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Medicinal Response of Broiler Chickens to Diets Containing Neem (*Azadirachta indica*) Leaf Meal, Haematology and Meat Sensory Analysis

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Abstract: The medicinal response, haematological parameters and meat sensory analysis of broilers to diets containing neem leaf meal (NLM) were studied in an 8-week trial. The experiment was in two phases: growing chicks phase (0-3 week) and finisher phase (4-8 weeks). In phase I, a total of 400 day-old unsexed cobb hybrid broiler chicks were randomly allotted in equal numbers to 4 treatments. Forty five chicks from each treatment were wing tagged to form 3 replicates of 15 chicks each. In phase 2, 180 birds were allotted to the 4-treatments which had 3 replicates made up of 15 birds each in a Completely Randomized Design (CRD). Four starter diets and finisher diets were formulated to contain the NLM at 0%, 1.5%, 2.0% and 2.5% dietary levels and were fed ad libitum in phases 1 and 2 respectively. Results showed that feed intake in both phases were not significantly ($P>0.05$) influenced by inclusion of the NLM in the diets. Body weight gain was significantly ($P<0.05$) depressed in birds fed the NLM diets when compared with the control which adversely affected the conversion efficiency ratio. The NLM inclusion controlled coccidiosis, worm infestation and respiratory infections effectively in those birds fed the NLM diets compared with the control. Carcass characteristics were not significantly ($P>0.05$) influenced by the NLM although mild bitter taste was sensed in the 2.5% inclusion level when the meat was not salted. The general haematological data was not significantly ($P>0.05$) influenced by the NLM. However, white blood cells (WBC) were significantly ($P<0.05$) higher in the control compared with those birds fed the NLM diets. Total profit was relatively higher for birds fed the NLM diets when compared with the control diet. It was concluded that NLM can be a good herbal medicine for broiler production aside its nutritional importance.

Key words: Antimicrobial resistance · Neem leaf meal · Herbal medicine · Chemical- free production

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. However, in most developing countries, the rate of population growth has not corresponded with the growth of the poultry industry and therefore raises food security concerns. The growth and expansion of the industry is confronted by high cost of feed and drugs [1] and their occasional shortages. Additionally, the use of antimicrobial agents as growth promotants are being discouraged due to human and animal health issues mainly resulting from development of antimicrobial resistance [2].

Attempts at addressing these challenges have focused on exploitation of non- conventional feed resources and herbal medicine motivated by recent consumer preference towards chemical- free production.

The neem (*Azadirachta indica*) belonging to the Meliaceae family and a fast growing evergreen tree has a potential to provide medicinal and nutritive value to broilers [3]. Various parts of the tree have been reported to contain chemicals like azadiractin, nimbin, nimbindin, quercetin among others [4-6] which have antimicrobial, antihelminth, antioxidant, antifungal, insecticidal, antiprotozoa and spermicidal [7] properties.

This experiment was conducted to investigate the medicinal value of neem leaf meal (NLM) in controlling coccidiosis, respiratory infections and worm infestation

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while assessing the nutritive value and effect of bitter neem taste on the broiler meat in a commercial poultry enterprise.

MATERIALS AND METHODS

Experimental Location: The experiment was conducted at the Poultry Section of the Animal Farm of the Department of Animal Science Education, University of Education, Winneba, Mampong-Ashanti, Ghana. Mampong-Ashanti is located at 07°03'N, 01°24'W and at altitude of 457 m above sea level. The mean annual rainfall was about 1500 mm. The mean monthly temperature was 32°C while the mean relative humidity was 90% in the morning (06.00h) and 55% in the afternoon (15.00h).

Experimental Design and Procedure

There Were Two Phases to this Experiment: In phase 1, a total of 400 day-old unsexed cob commercial broiler chicks were used. The chicks were distributed to 4 treatment groups, each made up of 100 chicks in a 4-brooder rooms. Forty five birds from each treatment were winged tagg forming 3 replicates of 15 birds each. Water and starter diet (Table 1) for the 4- respective treatments were offered *ad libitum*.

In phase 2, one hundred and eighty birds made up of 15 birds with a male:female ratio of 8:7 similar weight and were allotted to the replicates in a Completely Randomised Design (CRD). There were 4 treatments and 3 replicates, each replicate had 15 birds selected from their respective treatment from the previous phase which ensured continuity. Each bird had a space of 0.2 m^2. Finisher diet (Table 2) for the 4- respective treatments was offered *ad libitum*.

Processing of Neem Leaf Meal: Fresh leaves of the neem tree were harvested and sun-dried for four days on polythene sheets until they became crispy while retaining the greenish colouration. The leaves were turned regularly to prevent uneven drying and decay. The dried leaves were then hammer milled through a 2 mm sieve and stored in airtight bags until they were used.

Data Collection: Data were collected on growth parameters, health conditions, carcass characteristics, sensory analysis and haematological studies. Proximate composition of the NLM and diets were performed using the procedure outlined by the A.O.A.C [8].

Table 1: Composition and calculated analysis of dietary treatments (starter diet)

	Treatment			
Feed ingredients	T1	T2	T3	T4
Maize	58	58	58	58
Fish meal	15	15	15	15
Tuna fish meal	5.5	5.5	5.5	5.5
Soya bean meal	9	9	9	9
Wheat bran	10	8.5	8	7.5
Oyster shell	1	1	1	1
Dicalcium phosphate	0.5	0.5	0.5	0.5
Vitamin premix	0.5	0.5	0.5	0.5
Common salt	0.5	0.5	0.5	0.5
Neem leaf meal	0	1.5	2	2.5
Total	100kg	100kg	100kg	100kg
Calculated Analysis				
Crude protein (%)	23.08	23.08	23.08	23.08
Crude fibre (%)	2.15	2.15	2.15	2.15
Ether Extract (%)	3.7	3.7	3.7	3.7
Metabolisable energy (Kcal/kg)	2930.28	2903.82	2895	2886.18

*Vitamin mineral premix provided the following per kg of diet: Fe 100mg, Mn 110mg, Cu 20mg, Zn 100mg, I 2mg, Se 0•2mg, Co 0•6mg, sanoquin 0•6mg, retinal 2000mg, cholecalciferol 25mg, α-tocopherol 23000mg, menadione 1•33mg, cobalamin 0•03mg, thiamin 0•83mg, riboflavin 2mg, folic acid 0•33mg, biotin 0•03mg, pantothenic acid 3•75mg, niacin 23•3mg, pyridoxine 1•33mg.

Table 2: Percentage Composition of Experimental Diet (Finisher Diet)

	Dietary Treatment			
Feed ingredient	T1	T2	T3	T4
Maize	58	58	57	57
Fish meal	8	8	8	8
Tuna fish meal	7	7	7	7
Soya bean meal	8	8	8	8
Wheat bran	15.5	14	14.5	14
Oyster shell	2	2	2	2
Dicalcium phosphate	0.5	0.5	0.5	0.5
Vitamin premix	0.5	0.5	0.5	0.5
Common salt	0.5	0.5	0.5	0.5
Neem leaf meal	0	1.5	2	2.5
Total	100kg	100kg	100kg	100kg
Calculated Analysis				
Crude protein (%)	19.7	19.7	19.8	19.8
Crude fibre (%)	3.11	3.25	3.33	3.06
Ether Extract (%)	3.64	3.73	3.67	3.67
Metabolisable energy (Kcal/kg)	2834.22	2816.58	2370.16	2591.02

*Vitamin mineral premix provided the following per kg of diet: Fe 100mg, Mn 110mg, Cu 20mg, Zn 100mg, I 2mg, Se 0•2mg, Co 0•6mg, sanoquin 0•6mg, retinal 2000mg, cholecalciferol 25mg, α-tocopherol 23000mg, menadione 1•33mg, cobalamin 0•03mg, thiamin 0•83mg, riboflavin 2mg, folic acid 0•33mg, biotin 0•03mg, pantothenic acid 3•75mg, niacin 23•3mg, pyridoxine 1•33mg

Health Conditions: The general conditions of birds were observed visually for abnormality and symptoms of respiratory infections. Faecal samples were taken from the fourth to eighth week for laboratory detection of worms infestation and coccidian spp. The degree of presence was indicated with (+) for less severe, (++) for severe and (+++) for highly severe and (-) for absent.

Sensory Analysis: Chicken breast meat samples were steamed separately with 60 ml of water to a temperature of 100°C for 10 minutes to produce cooked meat samples. Equal quantities of salt (that is 2.0 g of granulated iodated salt) were added to chicken breast meat samples to produce salted cooked breast meat. The temperature-time treatment was monitored using a Digital Cooking Thermometer with Probe and Timer with 0°C to 200°C range. Cooked chicken breast meat samples were sliced into cubes of uniform sizes and served warm to 8 trained sensory panelists. Eight different meat cubes (representing 8 treatments) were wrapped in aluminium foil, labeled with random 3-digit codes and presented randomly to panelists in different orders.

The treatments for sensory evaluation were as follows:

- Unsalted (T1) and salted cooked breast meat (T2) from chickens fed neem-free diets.
- Unsalted (T3) and salted cooked breast meat (T4) from chickens fed 1.5% neem diet.
- Unsalted (T5) and salted cooked breast meat (T6) from chickens fed 2.0% neem diet.
- Unsalted (T7) and salted cooked breast meat (T8) from chickens fed 2.5% neem diet.

The trained sensory panelist evaluated meat colour, meat color intensity, aroma, juiciness, texture, flavour, abnormal flavour and overall acceptability of meat. The sensory qualities of the meat were evaluated with a 7-point category scale as follows:

Yellowish Meat Color Intensity: 1-extremely light; 2-very light; 3-slightly light; 4-moderate; 5-slightly intense; 6-very intense; 7-extremely intense.

Juiciness: 1-extremely dry; 2-very dry; 3-slightly dry; 4-moderate; 5-slightly juicy; 6-very juicy; 7-extremely juicy.

Tenderness: 1-extremely tender; 2-very tender; 3-slightly tender; 4-moderate; 5-slightly tough; 6-very tough; 7-extremely tough.

Chicken Flavour Intensity: 1-extremely weak; 2-very weak; 3-slightly weak; 4-moderate; 5-slightly intense; 6-very intense; 7-extremely intense.

Abnormal Flavour Intensity: 1-extremely weak; 2-very weak; 3-slightly weak; 4-moderate; 5-slightly intense; 6-very intense; 7-extremely intense.

Overall Acceptability of Meat: 1-extremely unacceptable; 2-very unacceptable; 3-slightly unacceptable; 4-moderate; 5-slightly acceptable; 6-very acceptable; 7-extremely acceptable.

Haematological Studies: After feed withdrawal for 12 h, blood samples were collected randomly from the jugular vein of two birds from each replicate into anticoagulant (heparin) bottles and analyzed for total red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), white blood cells (WBC) and serum cholesterol using a Haematological Auto Analyzer.

Statistical Analysis: Data collected were subjected to Analysis of Variance with Genstat [9]. The least significant difference (LSD) was used to separat treatment means at 5% significance level.

RESULTS AND DISCUSSIONS

Proximate Composition: The result of the proximate composition of the NLM is presented in Table 3. Result obtained is similar to that reported by Schmutterer [3]. The crude protein obtained in this study is lower than 20.68% and 24.06% respectively reported by Esonu *et al.* [10] Onyimonyi *et al*. [11]. The crude fibre obtained is however lower than 16.6% reported by [10] and higher than the 12% reported by Onyimonyi *et al*. [11]. Differences in nutrient composition are attributed to differences in soil nutrient composition and leaf age.

Growth Performance: The growth performance of the birds in the starter and the finisher phase are presented in Table 4&5 respectively. Mean feed intake for the starter and finisher broilers were not significantly influenced by the inclusion of NLM in the diet. Birds fed diets that contained NLM had similar higher feed intake compared with the control diet. Reduction in feed intake was expected for birds fed the NLM diet as a result of reduced palatability from the highly bitter neem taste but was not the case in this present study perhaps due to relatively lower levels of the NLM used. Mean body weight gain was significantly ($P<0.05$) higher in the

Table 3: Proximate composition of neem leaf meal

Components	Percentage
Crude protein	15.8
Crude fibre	14.6
Ether extract	8.5
Ash	4.5
Moisture	13.0
NFE	56.6

Table 4: Growth performance of broiler chicks fed with neem leaf meal

	Treatments				
Parameter	T1	T2	T3	T4	S.E.M
Initial body weight(g/bird)	60.00	60.00	60.00	60.00	0.00
Final body weight(g/bird)	680.0^{a}	640.4^{c}	628.6^{d}	659.6^{b}	1.63
Mean Feed Intake (g/bird)	1315	1413	1393	1413	24.90
Mean weight gain (g/bird)	620^{a}	580.4^{b}	583.6^{b}	599.6^{ab}	11.31
Feed Conversion Ratio	2.12^{b}	2.44^{a}	2.39^{ab}	2.36^{ab}	0.01

Values with different superscripts in a row differ significantly (P<0.05)

Table 5: Effects of NLM on growth performance of broiler finishers

	Treatments				
Parameters	T1	T2	T3	T4	S.E.M
Initial body weight (g/bird)	704.4	712.2	677.8	691.1	8.14
Final body weight (g/bird)	2330^{a}	2223^{ab}	2022^{c}	2028^{bc}	81.6
Mean Feed intake (g/bird)	4352	4292	4298	4292	83.7
Mean weight gain (g/bird)	1626^{a}	1511^{a}	1344^{b}	1337^{b}	31.4
Feed Conversion Ratio	3.15^{b}	3.61^{b}	4.03^{a}	4.01^{a}	0.19

Values with different superscripts in a row differ significantly (P<0.05)

control birds than in birds fed the diet that contained the NLM 1.5% and 2% but not 2.5% and therefore influenced the feed conversion ratio in favour of the control in the starter phase. Feed intake was not significantly influenced by NLM in the finisher phase. However, body weight gain was significantly (P<0.05) heavier for birds fed the control diet and the diet that contained NLM at 1.5% compared with birds fed the NLM diet at 2% and 2.5%. Feed conversion ratio followed a pattern similar to body weight gain. Depressed body weight gain at level above 0.5% NLM was reported and attributed to the bitter and astringent taste of the NLM [12].

Health of Birds: The health of birds fed diets that contained the NLM was generally good. Mortality that occurred was during the brooding phase and none occurred in the finisher phase. Coccidiosis attack was mainly observed on birds fed the control diet as evidenced by bloody droppings and laboratory determination (Table 6). A few of the birds fed the diet that contained NLM at 1.5% had the coccidiosis and respiratory problem but was completely absent from the birds that had the NLM included at 2% and 2.5%.

Table 6: Effects of NLM on health performance of broiler chickens

	Treatments			
Parameters	T1	T2	T3	T4
Coccidiosis infection	++	+	-	-
Respiratory infection	+	+	-	-
Worms infestation	+++	-	-	-

(+++) Highly Severe (-) Absent
(++) Severe (+) Less Severe

Table 7: Effect of NLM on carcass characteristics of broilers

	Experimental Diet				
Parameter	T1	T2	T3	T4	S.E.M
Live weight	2371	2320	2156	2170	112.3
Heart weight	12.00	12.00	10.00	10.67	1.06
Gizzard weight	40.50	39.67	38.50	34.33	3.59
Fat Isolated	62.33^{a}	54.17^{a}	40.67^{b}	50.00^{ab}	5.36
Intestine weight	99.5	108.3	100.3	100.5	8.96
Liver weight	47.80	41.50	45.17	43.67	9.07
Dressed weight	1816	1781	1639	1645	85.9
Percentage Dressed	76.61	76.76	76.04	75.82	0.74

Means with common letters in horizontal rows are significantly (P<0.05) different

Faecal samples taken were found to contain a number of round worms. Gizzard content contained a rather profuse quantity of the round worms in birds fed the control diet whereas those birds fed the diet that contained the NLM had no such worms. Some of the birds infected with coccidiosis and worms were isolated and given the diet that contained NLM at 2.5% and these birds recovered although minute traces of the coccidian were detected from laboratory analysis. This indicated that the NLM indeed has the potential aside nutritional value to control coccidiosis and worms infestation when used as part of feed ingredients in broiler diet. NLM could also serve as herbal medicine in poultry production as a way of reducing the cost of medication.

Carcass Characteristics: The overall carcass characteristics were not influenced by the NLM in the diets of the broilers (Table 7). Organ weights and the dressing percent were not significantly (P>0.05) influenced by the NLM indicating that inclusion at the present levels may not cause any detrimental effect on the organs. Fat deposition had a positive correlation with energy concentration of the diet. Fat isolated from the lower abdomen was significantly (P<0.05) higher in the control birds when compared with that of birds fed the NLM at 2% but did not differ significantly (P>0.05) from the NLM at 1.5% and 2.5%.

Table 8: Effect of NLM on haematological parameters

	Treatments				
Parameters	T1	T2	T3	T4	SEM
Hb (g/dl)	10.23	11.77	8.80	10.60	2.33
PCV (%)	28.03^{ab}	31.13^{a}	23.33^{b}	28.87^{ab}	2.89
RBC x 10^{12}/l	2.250	2.650	1.910	2.283	0.34
WBC x 10^{9}L	29.43^{a}	18.13^{ab}	7.03^{b}	16.83^{ab}	5.40
Serum					
Cholesterol (mg/dl)	103.67	91.33	102.67	87.33	9.66

Values with different superscript in a row differ significantly (P<0.05)

Table 9: Effect of NLM on cost benefit analysis

	Treatment			
Description	T1	T2	T3	T4
Total feed intake (kg)	5.667	5.705	5.691	5.705
Per unit feed cost (GH¢)	0.811	0.808	0.805	0.804
Total feed cost (GH¢/bird)	4.36	4.39	4.36	4.36
Total drug cost (GH¢ (0-8wk)/bird)	0.30	0.10	0.10	0.10
Other cost per bird (GH¢)	2.15	2.15	2.15	2.15
Total cost of production/ bird (GH¢)	6.81	6.64	6.61	6.61
Income/ bird (GH¢)	12.00	12.00	12.00	12.00
Profit on cost of production/bird (GH¢)	5.19	5.36	5.39	5.39

Haematological Studies: Blood Hb, RBC and packed cell volume were not significantly (P>0.05) influenced by the NLM at the present level of inclusion (Table 8). However, WBC were significantly higher for the control birds when compared with that of birds fed diets that contained NLM at 2% but not at 1.5% or 2.5% level although the control had a higher numerical value. The higher WBC in the control indicates a probable disease condition which might have stimulated the production of WBC to fight against the potential causative agent. The relatively lower WBC of the birds fed the NLM diets could be attributed to the inclusion of the NLM which fought against potential disease threats before the body's system could be stimulated to produce WBC. Serum cholesterol was not significantly affected by the inclusion of the NLM at the present level, although lower numerical values were recorded for those birds fed the NLM.

Cost Benefit Analysis: Per kg cost of feed was reduced by the inclusion of the NLM in the diet. However, due to relatively higher feed intake during the starter phase by birds fed the diet that contained the NLM, total feed cost was similar for all treatments. As a result of savings made from not providing drugs to birds fed the diets that contained the NLM, higher profit accrued because both morbidity and mortality rates were low or controlled(Table 9).

Table 10: Sensory attributes of cooked chicken breast meat

	Treatments								
Descriptor	1	2	3	4	5	6	7	8	SEM
Meat color intensity	3.8^{b}	3.9^{b}	5.1^{a}	5.3^{a}	5.0^{a}	5.3^{a}	5.2^{a}	5.3^{a}	0.19
Aroma	5.1	5.0	5.2	5.1	5.1	4.9	4.9	5.0	0.14
Juiciness	5.0	5.1	5.2	5.1	5.0	4.9	5.1	5.2	0.18
Texture	4.5	4.9	4.7	4.6	4.6	4.8	4.7	4.6	0.17
Flavour	5.1^{a}	5.0^{a}	5.0^{a}	5.1^{a}	5.2^{a}	5.1^{a}	4.0^{b}	5.1^{a}	0.09
Abnormal flavour	1.6^{b}	1.8^{b}	1.7^{b}	1.9^{b}	1.7^{b}	1.9^{b}	3.2^{b}	2.0^{ab}	0.24
Overall acceptability	6.2^{a}	6.1^{a}	6.3^{a}	6.0^{a}	6.2^{a}	6.3^{a}	5.2^{b}	6.1^{a}	0.17

Values with different superscript in a row differ significantly (P<0.05)

Meat Sensory Analysis: Meat colour and colour intensity: Visual appraisal of cooked chicken breast meat using the RAL Color Chart as a colour guide showed that breast meat from chicken fed on neem-free diets (T1 and T2; Table 10) had a pale colour for both the skin and breast meat while meat from chicken fed on neem-based diets had yellowish skin and breast meat. The variation in skin and meat colour could be attributed to the carotenes in the neem-based diets. There was however no appreciable difference in colour intensity as the levels of neem in diets was increased from 1% to 2.5%.

Aroma, Texture and Juiciness: There were no significant differences in the aroma, texture and juiciness of cooked meat samples. A few sniffs at cooked chicken breast meat samples did not show any differences in aroma of the treatments. They all had the characteristic "chicken smell". Cooked chicken breast meat did not show any differences for tenderness or toughness when they were bitten through. Likewise, cooked meat did not show any differences in exudation of juices or differences in dryness when they were lightly chewed. The inclusion of neem at rates of 1.0%, 2.0% and 2.5% did not alter the aroma, texture and juiciness of chicken meat.

Flavour of Meat: Sensory evaluation showed no differences among all cooked chicken breast meat samples once they were salted. However, unsalted cooked meat from chickens fed on 2.5% neem-based diets were found to be mildly bitter. The high rate of inclusion of neem at 2.5% might have resulted in the deposition of azadiratin in muscle fibres resulting in mild bitter taste.

Overall Acceptability: Generally, all the chicken breast meat (meat from broilers fed on neem-free diets and 1.0 % and 2.0 % neem diets) were acceptable for consumption. The mildly bitter taste of chicken breast meat from chickens fed 2.5% neem-based diet was masked and unnoticed if meat is adequately salted during cooking.

CONCLUSION

The present findings show that NLM has nutritional and health improvement potential as a feed ingredient in broiler production without compromising the haematological parameters, carcass characteristics and meat taste at present inclusion rate.

Further studies of the phytochemical properties of neem leaves in relation to toxicity level in broiler nutrition and thence quantification and acceptable inclusion levels is suggested in order to manage possible injury levels when fed to birds.

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