lymphocyte (%)	71.00±7.50	70.00±2.33	69.67±6.64	74.00±6.00
Eosinophil (%)	1.33±0.67	1.67±0.33	1.00±0.58	1.00±0.00
Basophil (%)	0.33±0.33	0.67±0.33	0.33±0.33	0.67±0.33
Monocyte (%)	2.33±0.88	2.00±0.00	1.33±0.67	2.00±0.58
MCV (µm ³)	173.98±32.26	141.37±11.01	167.30±22.55	145.44±1.48
MCH (pg)	59.38±3.61	48.56±5.81	47.72±3.18	52.93±5.11
MCHC (%)	31.21±1.37	32.22±1.08	31.52±0.44	31.73±1.00

MCV (Mean Corpuscular Volume) MCH (Mean Corpuscular Haemoglobin)
MCHC (Mean Cell Haemoglobin Concentration)

Table 4: Effect of air-dried neem leaf extract on serum biochemical parameters of broiler chickens

Parameters	Inclusion level of neem leaf extract (ml)			
	0	30	40	50
Starter phase				
Total protein (g/dl)	3.47±0.35	2.90±0.44	2.90±0.32	2.90±0.10
Albumin (g/dl)	1.90±0.20	1.73±0.32	1.67±0.27	1.70±0.15
Globulin (g/dl)	1.50±0.15	1.17±0.13	1.30±0.12	1.40±0.06
Uric acid (mg/dl)	9.90±0.38	9.50±0.85	8.93±0.84	9.60±0.10
Glucose (g/dl)	90.67±8.82	91.67±4.05	88.00±6.93	94.00±5.13
Creatinine (mg/dl)	0.67±0.29	0.77±0.13	0.73±0.09	0.80±0.28
Cholesterol (mg/dl)	124.00±3.06	124.00±8.74	119.00±6.56	119.00±5.49
Aspartate aminotransferase (U/l)	73.33±0.33	81.00±2.65	70.67±5.81	68.67±3.76
Alanine transaminase (U/l)	30.33±6.06	27.67±2.96	30.67±2.96	32.33±2.60
Alkaline phosphatase (U/l)	29.33±1.76	27.67±4.81	25.00±2.65	27.33±3.18
Finisher phase				
Total protein (g/dl)	3.30±0.26	3.80±0.29	2.97±0.27	3.03±0.29
Albumin (g/dl)	1.87±0.19	1.97±0.12	1.67±0.17	1.83±0.41
Globulin (g/dl)	1.43±0.18	1.83±0.22	1.30±0.10	1.20±0.15
Uric acid (mg/dl)	9.30±0.80	8.87±0.20	8.87±0.57	9.50±0.47
Glucose (g/dl)	82.33±4.98	86.00±7.02	72.33±2.67	86.33±6.44
Creatinine (mg/dl)	0.53±0.19	0.57±0.19	0.53±0.17	0.77±0.37
Cholesterol (mg/dl)	122.33±1.20	121.33±8.29	128.67±3.71	129.00±2.65
Aspartate aminotransferase (U/l)	69.00±7.64	66.33±4.33	67.67±4.33	66.00±1.53
Alanine transaminase (U/l)	25.00±3.21	34.00±4.16	32.00±4.36	28.67±1.86
Alkaline phosphatase (U/l)	21.67±2.67	20.67±1.76	26.00±1.00	25.33±3.84

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Discussion

The live weight and weight gains of the birds in this study were higher than the values reported by Egbeyale *et al.* (2015). The authors observed that neem leaf meal inclusion higher than 0.5% in the diet of broiler birds reduced weight gain. 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 was minimal to have depressed the growth of the birds. This corroborates the findings of Verma *et al.* (1998) who had earlier reported insignificant difference in weight gain of chicks fed raw and alkali treated neem kernel meal. The weight gain recorded in this study has further revealed the potentials in leaf extracts as source of nutrients needed by animals and as growth promoter. The results of present study disagree with the findings of Chakeravarty and Prasad (1991) and Durrani *et al.* (2008), on improved body weight gain in broiler chickens fed diets containing neem leaves and neem leaf infusion, respectively had. The similarity in water consumption observed in this study was not in accordance with the report of Durrani *et al.* (2008) who reported significant difference in the water intakes of the treated groups and the control. The differences in the results of the growth indices could be attributed to the processing method of the neem leaves before extraction. Durrani *et al.* (2008) oven dried the leaves while the leaves were air-dried in this present study. The difference in feed conversion ratio among the treatments shows that the neem leaves extract had effect on nutrient availability, digestion, absorption and utilization. The poor utilization of diets containing higher levels of neem leaf extract at starter phase might be related to the inability of the bird's enzyme to break down the active and improper metabolism associated with the

neem leaf as reported by Esonu *et al.* (2005).

The similarity in haematological indices across treatments indicate the wellness of the birds throughout the period of the experiment as normal haematological parameters of an animal are direct indication of absence of disease (Olafedehan, 2010). The values obtained were within the normal range for healthy birds as stated by Mitruka and Rawnsley (1977). This indicates that these animals were not stressed due to inclusion of the leaf extract. These results similar with those observed by Olugbemi *et al.* (2010) that packed cell volume was not affected due to the supplementation of Neem (*Azadirachta indica*) leaf extract. However, the result disagreed with that of Nagalakshmi *et al.* (1996) who reported that neem possesses strong influence on the haematological traits in broilers particularly haemoglobin. Sadre *et al.* (1984) and Gowda *et al.* (1998) also reported that neem preparations fed to laying hens significantly reduced 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.

Serum biochemical investigations have been explored extensively to distinguish normal state from stress and disease conditions in animals. The similarities in all the serum biochemical indices in this study did not agree with the earlier work of Biuet *et al.* (2009) who reported a significant increase in serum creatinine and urea levels on the administration of neem leaves aqueous extract on broiler birds intraperitoneally. He ascribed it to the fact that neem leaf aqueous extract has proven to be toxic to both the liver and kidney of chicken. In another study on rabbit,

Ogbuewu *et al.* (2008) attributed the significant effect of neem leaves on serum creatinine and urea level to the presence of some bioactive compounds (Azadirachtin, Nimbin, Salanin) which have been reported to block the energy metabolic pathway in animals, thus making it difficult for the animals to meet their energy requirement. The variation observed by the author might be due to the mode of application and basis of administration (dose per body weight). This finding also did not support the findings of Oyagbemi and Adejinmi (2012) who reported decline in glucose level using neem leaves extract in broiler production. The findings of the present study were not in line with the findings of Nagalakashmi *et al.* (1996) who used a diet containing different levels of urea ammoniated neem kernel cake as a protein supplement to replace peanut meal. The authors found that blood urea level of birds increased as the amount of urea treated neem was increased in the diet. The variation with this study could be as a result of the level or form of application (urea treated neem kernel against leaf extract).

Conclusion

It can be concluded from the result of this study that administration of 4% aqueous air-dried neem leaf extract in the drinking water of broiler chickens had no toxic effect on the growth response, haematological parameters and serum metabolites. Therefore, it can serve as alternative to antibiotics in commercial broiler production

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