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GROWTH PERFORMANCE AND BLOOD INDICES OF PULLETCHICKENS FED DIFFERENT INCLUSION LEVELS OF NEEM (*Azadirachta indica*) LEAF MEAL

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Abstract
This study was conducted to determine the growth performance and blood indices of pullet chickens subjected to varying levels of neem (*Azadirachta indica*) leaf meal (NLM. One hundred and seventeen 4-weeks old ISA brown chicks were randomly selected and allotted to three treatments. The birds were subdivided into three groups based on the neem inclusion levels (0, 0.5 and 1.0 % NLM). Each group consists of thirty nine randomly selected birds and three replicates of thirteen birds. The birds were weighed on weekly basis to determine growth performance. Blood analyses were carried out on the eight week. The experiment was arranged in a one-way analysis of variance. Inclusion of NLM up to 1.0% in the diet of pullets had no significant ($p>0.05$) effect on growth performance indices except protein intake. Hematological parameters of the birds were not influenced ($p>0.05$) by NLM inclusion level except mean corpuscular volume and mean corpuscular hemoglobin. It was concluded that up to 1.0% NLM could be included in the diets of pullet chickens without deleterious effect in their growth performance and blood constituents.
Key words: *Azadirachta indica*, pullets, performance, blood.

Background

Access to safe and healthy food products has now become an important public concern in the developed countries of the world since consumers now demand food that is not only economical, but also healthy, tasty, and safe [1]. As reported by [2], it is almost impossible to produce food of animals that is completely free from traces of drugs or chemicals. The use of antibiotics as feed additives was associated with residues in the meat and eggs consumed by consumers and has been banned or limited in many countries [3]. The major health risks associated with these groups of drugs are the development of drug resistance in the exposed individuals, hypersensitive reactions, and the development of resistant micro-organisms to antibiotics in human beings [4]. In the European Union, the inclusion of antibiotics in animal feed is forbidden. This has triggered the need for several non-conventional plant extracts with antimicrobial properties for possible incorporation into animal feeds for reducing the microbial load of animals and as natural alternatives to antibiotics due to their wide range of potential benefits [5]. Researchers have identified several beneficial chemical compounds in medicinal plants, which play important roles in improving the production and immune system of birds against different diseases they have strong medicinal value

and could be effectively utilized as natural growth promoters to replace antibiotics and other synthetic feed additives [6]

Azadirachta indica (neem) is a large evergreen fast-growing perennial tree that is native to Bangladesh and an inhabitant of Southeast Asia. Neem leaves have relieved so many different pains, fevers, infections, and other complaints that it has been called "the village pharmacy". [7] reported that neem leaf meal contains (on a dry matter basis) crude protein 185.1 to 206.8, crude fiber 174.2 to 245.7, ether extract 24 to 45, nitrogen-free extract 406 to 502, and ash 65 to 72 g/kg, respectively. Neem is known to increase the production of glutathione-transferase, thus improving the ability of the liver to detoxify itself from chemical contaminations [8].
The study therefore is geared towards determining the growth performance and blood indices of pullet chickens on neem leaf meal managed in two housing types.

Materials and Methods

Location of the Experiment

The experiment was carried out at the Poultry Research and Teaching farm of the Teko Livestock Research Centre, Makeni, Sierra Leone. It is located between latitudes 8° 53' 09" N and longitudes 12° 02' 39" W and at an altitude of 93m above sea level. The climate is typically tropical-humid, with annual ambient temperature ranges from 26° at night to 34°c during the day and averages of 26.9°c and a relative humidity of 61%. The vegetation is composed mainly of an interphase of grasslands, bolilands, and inland valley swamps with a mean annual rainfall range of 2500-3000mm [9]

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Experimental diets

Table1: Gross composition (%) of chick starter

	% Inclusion levels of neem leaf meal (NLM)		
Ingredients	0	0.5	1.0
Maize	40.00	40.00	40.00
Wheat offal	25.00	24.50	24.00
Fish meal (72%)	2.00	2.00	2.00
Soybean meal	18.00	18.00	18.00
Palm kernel cake	10.00	10.00	10.00
NLM	0.00	0.50	0.10
Bone meal	2.00	2.00	2.00
Oyster shell	2.00	2.00	2.00
Lysine	0.25	0.25	0.25
Methionine	0.25	0.25	0.25
Vit./trace mineral premix	0.25	0.25	0.25
Common salt	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated analysis (%)			
Crude protein	19.14	19.15	19.15
Crude fibre	4.55	4.55	4.55
Ether Extract	3.71	3.71	3.71
Calcium	0.13	0.13	0.13
Phosphorous	0.35	0.35	0.35
Methionine	0.39	0.39	0.39
Lysine	1.22	1.22	1.22
Ash	3.03	3.03	3.03
Energy (MJ/Kg)	3.56	3.56	3.56

Vit./Min. Premix contained: Premix (Embavit No 90) contained Vit. A, 10 000 000iu; D_3 , 2 000 000iu ; E, 12 500iu; K, 1.30g; B_1, 1.30; B_2, 4.00g; D Calcium-Pantothenate, 1.30g; B_6, 1.30g; B_{12}, 0.01g; nicotinic acid, 15.00g; folic acid, 0.05g; biotin, 0.02g; Co, 0.20g; Cu, 5.00g; Fe, 25.00g; I, 0.06g; Mn, 48.00g; Se, 0.10g; Zn, 45.00g; choline chloride, 200.00g; BHT, 50.00g.

The fresh green neem leaves were harvested from mature Neem trees within the environs of the Poultry Research farm. The leaves were cleaned, made free of stems, and sun-dried on a polyethylene sheet until they became crispy. They were milled and stored in sealed polyethylene bags until they were ready for diet formulation. Three experimental diets were formulated with neem leaf meal inclusion at 0, 0.5, and 1.0% to partially replace wheat offal and were offered to the birds from the start to the end of the experiment. The ingredient compositions of the experimental diets are shown in Table 1.

Experimental birds and management

One hundred and seventeen (117), four weeks-old ISA brown pullet chickens were placed in a Deep litter housing of three treatment groups, each comprising thirty-nine (39) randomly selected birds in three (3) replicates of thirteen (13) birds each. Birds in treatment 1 fed the control diet were given antibiotics from the start to the end of the experiment, while those on treatments 2 and 3 were offered diets with NLM inclusion at 0.5 and 1.0%, respectively. Newcastle, Infectious Bursal diseases, and coccidiosis vaccinations were carried out routinely via drinking water. Vitamins were given before immunization and at the end of each vaccination program. The experiment lasted for Thirteen weeks.

Data collection

Growth Performance Characteristics

The birds in each replicate were weighed in groups using a weighing balance. The initial body weights of the birds were taken with subsequent body weights recorded weekly. A known quantity of feed was given to the birds at the start of every week and the leftover feed at the end of the week was weighed to determine weekly feed intake and consequently daily feed intake. The feed conversion of each group of birds was determined by calculating the ratio of feed intake to weight gain. The Protein Efficiency Ratio (PER) was determined by dividing the weekly body weight gain by the weekly protein consumed for each group of birds. The mortality rate was calculated by subtracting the number of live birds at the end of the experiment from the total number of birds at the start of the experiment. The result was then divided by the total number of birds at the start of the experiment and was multiplied by 100 for the percentage mortality rate.

Blood analyses

Blood samples were collected at the end of week 8 of the experiment. Blood samples of 2.0 ml were drawn via jugular vein puncture with hypodermic syringes into labeled heparinized tubes containing 2mg ml^{-1} Ethylene diamine triacetate to prevent coagulation. Another 2.0ml of blood was drawn into labeled sample bottles without EDTA. Blood samples in the EDTA bottles were analyzed for packed cell volume (PCV), hemoglobin (Hb) concentration, Red blood cells (RBC), White blood cells (WBC), and differentials. Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were derived by calculation from the RBC, Hb, and PCV values. Serum was separated from the clotted blood by centrifuge where serum metabolites (total protein, albumin, globulin, cholesterol, glucose, uric acid, and, aminotransferase (ALT) were analyzed calorimetrically using a diagnostic reagent kit.

Statistical analysis

The experiment was arranged in a one-way analysis of variance. The data collected were (ANOVA). Significant ($p<0.05$) different means were separated using Duncan's New Multiple Range F-Test (DNMRT) of the software package.

Results:

The effect of Neem Leaf Meal on the growth performance of pullet chicks at 5 to 8 weeks is presented in Table 2. There were no significant ($P>0.05$) differences in final weights and weight gain. The results also showed that NLM elicited marked ($P<0.05$) variations in the protein intakes of the birds. Birds fed with a control diet with, a 0.5% inclusion level of NLM recorded the highest protein intake of 9.35, and 9.46g, respectively, while the birds fed with 1.0% inclusion recorded the lowest values (8.88g) of protein intake.

Table 2: Effects of NLM inclusion on growth performance of pullet chicks

	Neem Leaf Meal inclusion levels (%)		
Parameters	0	0.5	1.0
Initial weight(g)	187.31 ± 3.22	187.31 ± 3.22	186.67 ± 1.99
Final weight(g)	562.82±47.46	543.54±42.49	546.89±20.26
Total weight gain(g)	375.51±46.63	356.23±42.60	360.22±20.54
Weight gain /day(g)	13.41±1.67	12.72±1.52	12.87±0.73
Feed intake/bird/day(g)	47.01±2.01	47.01±1.25	48.68±1.67
Feed Conversion Ratio	3.54±0.39	3.74±0.49	3.79±0.18
Protein intake (g/day)	9.35±0.40^{a}	9.46±0.25^{a}	8.88±0.30^{b}
Protein Efficiency Ratio	1.43±0.15	1.35±0.18	1.45±0.07
Mortality (%)	0.00±0.00	2.56±3.97	0.00±0.00

^{a,b} Means in the same row with different superscripts differed significantly ($p<0.05$).

Table 3 shows the results for the effect of Neem Leaf Meal on the hematological parameters of pullet chicks at week eight of the experiment. All major hematological parameters were not significantly ($P>0.05$) influenced by NLM except for MCV and MCH.

Table 3: Effects of NLM concentration on hematological parameters of pullet at chick phase (week 8)

ab;

	Neem leaf meal inclusion level (%)		
Parameters	0	0.5	1.0
Packed Cell Volume (%)	23.17±3.37	25.17±1.83	23.00±3.52
Hemoglobin (g/dL)	7.73±1.14	8.40±0.59	7.80±1.17
Red Blood Cell (x10^{6}/L)	2.82±0.54	2.68±0.57	2.17±0.57
White Blood Cell (x10^{9}/L)	10.53±1.93	8.32±0.83	9.33±2.46
Heterophil (%)	30.67±6.31	35.00±3.52	37.67±10.01
Lymphocytes (%)	68.50±6.75	63.67±3.61	60.67±9.77
Eosinophil (%)	0.33±0.52	0.33±0.52	0.50±0.55
Basophil (%)	0.33±0.52	0.17±0.41	0.50±0.55
Monocytes (%)	0.17±0.41	0.83±0.75	0.67±0.82
MCV (fL)	83.48±10.47^{b}	97.92±25.45^{ab}	110.28±20.93^{a}
MCH (pg)	27.82±3.13^{b}	32.74±8.64^{ab}	37.50±7.55^{a}
MCHC (g/dL)	33.37±0.76	33.40±1.24	33.93±0.83

Means in the same row with different superscripts differ significantly ($p<0.05$).
MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration.

Table 4 displayed results for the inclusion levels of NLM on the Serum Biochemistry of pullet chicks at week 8 of the experiment. The results show that although protein and albumin numerically increased with increased levels of NLM inclusion, the values were not significant ($P>0.05$).

Table 4: Effects of NLM inclusion on serum biochemistry of pullet chicks

	Inclusion levels of NLM (%)		
Parameters	0	0.5	1.0
Total protein (g/dL)	4.20 ± 0.94	4.77 ± 0.71	5.22 ±0.82
Globulin (g/dL)	1.78±0.49	2.10±0.36	2.23±0.54
Albumin (g/dL)	2.42±0.61	2.67±0.50	2.98±0.31
Total cholesterol (mg/dL)	83.83±14.74	89.10±17.21	78.50±15.85
Glucose (mg/dL)	124.50±7.94	118.17±9.00	123.33±10.50
Uric acid (mg/dL)	2.38±0.37	1.83±0.39	2.20±0.54
ALT (u/L)	24.67±4.18	24.67±2.00	25.33±4.03

ALT=Alanine Aminotransferase, T. CHO= Total cholesterol; NLM= Neem Leaf Meal

Discussion:

The non-significant effect of NLM on weight gain of the birds could possibly be that the non-eliminated anti-nutritional factors in the diet containing NLM did not promote growth [10]. Earlier works reported by [11] also revealed the negative effects of nutrient imbalance and poor metabolism [10] on monogastric animals fed higher levels of unconventional feed ingredients. The results disagree with the findings of [6] who reported that boilers fed diets containing Neem leaves and Neem leaf infusion respectively, had higher body weight gains.

The similarities in feed intake for birds fed diets containing Neem leaf meal indicate that the quantum of toxic factors (terpenes and limonoids) [13]; [14] and the active ingredient Azadirachtin [15] were minimal to have had a depressing effect on the appetite of the birds. The results on feed intake corroborate the work of [13] who obtained no marked variations in feed consumption of broiler birds fed Neem leaf meal. However, the non-significant difference in voluntary feed intake could be an indication that the concentration of toxicants in the NLM was within the tolerable limits of the birds with the levels of inclusion of the ingredient. This work confirmed the findings of [16] who also reported no marked variations in feed intakes of broiler birds fed neem leaves at the inclusion levels adopted in the present study although the authors reported significant differences at the higher inclusion levels.

The non-significant effect of NLM on major hematological indices of the pullets was an indication that NLM inclusion levels used for this study are not detrimental to the formation and function of the blood cells and their constituents, except for some of the differential counts (heterophils, lymphocytes, eosinophil, and monocytes) that were influenced by the inclusion levels of the diet across the treatments. The non-significant differences between treatments for some of the major hematological indices observed in this study corroborate the findings of [17] who also reported non-significant differences in the hematological indices of laying hens fed with varying inclusion levels of NLM. This study is at variance with the findings of a previous study [8] which reported that PCV and hemoglobin were significantly higher in layers with higher inclusion levels (10%) of NLM across treatments. This suggest that dietary inclusion of NLM at lower level maintained the normal levels of the Hb, MCH and MCHC and did not cause iron deficiency anemia in the pullets. The significant differences for MCV and MCH with inclusion levels of NLM in this study indicated that NLM at 1.0% inclusion level could reduce macrocytic anemia, which is often cause by dietary deficiencies in vitamin B12 and other causes [18]. An elevated WBC count (leukocytosis) or a depressed WBC count (leucopenia) are indicators of an inflammatory or infectious process, autoimmune disorder, leukemia, allergy, bone marrow depression, malnutrition, or stress [19]. Mean values for WBC observed in this study fell below the range of reference values (9.20 – 28.60 x103/µl) for clinically normal chickens [20].

This indicated that high inclusion levels of NLM in the birds' diet did not create any room for Folate deficiency.

The results of this study showed that total protein and albumin were influenced. The depressive effect of NLM on total protein, albumin, and urea is in agreement with the findings of [21] and [22] who reported that serum urea and total protein contents depend on both the quantity and quality of the protein supplied in the diet. The values of globulin across treatments for grower pullet are indicators of better resistance and immune response to

disease infection. This is because the globulin values are within the literature values as reported by [20] for the normal range of chicken hematology.

The result on glucose concentration with increased inclusion levels of NLM in this study negates the findings of [22] who observed an increase in glucose concentrations of broiler chickens fed with varying inclusion levels of NLM. Glucose is one of the metabolites measured as an indicator of the energy status of the animal. Normal glucose levels in birds indicate adequate synthesis in the liver from propionate, a major glucose precursor [23]. [15] reported decreased concentration of glucose with an increase in NLM inclusion in rabbits fed 10% NLM. [24] also reported that aqueous neem root and leaves reduced blood glucose levels in rats exhibiting antidiabetic activity.

Conclusion

The study concludes that up to 1.0% NLM could be included in the diets of pullet chickens without deleterious effects on their growth performance and blood constituents.

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