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Effects of Varying Levels of Neem (Azadirachta indica) Leaf Meal in Layer Diets on the Haematological and Serological Indices, and Faecal Bacterial Counts of Layers

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Abstract
An experiment was conducted to investigate the effects of varying levels of Neem (*Azadirachta indica*) leaf meal in the diets of layers on the haematology, serology and faecal bacterial counts of laying pullets. Five layer diets formulated to contain 0%, 2.5%, 5.0%, 7.5% and 10.0% Neem Leaf Meal (NLM) were fed to a total of one hundred and fifty (150) 20-week old Shikka Brown layers. The birds were randomly assigned to 5 treatment groups of 30 birds each made up of 3 replicates of 10 birds per treatment. The diets were formulated to contain approximately 2500kcal/kg ME and 17% crude protein. NLM had no significant ($P>0.05$) effect on packed cell volume (PVC), red blood cell count (RBC), white blood cell counts (WBC), Haemoglobin, MCV, MCH, MCHC and plasma protein of the layers. NLM produced significant ($P<0.05$) differences between treatment means in the neutrophils, lymphocytes, basophils and monocytes counts but not in eosinophils. The neutrophil counts of layers fed diets containing 7.5% NLM were significantly ($P<0.05$) higher than those of the control, 2.5% NLM and 5.0% NLM treatment groups. Lymphocyte counts obtained from layers fed 5.0% NLM were significantly ($P<0.05$) higher than those of the control and other NLM treatment groups. Inclusion of NLM in the diets of the layers beyond 5% significantly ($P<0.05$) lowered basophil counts, while monocyte counts increased with increase in dietary NLM up to 5%, and declined thereafter. Serum albumins, cholesterol and total proteins were similar ($P>0.05$) between treatments, while total bacterial counts from cultures of faecal samples of the layers showed highly significant ($P<0.01$) progressive decline with increase in dietary level of NLM. It was concluded that as much as 10% dried Neem Leaf Meal could be included in the diets of laying chickens without deleterious effects on their blood constituents, serum biochemistry, and with the benefit of reducing possible risks of infection from pathogenic bacteria.
Keywords: Neem Leaf Meal, *Azadirachta indica*, haematology, serology, layers, bacterial counts

1. Introduction

The worldwide rise in the population of pathogenic microorganisms of livestock in general and poultry in particular has led to shortfalls in animal protein production and availability, particularly in the developing countries in Africa. This has necessitated investigations into several non-conventional plant extracts with antimicrobial properties for possible incorporation into animal feeds for reducing microbial loads of animals, and as replacements for synthetic antibiotic growth promoters.

In the course of producing livestock intensively in Nigeria, it has been estimated that feed costs constitute over 70% of total livestock production costs (Oluyemi & Robert 1988; Alu 2010). Current studies on Neem (*Azadirachta indica*) leaf extracts indicate an increasing interest in the plant owing to its versatile application and promising uses.

Neem (*Azadirachta indica*), popularly known as Indian neem (margosa) or Indian lilac of the family *Maliaceae,* is one of such non-conventional and available ingredients sources in the tropics with great potential in the 21st century. It is a tropical tree plant which is widely distributed in Africa, and available all year round (Ganguli 2002; Kabeh & Jalingo 2007; Ogbuewu *et al.* 2011; Koona & Budida 2011). The tree is well adapted to the climatic and soil conditions in the tropical rainforest regions, all the way to the Sahel savannah part of Nigeria. The leaves are very bitter to taste, and possess a garlic-like smell. The plant is popular because it is readily available, cheap, non-toxic to animals and humans, and efficacious against malaria.

Azadirachta indica leaves also contain compounds with proven antimicrobial activity (Makeri *et al.* 2007; Wikipedia 2007; Valarmathy *et al.* 2010). The antimicrobial activity of extracts of neem leaves against such micro-organisms as *Staphylococcus spp*, *Streptococcus spp, Pseudomonas spp and Escherichia coli*, and some fungal strains have been reported (Tuhin *et al.* 2007; Valarmathy *et al.* 2010; Koona & Budida 2011). Studies on the effects of neem on poultry production especially of broilers and laying hens also exist (Esonu *et al*. 2007; Obun *et al*. 2013). Antimicrobial studies on the effects of neem leaves and their extracts on cultured micro-organisms *in vitro* have also been carried out (Koona & Budida 2011).

Although the beneficial effects of incorporating neem leaf meal into livestock feeds in terms of increased feed value, protein utilization and antimicrobial activity have been reported, data on the effects of dried neem leaf meal on the health status of layers are still scanty. An understanding of the antimicrobial effects

of neem as reflected in the health status and growth performance of birds fed neem leaf meal is important (Esonu *et al*. 2007).

The present study was therefore undertaken to investigate the effects of varying levels of dried neem (*Azadirachta indica*) leaf meal (NLM) in layers diets on the hematological and serological indices, and faecal microbial counts of layers.

2. Materials and Methods

2.1 Location

This study was carried out at the Poultry Unit of the Teaching and Research farm of the Faculty of Agriculture, Delta State University, Asaba Campus, Asaba, Nigeria. Asaba is situated in south-western Nigeria, and has an equatorial rain forest vegetation type, and coordinates of 6^0 12'N and 6^0 45'E. The area experiences a hot dry season (November - March) and a wet season (April – October). Maximum day temperatures range from 27^0C to 35^0C, while an average annual rainfall of 1800 to 3000mm is prevalent.

2.2 *Processing of the Leaves, and Formulation of the Experimental Diets*

Fresh leaves of *Azadirachta indica* were harvested from mature neem plants in and around the Delta State University, Asaba Campus, Asaba, Nigeria, and spread out evenly to dry under sunlight for five (5) days until they became crispy to touch.

Table 1: Percent Composition of the Experimental Diets

	Dietary Treatments				
Ingredient	**NLM0**	**NLM2.5**	**NLM5.0**	**NLM7.5**	**NLM10.0**
Maize	57.98	56.37	54.75	53.14	51.50
Neem Leaf Meal	-	2.50	5.00	7.50	10.00
Soya bean meal	16.92	16.03	15.15	14.23	13.40
Fish meal	3.00	3.00	3.00	3.00	3.00
Wheat offal	10.00	10.00	10.00	10.00	10.00
Rice offal	1.00	1.00	1.00	1.00	1.00
Oyster shell	7.50	7.50	7.50	7.50	7.50
Bone meal	3.00	3.00	3.00	3.00	3.00
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20
Common salt	0.20	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated					
Crude protein (%)	17.06	16.99	16.93	16.97	16.90
ME (kcal/kg)	2581.23	2545.09	2509.16	2520.46	2503.60

The dry leaves were milled to a fine-powdered state using a hammer mill to produce the leaf meal. Samples of the leaf meal were subjected to laboratory analysis to determine their proximate composition according to the methods of the Association of Official Analytical Chemists (AOAC, 1990). Thereafter, five experimental layers diets were formulated such that they contained milled Neem Leaf Meal (NLM) at 0%, 2.5%, 5%, 7.5%, and 10% inclusion levels, and were designated as NLM0, NLM2.5, NLM5.0, NLM7.5 and NLM10.0 respectively (Table 1). NLM0 had no NLM, and served as the control. The five diets were formulated to be as isocaloric and isonitrogenous as possible, and to contain approximately 2500 kcal/kg Metabolizable Energy (ME) and 17% Crude Protein (CP).

2.3 Experimental Design, and Management of Experimental Birds

One hundred and fifty (150) Shikka Brown layers, aged 20 weeks, were used for the experiment. The birds were weighed individually on arrival at the farm, and at weekly intervals thereafter. The birds were randomly assigned to the five treatment dietary groups (NLM0, NLM2.5, NLM5.0, NLM7.5 and NLM10.0) of 30 birds each. Each treatment was further divided into three (3) replicates of ten (10) birds each, and placed in pens in line with the design of the experiment. The experimental design was a one-way classification in a Completely Randomized Design (CRD) with the model in Equation (1):

$$X_{ij} = \mu + a_i + e_{ij} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where X_{ij} = the observed value of each of the response variable,

μ = the overall population mean,

a_i = observed effect of the *i*th dietary treatment, and

e_{ij} = random residual error.

The birds were managed on deep litter, and provided feed and water *ad-libitum*. Good sanitation practices and

routine vaccinations were carried out.

2.4 Data Collection:
Data were collected and recorded on replicate basis for a period of ten (10) weeks of the experiment. The parameters measured or determined were as follows:
2.4.1 Haematological Parameters:
At the end of 10 weeks of feeding the experimental diets, one bird with similar body weight to the average body weight from each replicate was selected for bleeding. With a 5mL syringe fitted with a 24-gauge sterile hypodermic needle, 5mL of blood was carefully drawn from the left wing at the point of bifurcation of the vein. 3mL of the blood was put into a vacutainer containing EDTA as anticoagulant, and shaken gently to prevent coagulation, while the rest of the blood was put into a vacutainer which had no anticoagulants in it for serum biochemical analysis. All blood samples were immediately sent to the Physiology Laboratory of the Department of Animal Science, Delta State University, Asaba Campus for analysis using routine laboratory procedures as described by Schalm *et al*. (1975), Saror & Schillhorn van Veen (1977), Friedman & Young (1997), Iyayi & Tewe (1998). The heamaglobin (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 Winthrose microhaematocrit technique, and expressed in percentages (%). Red blood cell (RBC) counts were obtained with a Coulter Electronic counter (Model ZF by Coulter Electronic Ltd. London), and the values expressed in millions per microlitre (µL) of blood ($x10^6/\mu L$). The total leucocytes or white blood cells (WBC) were counted with an improved Neubauer haemocytometer and expressed in thousands per microlitre (µL). Plasma protein was obtained with a hand refractometer in gram per litre (g/L) of blood. Other haematological parameters computed or evaluated included mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophil, lymphocyte, oesinophil, basophil, and monocyte counts.
2.4.2 Serological Parameters
Total protein was determined by the Biuret method, albumins by the bromcresol green method, and serum cholesterol by the enzymatic colorimetric technique.
2.4.3 Antimicrobial Activity:
At the end of experimentation, faecal samples were collected from each replicate group by placing dropping trays covered with aluminum foil under their cages, and processed for antimicrobial activity evaluation. Sterile forceps were used to pick up the faeces from the dropping tray into clean, labeled petri-dishes, and sent to the Animal Science Laboratory of Delta State University Asaba Campus, for analysis. The samples were oven-dried at $60^0C - 80^0C$, transferred into labeled sterilized bottles and stored in a refrigerator (at 5^0C) until required for further analysis. Microbial identification and population were done using routine laboratory procedures outlined by John (1984), Collins *et al.* (1995), Prescott *et al.* (1999), Baker *et al.* (2001), Duraipandiyan *et al.* (2006), Tuhin *et al.* (2007), and Valarmathy *et al.* (2010). The analysis included preparation of culture media, microscopic and macroscopic examination of the faecal samples, and growth/population counts. Total Bacteria Counts were then determined.
All glassware used were thoroughly washed with detergent, rinsed with distilled water, wrapped in aluminum foil and sterilized in a hot air oven at 170^0C for 1 hour.
2.4.4 Serological Parameters
Total protein was determined by the Biuret method, albumins by the bromcresol green method, and serum cholesterol by the enzymatic colorimetric technique.

2.5 Data Analysis
Data collected and observations made were subjected to one-way analysis of variance (ANOVA) according to the procedure of Steel & Torrie (1980). Where ANOVA indicated significantly different treatment means, they were separated using the Duncan's New Multiple Range Test (DNMRT) at 5% probability (Duncan 1955; Snedecor & Cochran 1978; Obi 1990).

3. Results
Proximate analysis performed on samples of the test ingredient revealed that it contained approximately 8.33% moisture, 21.87% crude protein, 14.33% crude fibre, 6.33% ether extract, 9.16% ash and 39.96% nitrogen-free extract.

Mean (± SE) values of haematological characteristics and plasma proteins of the layers fed varying levels of dietary NLM are presented in Table 2. The result indicate that NLM had no significant ($P>0.05$) effect on PVC, RBC, WBC, haemoglobin, MCV, MCH, MCHC and plasma proteins.

Table 2. Effects of Feeding Varying Levels of Dried Neem Leaf Meal to Laying Pullets on their Heamatological Characteristics and Plasma Protein (Mean ± SE)

Parameters	Dietary Treatments				
	NLM0	NLM2.5	NLM5.0	NLM7.5	NLM10.0
Packed Cell Volume (%)	27.67±2.67	27.33±1.20	28.67±2.60	27.67±0.88	29.00±1.52
Red Blood Cells ($x10^6$ /µL)	2.80±0.50	2.53±0.85	4.00±0.20	2.93±0.77	4.13±1.01
White Blood Cells ($x10^3$/µL)	14.50±1.93	14.03±5.32	16.22±6.30	10.94±0.81	10.14±2.58
Haemoglobin (g/L)	60.00±18.87	65.07±16.39	60.00±11.45	69.50±0.10	67.50±1.05
MCV fL	108.72±35.77	161.79±56.40	77.00±18.98	144.52±18.98	95.90±29.15
MCH (pg)	26.43±5.28	33.25±10.18	18.03±1.87	29.10±10.49	20.87±6.78
MCHC (g/dL)	31.03±11.03	32.26±9.50	28.05±5.06	33.32±1.08	31.22±1.72
Plasma protein (g/dL)	8.07±0.94	8.60±0.70	8.13±0.18	8.97±0.33	7.53±0.35

Results of the effects of treatment on the differential white blood cell counts of the layers are shown in Table 3. NLM produced significant ($P<0.05$) differences between treatment means in the neutrophils, lymphocytes, basophils and monocytes counts but not in eosinophils. The neutrophil counts of layers fed diets containing 7.5% NLM were significantly ($P<0.05$) higher than those of the control, 2.5% NLM and 5.0% NLM treatment groups. Lymphocyte counts obtained from layers fed 5.0% NLM were significantly ($P<0.05$) higher than those of the control and other NLM treatment groups. Inclusion of NLM in the diets of the layers beyond 5% significantly ($P<0.05$) lowered basophil counts, while monocyte counts increased with increase in dietary NLM up to 5%, and declined thereafter.

Planned contrasts on the data aimed at comparing the average of the NLM groups with the control group showed that feeding NLM significantly lowered lymphocyte counts, ($t = -2.64$, $df = 10$, $P < 0.05$), compared with the control birds. Eosinophil counts were, however, not significantly ($P>0.05$) altered by NLM.

Table 3. Effect of Feeding Varying Levels of Dried Neem Leaf Meal to Laying Pullets on their Differential White Blood Cell Counts (Mean ± SE)

Parameters (%)	Dietary Treatments				
	NLM0	NLM2.5	NLM5.0	NLM7.5	NLM10.0
Neutrophils	$47.43±1.38^{c}$	$49.98±0.58^{bc}$	$40.54±1.36^{d}$	$54.98±1.45^{a}$	$53.20±0.49^{ab}$
Lymphocytes	$39.50±1.86^{a}$	$35.45±1.27^{b}$	$41.02±1.06^{a}$	$33.41±1.14^{b}$	$33.57±0.32^{b}$
Eosinophils	9.02±3.27	9.02±1.77	11.25±1.92	9.84±0.72	10.83±0.61
Basophils	$3.07±1.06^{ab}$	$3.14±0.34^{ab}$	$4.50±0.39^{a}$	$1.45±0.32^{bc}$	$1.08±0.01^{c}$
Monocytes	$1.00±1.00^{c}$	$1.57±0.36^{ab}$	$2.71±0.04^{a}$	$1.10±0.03^{b}$	$1.09±0.01^{b}$

$^{a, b, c, d}$: Means with similar superscripts within the same row are not significantly ($P>0.05$) different.

Serum albunins, cholesterol and total proteins were similar ($P>0.05$) between treatments, while total bacterial counts from cultures of faecal samples of the layers showed highly significant ($P<0.01$) progressive decline with increase in dietary level of NLM (Table 4). Planned contrasts on the bacterial counts data showed that feeding NLM drastically lowered bacterial populations in the culture media, ($t = -57.96$, $df = 10$, $P < 0.01$) compared with the control birds.

Table 4. Effect of Feeding Varying levels of Dried Neem Leaf Meal to Laying Pullets on their Serological Characteristics and Total Bacterial Counts (TBC) (Mean ± SE)

	Dietary Treatments				
Parameters (%)	**NLM0**	**NLM2.5**	**NLM5.0**	**NLM7.5**	**NLM10.0**
Albumins (g/dL)	2.81±0.46	2.84±0.10	2.81±0.35	1.97±0.24	2.12±0.17
Cholesterol (mg/dL)	71.85±3.73	70.82±8.35	91.01±13.97	83.24±16.88	78.84±18.46
Total Protein (g/dL)	4.91±1.48	4.22±0.26	6.05±0.47	3.92±0.64	5.56±0.39
TBC (x10^6/mL)	206.67±6.11^a	140.00±11.14^b	93.33±8.02^c	50.00±6.25^d	26.67±2.89^e

^{a,b,c,d,e} Means with similar superscripts within the same row are not significantly (P>0.05) different.
TBC = Total Bacterial Counts

4. Discussion

4.1 Proximate Composition of the Test Ingredient

The crude protein (CP) content of 21.87% obtained for dried NLM used in this study compares favourably with that reported by Esonu *et al.* (2007), is higher than those obtained by Sokunbi *et al.* (2003) (18.90%) and by Kudke *et al.* (1999) (13.4% and 15.4% in India and Pakistan respectively), and slightly lower than the value of 24.06% reported by Onyimonyi *et al.* (2009). Its high fibre content (14.33%) may, however, be a limiting factor to its use in high amounts in non-ruminant nutrition.

4.2 Haematological Characteristics

Packed cell volume (PCV) and haematological variables give an insight into the physiological state of animals in terms of blood cells formation and function. The non-significant effect of dietary NLM on the major heamatological indices of the layers is an indication that NLM, at the inclusion levels employed in this study, is not detrimental to the formation and function of the blood cells and their constituents, including haemoglobin. Although this is at variance with the findings of a previous study (Esonu *et al.* 2007) in which PCV and the haemoglobin values of layers were reported to be significantly higher at 10% than at lower levels of NLM inclusion, differences between treatment means, in this study, were not significant (P>0/05), while values obtained fell within the ranges of reference values for clinically normal chickens (24.9-40.7% PCV; 1.58-3.82 x10^6/µL RBC; 9.2-28.6 x10^3/mm^3 WBC; 7.4-12.2g/dL Hb; 102-129fL MCV; 31.9-40.7pg; 25.9-33.9% MCHC) by Mitruka & Rawnsley (1981). The implication of normal levels of the haemoglobin indices (Hb, MCH and MCHC) is that feeding as much as 10% neem leaf meal (NLM) did not cause iron deficiency anaemia in the layers. Normal MCV values obtained in this study are an indication of the absence of macrocytic anaemia, which is often caused by dietary deficiencies in vitamin B_{12} and folate among other causes (Hoffbrand & Provan 2007).

4.3 White Blood Cell Counts (WBC) and Differential WBC Counts

This study found that dietary NLM had no significant (P>0.05) effects on WBC and eosinophil counts, but significantly (P<0.05) affected the neutrophil, lymphocyte, basophil and monocyte counts. An elevated WBC count (leukocytosis) or a depressed WBC count (leucopenia) are indicative of an inflammatory or infectious process, autoimmune disorder, leukemia, allergy, bone marrow depression, malnutrition or stress (Cavanaugh 2003). Mean WBC values observed in this study ranged from 10.14±2.58 to 16.22±6.30 x10^3/µL, and fell within the range of reference values for clinically normal chickens by Mitruka and Rawnsley (1981) (9.20 – 28.60 x10^3/µL).

Eosinophils are commonly believed to play a role destroying invading pathogens, protecting the lungs against asthma and defending the host against helminthic parasites in addition to other important but recently identified immune regulatory functions performed both in health and disease (Jacobsen *et al.* 2012). Lymphocyte counts recorded in this study declined slightly but significantly (P<0.05) at the 7.5% and 10.0% dietary NLM levels when compared with the control and 5.0% NLM treatment groups, as was also reported in an earlier study by Esonu *et al.* (2007) in which 10% or 15% NLM significantly decreased the proportion of circulating lymphocytes in layers. The slight lymphopena at the higher levels of NLM feeding may have slightly compromised the affected layers' ability to ward off infections since values obtained (33.41 and 33.57% for 7.5 and 10.0% NLM respectively) fell below the range of reference values for normal chickens of 47.2 – 81.2% recommended by Mitruka & Rawnsley (1981). This may explain the slightly elevated neutrophil counts at the 7.5% and 10.0% levels of NLM inclusion, which, according to von Vietinghoff & Ley (2008) may be an indication of infection, inflammation or stress.

At dietary inclusion levels exceeding 5.0%, NLM significantly reduced the number of circulating

basophils and monocytes. Basopenia (low basophil count) is an indication of allergies and possible hyperthyroidism. Diet-related causes of low monocyte counts are vitamin B_{12} and folate deficiency, and oral ingestion of corticosteroids. It is not unlikely that one or more of the over 300 bioactive compounds which have been isolated from neem (Tiwari *et al.* 2014), and which include several steroids, may have accounted for the low basophils and monocytes.

4.4 Serological Characteristics of Layers Fed the Experimental Diets

The absence of significant differences between treatments in mean serum albumins, serum cholesterol and total proteins (Table 4) was an indication that 5% to 10% dietary levels of NLM had no significant ($P>0.05$) effect on these parameters. The cholesterol level was lower than the report of Ogbuewu *et al.* (2010). The serum albumins and total proteins are similar to the findings of Ogbuewu *et al.* (2010). The serum albumins level indicates that the representative diets had no detrimental effect on the immunity of the birds and the albumin level and its functions on the birds.

Serum cholesterol in NLM-fed layers was numerically, though non-significantly ($P>0.05$), higher than those of the control birds contrary to the findings of Ogbuewu *et al*. (2011), Uko *et al.* (2006) and Bonsu *et al*. (2012) who reported hypocholesmic effects of NLM in rabbits and broilers respectively. Values obtained in this study, however, were still within the range of reference values (52.0-148.0mg/dL) for normal chickens (Mitruka & Rawsley 1981), and so pose no serious risk of cardiovascular disease to humans at 10% inclusion in layer diets.

The non-significant effects of treatment on total serum proteins including albumins (Table 4) indicate that the inclusion of NLM in the layer diets did not significantly alter dietary protein utilization and synthesis in the liver, or suppress the immune system of the birds. Serum proteins are involved in transport of important body substances, and maintenance of normal distribution of water between blood and tissues through osmotic pressure (Polat *et al*. 2011). Low protein is evidence of malnutrition, impaired protein synthesis in the liver, loss (as by haemorrhage) or excessive protein catabolism while elevated protein levels may result from dehydration.

4.5 Total Bacteria Counts (TBC) of the Faecal Bacterial Culture

Bacterial counts of colonies of faecal samples declined significantly ($P<0.01$) and progressively with increase in the proportion of dietary NLM (Table 4) thus confirming the findings of a previous study by Divya *et al.* (2013) who demonstrated the antibacterial effect of neem oil on various drug-resistant bacteria isolated from humans. In another study, Abalaka *et al.* (2012) also showed that a minimum inhibitory concentration (MIC) of 5mg, and a minimum bactericidal concentration (MBC) of 50mg were potent against *Pseudomonas aeruginosa, Klebsiella ozanae*, *Staphylococcus aureus* and *Escherichia coli*. It appears, therefore that at 10% dietary inclusion, NLM can safely serve as an antimicrobial and natural growth-promoting agent in layer diets.

5. Conclusion

As much as 10% dried Neem Leaf Meal can be included in the diets of laying chickens without deleterious effects on their blood constituents and serum biochemistry, and with the beneficial effect of reducing possible risks of infection from pathogenic bacteria.

References

Abalaka, M., Oyewole, O.A., Kolawole, A.R. (2012), "Antibacterial Activities of *Azadirachta Indica* against Some Bacterial Pathogens". *Advances in Life Sciences* **2**(2), 5-8

Alu, S.E. (2010), "Replacement of Bone Ash with Eggshell Meal on Growth Performance and Carcass Characteristics of Broiler Chickens". *International Journal of Food and Agricultural Research* **7**(1), 229-238.

A.O.A.C, (1990), "*Official Methods of Analyis"*. Association of Official Analytical Chemists. 15th Edition, Washington DC.

Baker, F.J., Silverton, R.E. & Pallister C.J. (2001), "*Introduction to Medical Laboratory Technology"*. Seventh Edition. Baunty Press Ltd pp. 258 – 265. ISBN 0750621907 Nigerian Edition. Ibadan.

Bonsu, F.R.K., Kagya-Agyemang, J.K., Kwenin, W.K.J. & Zanu, H.K. (2012), "Medicinal Response of Broiler Chickens to Diets Containing Neem (*Azadirachta indica*) Leaf Meal, Haematology and Meat Sensory Analysis". *World Applied Sciences Journal* **19**(6), 800-805.

Cavanaugh, B.M. (2003), "*Nurse's Manual of Laboratory and Diagnostic Tests"*. 4th Edition, F.A. Davis Company, Philadelphia, PA 19103.

Collins, C.H., Lynes, P.M. & Grange, J.M. (1995), "*Microbiological Method"*. 7th Edition. Butterworth – Heinemann Limited, Britain, pp. 175–190.

Divya, J., Lakshmi, J., Venkatraya, P.M. & Gopalkrishna, B.K. (2013), "Antibacterial Effect of Neem (*Azadirachta indica*) Oil on Multidrug Resistant Bacteria Isolated from Human Infections". *International Journal of Biological & Medical Research,* **4**(4), 3544-3546.

Duncan, D.B. (1955), "New Multiple Range Test". *Biometry*, **11**: 1 – 48.
Duraipandiyan, V., Muniappan, A. & Savarimthu, I. (2006), "Antimicrobial Activity of some Ethnomedicinal Plants Used by Paliyer Tribe from Tamil Nadu, India". Entomology Research Institute Loyola College Chennai. 60034, India.
Esonu, B., Opara, M.N., Okoli, I.C., Obikaonu, H.O., Udedibie, C. & Iheshiulor, O.O.M. (2007), "Physiological Response of Laying Bird to Neem (*Azadirachta Indica)* Leaf Meal-based Diets: Body Weight Organ Characteristics and Haematology". *Life Science Journal* **4**(2), 37-41.
Friedman, R.B. & Young, D.S. (1997), "Effect of Disease on Clinical Laboratory Tests". 3rd Edition. AACC Press, Washington DC.
Ganguli, S. (2002), "Neem: A Therapeutic for all Seasons". *Current Science* **82** (11), 1304.
Iyayi, E.A. & Tewe, O.O. (1998), "Serum Total Protein, Urea Creatinine Levels as Indices of Quality of Cassava Diets for Pigs". *Trop. Vet.***16**, 59-67.
Jacobsen, E.A., Helmers, R.A., Lee, J.J. & Lee, N.A. (2012), "The Expanding Role(s) of Eosinophils in Health and Disease. *Blood* **120**(19), 3882-3890.
Hoffbrand, A.V. & Provan, D. (2007), "Macrocytic Anaemias". In. D. Provan (Ed.) *ABC of Clinical Haematology* 3rd Edition, Blackwell Publishing Ltd., Oxford, OX4 2DQ, UK.
John, B.H, MD. (1984), "Clinical Diagnosis and Management by Laboratory Methods". 17 Edition. W.B. Saunders & Company, pp.1322- 1350.
Kabeh, J.D. & Jalingo, M.G.D.S.S. (2007), "Exploiting Neem (*Azadirachta indica)* Resources for Improving the quality of Life in Taraba State, Nigeria". *International Journal of Agriculture and Biology* **9**(3), 530 – 532.
Koona, S. & Budida, S. (2011), "Antibacterial Potential of the Extracts of the Leaves of *Azadirachta indica* Linn.". *Notulae Scientia Biologicae* **3**(1), 65–69.
Kudke, R.J., Kalaskar S.R., & Nimbalkar, R.V. (1999), "Neem Leaves as Feed Supplement for Livestock". *Pushudhn* **14**:12.
Makeri, H.K., Maikai, V.A, and Nok, J.A. (2007), "Effect of Topical Application of Neem Seed (*Azadirachta indica*) Extract on Sheep Infested with *Amblyomma variegatum, African Journal of Biotechnology* **6**(20),2324 – 2327.
Obi, I.U. (1990). "*Statistical Methods of Detecting Differences Between Treatment Means"*. 2nd Edition. Snaap Press, Enugu, Nigeria.
Ogbuewu, I.P, Okoli I.C, & Iloeje M.U. (2010), "Evaluation of Toxicological Effects of Leaf Meal of an Ethnomedicinal Plant-Neem on Blood Chemistry of Puberal Chinchilla Rabbit Does". *Report and Opinion* **2**(2), 29-34.
Ogbuewu, I.P., Odoemenam, V.U., Obikaonu, H.O., Opara, M.N., Emenalom, O.O., Uchegbu, M.C., Okoli I.C., Esonu, B.O. & Iloje, M.U. (2011), "The Growing Importance of Neem (*Azadirachta indica A. Juss)* in Agriculture, Industry, Medicine and Environment: A Review". *Research Journal of Medicinal Plants* **5**(3), 230–245.
Oluyemi, J.A. & Robert, F.A. (1988), *Poultry Production in Warm Wet Climates* (2nd Edition) Published by Machmillian Press Limited, Hong Kong.
Onyimonyi A.E., Adeyemi, O. & Okeke, G.C. (2009), "Performance and Economic Characteristics of Broilers Fed Varying Dietary Levels of Neem Leaf Meal (*Azadirachta indica)". International Journal of Poultry Science* **8**(3), 256 – 259.
Polat, U., Yesilbag, D. & Eren, M. (2011), "Serum Biochemical Profile of Broiler Chickens Fed Diets Containing Rosemary and Rosemary Volatile Oil". *Journal of Biology and Environment Science* **5**(3), 23-30.
Prescott, M.L., Harley, J.P. & Klein, D.A. (1999). "Microbial Diseases and Their Control". *Microbiology*. 4th Edition. McGraw-Hill Book Company, New Delhi, India. ISBN 0-697-35439-3. Pp. 702 –709.
Saror, D.I. & Schillhorn van Veen, T.W. (1977), "Haematological Values of Udah and Yankasa Sheep in the Northern Guinea Savanna of Nigeria". *Tropical Animal Health and Production* **9**(4), 245-248.
Schalm O.W., Jain N.C. & Carol, E.J. (1975) *Veterinary Hematology*. 3rd Edition. Lea and Febiger, Philadelphia. Pp 51 - 81.
Snedecor, G.W. & Cochran, W.G. (1978). *Statistical Methods*. 6th Edition. The Iowa State University Press, Ames Iowa.
Sokunbi, O.A, Egbunike, G.N., Salako, A.O., & Babadoye A.O. (2003), "The Performance of Pre-Pubertal Female Swine Fed Diet Containing Sun-cured Neem (*Azadirachta indica)* Leaf Meal". *Proceedings of the 8th Annual Conference of the Animal Science Association (ASAN)*, pp: 28 – 31.
Steel, G.D. & Torrie, J.H. (1980). *Principles and Procedures of Statistics*. McGraw Hill Co. Inc. New York.
Tiwari, R., Verma, A.K., Chakraborty, S., Dhama, K. & Singh, S.V. (2014), "Neem (*Azadirachta indica*) and its Potential for Safeguarding Health of Animals and Humans: A Review". *Journal of Biological Sciences* **14**, 110-123.
Tuhin, J., Zinnat, A.B., & Sayeeda, S. (2007), "Effect of Neem Oil on Some Pathogenic Bacteria". *Bangdalesh*

Journal of Pharmacology **2**(2), 71 – 72.
Uko, O.J., Kamalu, T.N., Pindaga, U.H. & Rabo, J.S. (2006), "Studies on Toxicity to Cockerel Chicks of Raw Full-Fat Neem (*Azadirachta indica*A. Juss) Seed Kernel". *Veterinarski Arhiv* **76**(2), 135-144
Valarmathy, K., Gokulakrishnan, Salma, K.M., & Kusum D.P. (2010), "A Study of Antimicrobial Activity of Ethanolic Extracts of Various Plant Leaves Against Selected Microbial Species". *International Journal of Pharm Sciences and Research* **1**(8), 293 – 295.
von Vietinghoff, S. & Ley, K. (2008), "Homeostatic Regulation of Blood Neutrophil Counts". *Journal of Immunology* **181**, 5183-5188.
Wikipedia, Free Encyclopedia. (2007), "Azadirachtin". Wikipedia Foundation Inc., U.S.A. *http://enWikipedia.org.wiki/Azadirachtin*. p-ISSN: 2163-1387 e-ISSN: 2163-1395.

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