EFFECT OF FREQUENCY OF ADMINISTRATION OF NEEM *(Azadirachta indica)* LEAF AQUEOUS EXTRACT ON HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF BROILER CHICKENS

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ABSTRACT
A 56-dy experiment was conducted to evaluate the effects of administration of neem (*Azadirachta indica*) leaf aqueous heat treated extract on the haematological and serum biochemical indices of broiler chickens. One hundred and fifty one week-old broiler chicks (Arbor acres) were weighed and randomly distributed to five dietary treatments in a completely randomized design and each treatment was replicated three times. There were thirty birds in each treatment. The birds in treatment A were given vaccination and conventional drugs without neem leaf aqueous extract (NLAE) (control). Birds in treatment B were given vaccination and 10ml of NLAE in 4days interval without conventional drugs, while birds in treatments C, D and E were given 10ml of NLAE in 4, 5 an 6 days interval, respectively with the required vaccination and conventional drugs. The birds al the treatments were with fed the same starter and finisher diets *ad-libitum*. The experiment lasted for eight weeks. Blood constituents were determined using standard haematological and serum biochemical procedures. The results revealed that administration of NLAE at 10ml per 250ml of water at 4,5, and 6days interval significantly ($P<0.05$) influenced the concentrations of haemoglobin (Hb), packed cell volume (PCV), red blood cell RBC), mean corpuscular volume (MCV), lymphocytes (LY) and platelets (PL). The Hb, PCV, RBC, WBC and LY concentrations reduced with the administration of 10ml NLAE at 4, 5 and 6days interval, while MCV and PL had the highest concentrations at 6days interval. The least concentrations of Hb, PCV and RBC were at 6 days interval of NLAE administration. The total serum protein (TSP), albumin (AB, globulin (Bd), cholesterol (CH), aspartate amino transaminase (AST), alanine amino transaminase (ALT) and urea (UR) concentrations were significantly ($P<0.05$) influenced by the administration of NLAE at 4, 5, and 6days interval. Administration of 10ml NLAE per 250ml of water at 5 and 6days interval reduced the concentration of CH by 13.94 and 14.59% respectively. Broiler chickens can be administered 10ml NLAE per 250ml of water at 4-5 days interval for adequate blood formation and reduction of CH level.
Keywords: Frequency, neem leaf, aqueous blood, broiler.*Azadirachta india*, broiler, haematology.

INTRODUCTION
The drive for attainment of self-sufficiency in meat and other animal products in Nigeria calls for total exploitation of the potential sources of animal protein to ensure a satisfactory level of human protein intake (Nworgu, 2015), The FAO (2015) reported high level of malnutrition in developing countries of the world. Medicinal plants play important roles in treatment of poultry diseases, poultry nutrition and help in reduction of cost in poultry production (Nworgu *et al*., 2013). Neem is one of the medicinal plants that has anti-coccidial effects on broilers (Tipu *et al*., 2002) and has ability to improve feed efficiency of birds because of its antibacterial properties (Prasannabalaji *et al*., 2012) and neem plant has the ability of lowering blood cholesterol level of chickens (Biovet, 2005). Neem leaves contain bioactive compounds which may affect nutrient utilization, haematological and serum biochemical parameters of farm animals (Kausik *etal*., 2008). Blood profiles are important indices for ascertaining physiological state of farm animals (Khan and Zafar, 2005).

MATERIALS AND METHODS
A 8 –week experiment was carried out in late dry season of 2015 in the Federal College of Animal Health and Production Technology Moor Plantation, Ibadan, Nigeria. A hundred and fifty one week old broiler chicks (Arbor acres) were

weighed and randomly distributed to five dietary treatment groups A, B, C, D and E in a completely randomised design and each treatment was replicate three times on deep litter system with 30 broiler chicks per treatment. The birds in treatment A (Control) were given vaccination and conventional drugs required for broiler chicks production without neem leaf aqueous extract (NLAE). The birds in treatment B were given vaccination and 10ml of NLAE in 4 days internal without conventional drugs, while the birds in treatments C, D and E were given 10ml of NLAE at 4, 5 and 6 days interval, respectively with required vaccination and conventional drugs. The birds in all the treatments were fed the same broiler starter and finisher diets (Table 1) *ad-libitum*. Four hundred grammes of neem leaves were used n the preparation of the NLAE and 10ml of the NLAE was added to 250ml of water for the birds in treatments B,C, D and E each and 250ml of water was served to birds in control, after which more water was served to all the birds in all the treatments *ad-libitum*. NLAE was prepared based on Nworgu *et al.* (2013) method. The following data were collected: packed cell volume (PCV), hemoglobin (Hb), mean corpuscular concentrated (MCHC), mean corpuscular heamoglobin (MCH), red blood cell (RBC), white blood cell (WBC), lymphocyte (LY), cholesterol (CHl), albumin (ALB), urea (UR) globulin (GB), alanine transaminase (ALT), aspartate transminase (AST), total serum protein (TSP). Blood collection was carried out at the 8th week of the experiment. Three birds per treatment were randomly selected and bled via the wing veins. For heamatology, about 5ml of blood samples were collected into two set of sterilized bottles containing ethylene diamenetetra-acetic acid (EDTA). Blood samples of serum biochemical analysis were collected into plain vacutainers (i.e. without anti coagulant) for serum separation. The packed cell volume (PVC), haemoglobin (Hb), red and white blood cells (RBC and WBC) were estimated by Schlam *et al.* (1975) methods. Serum total protein was determined by the biurette method (Reinhold, 1953), while and all other serum metabolites were determined using standard procedures. All data were subjected to one-way analysis of variance (ANOVA). Significant differences were separated by Duncan's Multiple Range Test and probability was estimated at $P<0.05$.

RESULTS AND DISCUSSION

The concentration of haemoglobin (Hb), packed cell volume (PVC), red blood cells (RBC), while blood cells (WBC),mean corpuscular volume (MCV) and lymphocytes (LY), were significantly ($P<0.05$) different among the treatment groups, unlike mean cell haemoglobin (MCH). The results of the serum biochemistry of the broiler chickens (BC) served neem leaf aqueous extract (NLAE) revealed that total serum protein (TSP), albumin (AB), globulin (GB), cholesterol (CH), aspartate aminotransaminase (AST), alanine aminotransaminase (ALT) and urea were significantly ($P<0.05$) influenced by the administration of 10ml NLAE in 25ml of water at different frequency (Table 2). The highest concentrations of Hb, PCV, RBC, WBC and LY were recorded on the BC placed in control, while the least of these parameters were recorded on the birds placed in treatment E for Hb, PCV, RBC and LY, but for WBC on the birds placed in treatment B (1.87×10^4l).The Hb (6.40-8.30%) concentrations in the preset study are similar to the reports of Islam *et al.* (2004) (7.06-9.37%) for commercial and local chickens in Bangladesh. The Hb and PCV value in the present work are slightly lower than the revelations of Nworgu *et al.* (2013) (8.55-12.50%) for Hb and 23.50-28.00% for PVC. The Hb (9.0-13.0%), RBC (3.0×10^6l) and PVC (30.0-40.0%) reported by MVM (1986) are higher that the present results. Odoh and Bratte (2015) reported PCV of 27.33-29.10% when the authors fed laying hens graded levels of neem lea meal and their value is in harmony with the result herein. Obikaonu *et al.* (2012) reported that broiler starter fed graded levels of neem leaf meal had reduced Hb (8.00-8.40%) and PVC (23.33-27.33%). Similar observation was made in the present study, as birds served 10ml NLAE in 250ml of water at 4,5 and 6 days interval had reduced concentrations of WBC ($1.58\text{-}2.17 \times 10^6$l) and PCV (20.00-23.00%). The TSP, AB and GB reported in the present study are similar with the reports of Nworgu *et al.* (2008) whose value were 5.39 – 5.41, 3.21-3.36 and 1.94-2.22g/dl, respectively. Birds served 10ml of NLAE at 5 and 6 days interval had reduced concentration of

CH, but serum urea could not follow any definite pattern. The CH in this study agrees with the values reported by Sturkie *et al.* (2000) (100-150mg/dl), but is slightly higher than the submission of Okorie-Kanu *et al.* (2016) (86.67-92.38mg/dl) when the blood sample of 15days old cockerels were examined. The ALT and AST in this report is lower than the reports of Nworgu *et al.* 2007. However, Obikaonu *et al.* (2012) reported that broiler chickens fed neem leaf meal had decreased concentrations of ALT, AST and Alkaline phosphate at ($P<0.05$).

CONCLUSION

The concentration of 10ml NLAE at 4, 5, and 6 days interval might have maintained the integrity of the kidneys in their functions. The concentration of 10ml of NLAE in 250ml of water at the interval used had no deleterious effects on the internal physiology of the BC. The BC served heat treated NLAE had reduced concentration of PCV and CH at 5 and 6 days interval. The lymphocytes decreased with increased frequency, unlike the heterocytes. The BC served NLAE at 6 days interval had the least concentration of urea (1.32mg/dl). The BC could be served 10ml of NLAE at both starter and finisher phases at 4 days interval. The relative low mortality rate (3.45-6.90%) for the birds in treatment B revealed that NLAE has antipathogenic effects on the BC.

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Table 1: Gross composition of broiler starter diet (%)

Ingredients	Broiler starter	Broiler Finisher (%)
Maize	55.00	60.25
Corn bran	6.85	6.00
Wheat offal	5.00	5.00
Soybean meal	20.00	15.00
Groundnut cake	10.00	7.00
Fish meal (72%)	4.00	2.00
Bone meal	2.00	3.00
Oyster shell	1.10	1.00
Salt	0.25	0.25
Methionine	0.15	0.10
Lysine	0.20	0.15
Vitamin/premix	0.30	0.25
Calculated analysis (%)		
Crude protein	22.76	18.83
Crude fibre	3.60	4.20
Ether extract	3.77	3.66
Calcium	1.47	1.67
Phosphorus	0.36	0.28
Lysine	1.21	0.84
Methionine	0.35	0.84
Metabolizable energy (kcal/kg)	2900	3000

Table 2: Some haematological and serum biochemical parameters of broiler chickens administered neem leaf aqueous extract

	Experimental Diets					
Parameters	**A**	**B**	**C**	**D**	**E**	**SEM±**
Haematological indices(%)						
Haemoglogin (%)	8.30^{a}	$7.30b^{c}$	7.70^{ab}	7.70^{a}	6.40^{c}	0.21
Packed cell volum (%)	25.50^{a}	25.50^{a}	24.00^{a}	23.00^{ab}	20.00^{b}	0.58
Rred Blood Cell ($x10^{6}/_{L}$)	2.42^{a}	2.68^{a}	2.75^{a}	2.17^{ab}	1.58^{b}	0.11
White blood cell ($x10^{4}/_{L}$)	2.89	1.87	1.99	2.18	2.50	0.18
Mean corpuscular cell volume (fl)	104.82^{b}	112.53^{b}	118.74^{ab}	107.43^{b}	130.16^{a}	3.21
Mean cell haemoglohin (pg/cell)	34.35^{b}	36.57^{ab}	37.92^{ab}	35.82^{ab}	40.58^{a}	0.89
Mean cell haemoglobin Concentration (%)	32.55^{ab}	33.44^{a}	31.94^{bc}	33.34^{a}	31.20^{c}	0.24
Lymphocyte (%)	61.50^{b}	72.00^{a}	59.00^{b}	59.00^{b}	54.50^{c}	1.59
Heterocytes (%)	33.00^{b}	23.00^{c}	34.50^{b}	34.50^{b}	39.50^{a}	1.48
Monocytes (%)	3.50	2.50	2.50	2.50	2.00	0.24
Eosinophils (%)	2.00^{b}	2.00^{b}	4.00	4.00^{a}	3.50^{a}	0.30
Basophils (%)	0.00	0.50	0.00	0.00	0.50	-
Platelet (x 105L)	2.98^{b}	1.88^{c}	3.43^{a}	3.42^{a}	3.37^{a}	0.13
Mortality starter phase (%) (*)	3.45(1)	6.90(2)	13.90(4)	13.80(4)	20.70(6)	-
Mortality finisher phase (%) (*)	3.45(1)	3.45(1)	0.00(0)	3.45(1)	3.45(1)	-
Serum Biochemical indices						
Total serum protein (g/dl)	4.16^{ab}	3.68^{c}	4.41^{a}	3.77^{bc}	3.90^{abc}	0.01
Albumin (g/dl)	1.56^{b}	1.44^{b}	2.07^{ab}	1.77^{ab}	3.78^{a}	0.15
Globulin (g/dl)	2.60^{a}	2.16^{ab}	2.85^{a}	2.01^{b}	2.02^{b}	0.10
Cholesterol (mg/dl)	128.15^{b}	125.9^{3b}	142.22^{a}	110.38^{c}	109.45^{c}	3.44
Aspartate transaminase (i.u./1)	129.58	140.31	146.85	12513	143.97	3.96
Alaninee transaminase (i.u/1)	5.04^{a}	1.39^{c}	2.06^{c}	3.12^{b}	1.44^{c}	0.38
Urea (mg/dl)	1.60^{ab}	2.00^{ab}	1.54^{ab}	2.56^{a}	1.32^{b}	0.17

a,b,c,d, e Means with different superscripts on the same horizontal row differ significantly ($P<0.05$); (*) = Number of birds that died