HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF FINISHER BROILERS FED NEEM (*Azadirachta indica*) LEAF MEAL

[1]H. O. OBIKAONU, [2]V. M, MMEREMIKWU, [1]V.U., ODOEMENA,[1] I.C. OKOLI AND [1]A.B.I UDEDIBIE
[1]Department of Animal Science and Technology
Federal University of Technology,
P. M. B. 1526, Owerri, Imo State, Nigeria
[2]Cross River State University of Technology
P.M.B. 102
e-mail: helenfuto_nig@yahoo.com
phone: 08035447997

ABSTRACT
A 28-day feeding trial was conducted to evaluate the effects of dietary inclusion of neem (*Azadirachta indica*) leaf meal on the haematological and serum biochemical indices of finisher broilers. The neem leaves used in the experiment were manually harvested, air-dried and milled to become neem leaf meal. The neem leaf meal was included in broiler finisher diets at 0 (T_0), 2.5 ($T_{2.5}$), 5.0 ($T_{5.0}$), 7.5 ($T_{7.5}$) and 10%(T_{10}) levels, respectively and each diet fed to a group of 30 broilers at finisher phase for 28 days in a completely randomized design (CRD). Each group was sub-divided into 3 replicates of 10 birds each and each replicate housed in a 2m x 2m pen. Feed and water were given to them *ad libitum.* Proximate analysis of the neem leaf meal displayed same characteristics as leaf meals from other tropical browse plants - high crude fibre (15.56%) and moderate crude protein content (18.10%).
At the end of the feeding trial blood was collected from the birds, 4 per treatment and analyzed for haematological and serum biochemical indices. Haemoglobin (Hb), RBC parked cell volume (PCV) MCV, MCH, MCHC, TWBC, neutrophils and lymphocytes were not affected by the treatments ($P>0.05$). there were no traces of eosinophils and monocytes but ESR and platelets were significantly ($P<0.05$) enhanced by the leaf meal. The leaf meal did not produce any consistent effects on most of the serum biochemical indices but significantly ($P<0.05$) reduced total bilirubins, conjugated bilirubins, ALP, ALT, AST and urea, showing that up to 10% dietary level, the integrity of the kidney and liver was maintained.

Keywords: Neem leaf meal, finisher broilers, haematological indices, serum biochemical indices.
Corresponding author: Dr. (Mrs) H. O. Obikaonu, e-mail: helenfuto_nig @yahoo.com

INTRODUCTION:

The use of leaf meals of plants as feed ingredients as alternative to conventional feed resources is a novel area of research in animal nutrition. Leaf meals of some tropical legumes and browse plants are rich in nutrients like vitamins, minerals and carotenoids (Vohra *et al,* 1972; Udedibie, 1987; Udedibie and Opara, 1996). One of the tropical plants that have attracted attention of animal nutritionists in recent time is neem (*Azadirachta. indica).* Various parts of the tree have medicinal value (Chakraborty *et al*, 1989). There is need therefore to evaluate its effects on the haematological and serum biochemical constituents of poultry. Haematological changes are routinely used to determine various status of the body and to determine stress due to environment, nutritional and/or pathological factors (Islam *et al*, 2004; Ozbey *et al*, 2004). Nwosu and Stephen (2005) agreed and reported that there is a normal range for the PCV (haematocrit) which is the relative quantity of cells compared to the total volume of blood. James (1979) reported that an increase in haematocrit indicates an increasing blood viscosity because it is the concentration of erythocytes that results in disturbed blood flow while low RBC reduces viscosity and increases blood flow but lowers blood plasmas. Low (restricted) energy intake results in elevated MCHC; also Hb, PCV are very sensitive to levels of protein intake as the value increases with increase in dietary protein concentration (Edozien and Switzer, 1977). Elevated WBC values may however indicate an infection and cell mediated immunity (authority).

According to (Edozien and Switzer, (1977) packed cell volume (PCV) values vary between sexes, ages and species and deviation from the normal range may support abnormalities in function. Total serum cholesterol according to Elietson and Caraway (1976) varies with age, sex and diet. Iheukwumere and Herbert (2003) reported that water restriction significantly ($P<0.05$) influences the biochemical constituents of Na, K, Cl, HCO3 and Ca in the sera of broiler chickens. Reduction in total protein, albumin and globulin in the serum suggests an alteration in protein metabolism (Maja_Villa *et al*, 1977). A study conducted by Obikaonu *et al* (2011_b) on the haematological and serum biochemical indices of starter broilers fed neem (*Azadirachta indica*) leaf meal suggested that neem leaf meal can be included in the diets of young broiler chicks up to 10% without any deleterious effects on their haematological and serum biochemical constituents. The study also showed that neem leaf diets reduce

blood cholesterol and tend to maintain the integrities of both the kidney and liver.

The studies herein reported were designed also to examine the haematological and serum biochemical indices of finisher broilers as affected by dietary Neem leaf meal.

MATERIALS AND METHODS

Study sites: The study was carried out in the Poultry Unit of the Teaching and Research Farm of the School of Agriculture and Agricultural Technology and Animal Science Laboratory of the Federal University of Technology, Owerri, Imo State, Nigeria. Imo State lies between latitude 4˚4' and 6˚3' N and longitude 6˚15' and 8˚15' E. Owerri ais about 100m above sea level. The climatic data of Owerri as summarized in Ministry of Lands and Survey Atlas (1984) of Imo State are as follows: mean annual rainfall, 2500 mm; temperature range, 26.5 – 27.5˚C and humidity range of 70 – 80 %. Dry season duration (i.e. months with less than 65 mm rainfall) is 3 months. The annual evapo-transpiration is 1450 mm and the soil type is essentially sandy loam with average pH of 5.5.

Source and processing of Neem leaves: Fresh green Neem leaves used for the experiment were harvested within the University environment. Each batch of collection was air-dried. They were considered adequately dried when they became crispy to the touch. They were then milled using a hammer mill with 2 mm sieve, to produce Neem leaf meal (NLM). Samples of the leaf meal were subjected to proximate analysis according to AOAC (1995).

Experimental Diets:

Five white maize-based experimental broiler finisher diets (19 % CP) were made, incorporating the leaf meal at 5 levels of 0.00, 2.50, 5.00, 7.50 and 10% designated as $T_{0,}$ $T_{2.50,}$ $T_{5.0,}$ $T_{7.5}$ and T_{1O}, respectively. The ingredient composition of the experimental diets is shown in Table 1. The diets were balanced for crude protein and caloric content as per the requirements of this class of birds in the tropics (Sansbury, 1980)

Experimental Birds and Design:

One hundred and fifty (150) 5-week old Anak broiler birds were used for the experiment. They were divided into 5 groups of 30 birds each and each group randomly assigned to one of the 5 experimental diets in a completely randomized design (CRD). Each group was further sub-divided into 3 replicates of 10 birds each and each replicate housed in a 2m x 2m pen. Feed and water were given to them *ad-libitum*. The feeding trial lasted 4 weeks.

Blood collection and Analysis: At the end of the feeding trial blood samples were collected from 4 birds per treatment. Bleeding was done from the punctured wing vein with a 5 ml scalp vein needle set. About 2 ml of blood was collected from each bird into two sets of sterilised bottles, one containing ethylene diamine tetra acetic acid (EDTA) as the anti–coagulant for determination of haematological parameters, viz: Hb using Sahli method and the value recorded in g/100mls (WHO, 1980), RBC and WBC using the improved Neubauer haemocytometer as described by Dacie and Lewis (1991); PCV was determined by the Microhaematocrit method, while mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the appropriate formulae.

The second set of bottles without EDTA was centrifuged in a macro centrifuge to generate serum for biochemical analysis. Total protein was determined using the burette method as described by Daumas (1975); urea by di-methethyl monoxide method as described by Varley *et al* (1980). Creatinine was determined by Jaffe reaction method as described by Henry *et al (*1974). Albumin was measured using dye–binding technique with bromocresol green as described by Doumas and Bigger (1972). Serum potassium and sodium were determined by the calorimetric method, while serum cholesterol was by a modification of the Liebermann Burchard reaction.

Data Analysis

Data collected were subjected to analysis of variance (ANOVA). Where analysis of variance indicated significant treatment effects, the means were separated using Duncan's New Multiple Range Test as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION:

Proximate composition of the leaf meal is presented in Table 2.

The leaf meal contained 18.10% crude protein, 15.56% crude fibre, 2.50% ether extract, 5.26% ash and 58.22% nitrogen-free extract. The leaf meal displayed same characteristics as leaf meals from other tropical browse plants – high crude fibre and moderate crude protein content as reported for *Jacaranda mimosifolia* (Okorie, 2006) and for *Microdesmis puberula* (Esonu *et al*, 2002). With relatively high crude fibre content (15.56%), the metabolizable energy must be low even though its gross energy content was high (4.16 Kcal/g).

Haematological Indices:

The haematological indices of the finisher broilers fed Neem leaf meal is presented in table 3. There were no significant differences (P>0.05) between the Hb values of the control group and those of $T_{5.0}$, $T_{7.5}$ and T_{10} with $T_{5.0}$ recording the highest value among the treatments, while $T_{2.5}$ recorded the lowest. The values were, however, in agreement with the work of Iheukwumere and Herbert (2003) and Islam *et al* (2004). The RBC values for all the treatment compared favourably with the control. There were no

significant differences (P>0.05) among the treatment which shows no form of RBC damage in the accused birds. WHO (1975) stated that RBC destruction could result in drop in its value. There were no significant differences (P>0.05) between the control and $T_{2.5}$, $T_{7.5}$ and T_{10} in PCV values. The values of PCV in this study are in harmony with the reports of Iheukmumere and Herbert (2003). TWBC increased as the level of Neem leaf meal increased with $T_{7.5}$ recording the highest value. Chickens and domestic turkeys have a WBC distribution with lymphocytes as the most numerous leucocytes (Okeudo *et al* (2003). No traces of eosinophils and monocytes were observed in all the treatments. The eosinophils and monocytes values therefore indicated that values of ESR recorded in this study are within the normal values and show no toxin or bacterial infection (Franson 1974).

Serum Biochemical Indices

The serum biochemical constituents of the birds are shown in table 4.

The values of the serum sugar in the treatment birds fall within the normal values of 227.83g/dl for native chickens (Rampori and Igbel, 2007,). However, there was a significant difference (P<0.05) between the control and $T_{5.0}$ which had highest value of 292.03 g/dl but recorded the lowest value for serum cholesterol. Similar low value was also observed for cholesterol in the broiler starter experiment (Obikaonu *et al* 2011_b).

Calcium level increased as the level of Neem leaf meal increases uptil $T_{5.0}$. The phosphate values for T_0 were also high but T_0 recorded highest value for phosphorus. Even though statistically there was no significant difference between the values of the control and that of the birds on T_{10}. The $T_{2.5}$ group recorded the highest value for sodium. Varying dietary phosphorus levels as wide as 0.24 - 0.64% have been shown not to significantly affect plasma phosphorus (Keshavarz, 1986). Bicarbonate values were stable in T_0 and $T_{5.0}$ (P>0.05) while significant variations occurred (P<0.05) between $T_{2.5}$, $T_{7.5}$, and T_{10}. Chloride values were similar (P>0.05) for the birds on T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$.

Serum total protein decreased as the level of Neem leaf meal increased uptil $T_{7.5}$. The high level of protein by T_0 was attributed to the high PER and better feed conversion ratio recorded in the treatment birds (Obikaonu *et al* 2011a; Hoffenberg *et al*, 1966). Urea levels decreased as the dietary levels of neem leaf meal increased. The level of urea is reported to be influenced by dietary protein quantity, quality and bleeding time(Karasawa 1989). Creatinine levels also followed the same trend since creatinine is the bye-product of protein metabolism (Iyayi and Tewe, 1998). Alanine transaminase (ALT), alkaline phosphase (ALP) and aspartate transaminase (AST) were depressed as the level of dietary Neem leaf meal increased indicating no toxicity. However age of birds is a factor influencing serum biochemical indices of the birds. Oluyemi *et al* (2002) reported that young chickens (8 - 10weeks) had significantly greater aspratate amino transferase and alanine tranferase values than adult (50 - 80weeks) birds. The slight increase in ALP values in the study might therefore be in response to age (Arslam *et al* 2001).

CONCLUSION

The haematological indices recorded in this study were within the levels reported from earlier studies on Neem. Serum biochemical parameters show reduction in cholesterol level at 5%. Also at 5% level, serum minerals were observed to compare favourably with the control showing that Neem leaf meal at 5% did not affect the integrity of the kidney. The liver enzymes (ALP, ALT and AST) decreased as the level of Neem leaf meal increased up to 10% level showing no toxicity. It is therefore concluded that the haematological and serum biochemical indices recorded in this study were within normal ranges, showing that neem leaf meal up to 10% dietary level poses no threat to finisher broilers.

Table 1: Ingredient composition of broiler finisher experimental diets

	Dietary levels of NLM (%)				
Ingredients (%)	**$T_{0.00}$**	**$T_{2.50}$**	**$T_{5.00}$**	**$T_{7.50}$**	**$T_{10.00}$**
White maize	60.00	59.00	57.00	56.00	55.00
Neem leaf meal	-	2.50	5.00	7.50	10.00
Soybean meal	20.00	20.00	20.00	20.00	20.00
Wheat offal	9.00	7.50	7.00	5.50	4.00
Palm kernel cake	3.00	3.00	3.00	3.00	3.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Blood meal	2.00	2.00	2.00	2.00	2.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Common salt	0.25	0.25	0.25	0.25	0.25
Vitamin/Trace min. premix *	0.25	0.25	0.25	0.25	0.25
L- lysine	0.25	0.25	0.25	0.25	0.25

L. methionine	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis (% of Dm)					
Crude protein	18.99	18.98	18.96	18.95	18.93
Crude fibre	4.32	5.06	4.82	5.07	5.32
Ether extract	3.94	3.94	3.94	3.94	3.93
Calcium	1.99	2.04	2.58	2.07	2.18
Phosphorus	1.07	1.04	1.01	0.98	0.95
Methionine	0.57	0.59	0.59	0.57	0.57
Lysine	1.24	1.22	1.20	1.20	1.20
Methabolisable Energy (kcal/kg)	3019.20	2978.10	2964.36	2915.00	2906.60

* To provide the following per kg of feed: vit. A, 1500 iu; vit. D_2 1600 iu; riboflavin, 90 mg; biotin, 0.25 mg; pantothenic acid, 11.0 mg; vit. K, 3.0 mg; vit. B, 22.5 mg; vit. B_1, 60.3 mg; vit B_{12}, 8.0 mg; nicotinic acid, 8.0 mg; Fe, 5.0 mg; Zn, 4.5 mg; Mn, 10.0 mg; Co, 02 mg; Se, 0.01 mg.

Table 2: Proximate Composition of Neem Leaf Meal (100% DM basis)

Components	% of dm
Crude protein	18.10
Crude fibre	15.56
Ether extract	2.50
Ash	5.62
Nitrogen free extract	58.22
Gross energy (Kcal/gm)	4.16

Table 3: Effects of graded dietary levels of neem leaf meal on the haematological indices of finisher broilers

	Dietary Levels of Neem Leaf Meal					
Indices	**T_0**	**$T_{2.5}$**	**$T_{5.0}$**	**$T_{7.5}$**	**T_{10}**	**SEM**
Hb (g/dl)	9.7^{a}	8.83^{b}	9.87^{a}	9.10^{a}	9.53^{a}	0.22
RBC ($x10^6$ uL)	2.30^{a}	2.26^{a}	2.45^{a}	2.36^{a}	2.36^{a}	0.04
PCV (%)	29.9^{a}	27.06^{b}	30.27^{a}	28.93^{a}	29.13^{a}	0.55
MCV (fL)	130.17^{a}	119.07^{a}	123.50^{a}	122.47^{a}	123.60^{a}	3.87
MCH (Pg)	42.13^{a}	39.10^{b}	41.10^{c}	38.50^{b}	40.43^{bc}	0.49
MCHC (g/dl)	32.40^{a}	32.87^{a}	32.70^{a}	32.70^{a}	31.47^{a}	0.38
TWBC ($x10^6$ uL)	10.42^{a}	11.49^{a}	12.65^{a}	14.68^{b}	10.95^{a}	0.76
Neutrophils (%)	3.33^{a}	3.67^{a}	4.33^{a}	5.00^{b}	3.33^{a}	0.36
Lymphocytes (%)	96.67^{a}	96.33^{ac}	95.67^{bc}	95.00^{b}	96.67^{a}	0.33
Eosinophils (%)	0.00	0.00	0.00	0.00	0.00	0.00
Monocytes (%)	0.00	0.00	0.00	0.00	0.00	0.00
ESR	4.63^{a}	4.20^{a}	7.00^{bc}	7.67^{bc}	11.00^{b}	0.94
Platelets ($x10^3$uL)	27.33^{a}	53.00^{b}	73.00^{bc}	93.67^{bc}	76.67^{bc}	7.91

^{abc} Means within the same row with different superscripts are significantly different ($P < 0.05$)

Table 4: Serum biochemical indices of finisher broilers fed graded levels of neem leaf meal

	Dietary Levels of Neem Leaf Meal (%)					
Indices	**T_0**	**$T_{2.5}$**	**$T_{5.0}$**	**$T_{7.5}$**	**T_{10}**	**SEM**
Sugar (g/dl)	249.32[a]	241.71[a]	292.03[b]	246.72[a]	285.31[a]	12.21
Calcium (g/dl)	9.33[a]	8.14[b]	8.81[b]	9.03[a]	8.91[a]	0.25
Inorganic phosphate (g/dl)	2.70[a]	1.91[b]	2.42[a]	2.81[a]	2.53[a]	0.12
Total serum protein (g/dl)	2.31[a]	2.03[b]	1.71[bc]	1.62[c]	2.12[a]	0.09
Albumin (g/dl)	1.74[a]	1.32[bc]	1.12[c]	1.11[c]	1.53[a]	0.10
Globulin (g/dl)	0.62[ab]	0.74[a]	0.63[ab]	0.51[b]	0.63[ab]	0.05
Cholesterol (g/dl)	165.22[a]	201.21[b]	162.93[a]	167.21[ac]	181.22[c]	4.14
Creatinine (mol/l)	0.71[a]	0.80[b]	0.81[b]	0.73[a]	0.72[a]	0.02
Sodium (mol/l)	151.30[a]	153.12[a]	140.33[c]	137.01[c]	147.21[b]	1.66
Potassium (mol/l)	4.42[a]	3.73[b]	4.11[ac]	4.03[c]	4.42[a]	0.10
Chloride (mol/l)	88.91[a]	80.63[a]	80.22[a]	80.41[a]	123.11[b]	4.44
Bicarbonate (mol/l)	19.4[a]	17.23[b]	19.40[a]	22.56[c]	17.07[b]	0.56
Total bilirubin	1.57[a]	1.10[b]	0.90[b]	1.03[b]	0.87[b]	0.08
Conjugated bilirubin	1.03[a]	0.77[b]	0.63[bc]	0.73[b]	0.53[c]	0.05
ALP (iu/L)	533.20[a]	540.17[a]	583.60[c]	518.57[a]	453.73[b]	12.46
ALT (iu/L)	17.00[a]	23.00[b]	12.67[c]	11.00[c]	16.33[a]	1.15
AST (iu/L)	47.67[a]	51.00[a]	9.00[c]	38.33[a]	18.67[b]	4.45
Urea (mg/dL)	32.67[a]	26.33[b]	25.00[bc]	23.67[bc]	22.33[c]	1.07

[abc] Means within the same row with different superscripts are significantly different ($P < 0.05$)

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