

## Effect of strain and sex on haematological and serum biochemical indices of tropical indigenous chickens

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### Abstract

A thirteen-week experiment was conducted to determine the effect of sex and strain on the haematological and serum biochemical indices of two indigenous chicken strains (FUNAAB Alpha and Yoruba ecotype) in the African subtropics. One hundred and twenty chickens comprising thirty (30) each of FUNAAB Alpha males, FUNAAB Alpha females, Yoruba ecotype males and Yoruba ecotype females were assigned to four treatments consisting of three replicates of ten (10) chickens each, in a 2 x 2 factorial arrangement of a completely randomized design. Data collected were analyzed for simple descriptive and inferential statistics using SAS. Significantly ( $p < 0.05$ ) higher white blood count (13.55 g/100ml) was observed with the FUNAAB Alpha compared with 10.10 g/100ml of the Yoruba ecotype chicken strain. Both the mean corpuscular volume and corpuscular haemoglobin were higher ( $p < 0.05$ ) in the Yoruba ecotype than FUNAAB Alpha. Similarly, glucose and phosphorus values were significantly ( $p < 0.05$ ) higher in FUNAAB Alpha than the Yoruba ecotype. Aspartate amino transferase (AST), Alanine amino transferase (ALT) and calcium concentrations were significantly higher ( $p < 0.05$ ) in the Yoruba ecotype than FUNAAB Alpha. The males of Yoruba ecotype recorded higher volume of white blood count while FUNAAB Alpha females recorded higher concentration of Heterophil than their male counterparts. The values of glucose, total protein, globulin and uric acid were observed higher in female than male chickens. The study showed that strain and sex had significant effects on both haematological and serum biochemical indices of tropical indigenous chickens.

**Keywords:** Tropical indigenous chicken; strain; sex; blood indices

## L'Effet de la tension et du sexe sur les indices biochimiques hématologiques et sériques des poulets indigènes tropicaux



### Résumé

Une expérience de treize semaines a été menée pour déterminer l'effet du sexe et de la tension sur les indices biochimiques hématologiques et sériques de deux souches indigènes de poulet (FUNAAB Alpha et Yoruba ecotype) dans les sous-tropiques africains. Cent vingt poulets comprenant trente (30) mâles FUNAAB Alpha, des femelles FUNAAB Alpha, des mâles ecotypes Yoruba et des femelles ecotypes Yoruba ont été affectés à quatre traitements composés de trois répliques de dix (10) poulets chacun, dans un arrangement factoriel de 2 x 2 d'une conception complètement randomisée. Les données recueillies ont été analysées à l'aide de statistiques descriptives et inférentielles simples à l'aide du SAS. Significativement ( $p < 0.05$ ) le compte plus élevé de sang blanc (13.55 g/100ml) a été observé avec le FUNAAB

*Alpha comparé à 10.10 g/100ml de la souche de poulet d'écotype de Yoruba. Le volume corpusculaire moyen et l'hémoglobine corpusculaire étaient plus élevés ( $p < 0,05$ ) dans l'écotype de Yoruba que FUNAAB Alpha. De même, les valeurs de glucose et de phosphore étaient significativement ( $p < 0,05$ ) plus élevées dans FUNAAB Alpha que l'écotype de Yoruba. L'aspartate aminotransferase (AST), l'Alanine aminotransferase (le 'ALT') et les concentrations de calcium étaient significativement plus élevées ( $p < 0,05$ ) dans l'écotype de Yoruba que FUNAAB Alpha. Les mâles de l'écotype de Yoruba ont enregistré un volume plus élevé de sang blanc, tandis que les femelles FUNAAB Alpha ont enregistré une concentration plus élevée d'hétérophiles que leurs homologues masculins. Les valeurs du glucose, de la protéine totale, de la globuline et de l'acide urique ont été observées plus élevées chez les poulets femelles que chez les poulets mâles. L'étude a montré que la tension et le sexe avaient des effets significatifs sur les indices biochimiques hématologiques et sériques des poulets indigènes tropicaux.*

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**Mots-clés:** Poulet indigène tropical; tension; sexe; indices sanguins

## **Introduction**

Nigeria is the second largest producer of poultry in Africa (FAOSTAT, 2018). The larger percentage of about 60% of poultry population in Nigeria are indigenous chicken ecotypes (Ogie *et al.*, 2013). In developing and underdeveloped countries, indigenous chickens are more abundant with Nigeria having the highest in the sub-Saharan countries (Manyelo *et al.*, 2020). However, the potentials of these indigenous chickens to mitigate the inadequate supply of quality animal protein in average Nigerian meal is grossly untapped. These indigenous chickens are known to have comparable advantage over their exotic counterparts in the areas of disease resistance, adaptation to the climatic conditions and acceptability of the meat and eggs by the local populace. The meat flavour, taste and leanness is preferred to the exotic breeds. Indigenous chickens are however low yielding and take longer period to be ready (Choo *et al.*, 2014). Deliberate efforts towards developing and populating the indigenous breeds will be an effort in the right direction. In Nigeria, notable among the various ecotypes reported are Forest or Yoruba ecotype and Savannah or Fulani ecotype (Lasagna *et al.*,

2020). Animal breeding programs in Nigeria has been used to increase the genetic potential of indigenous chickens. Intensive selection over the years and crossbreeding programmes has led to the development of some chicken populations in Nigeria including Shika brown and FUNAAB Alpha breeds from Nigerian Animal Production Research Institute, Zaria and Federal University of Agriculture, Abeokuta, respectively. This achievement has led to improved meat and egg production without sacrificing adaptation to tropical environment well known for heat stress and infectious diseases (Ilori *et al.*, 2016). With due attention paid to the improvement and multiplication of the indigenous chickens, these birds are able to play important roles in solving the food security challenges of the country in the future and also be used in animal breeding programs in the world. According to Mpenda *et al.* (2019), many selections have been made due to phenotypic characteristics and adaptation to environment as well as resistance to prevalent diseases and these indigenous chickens are able to improve quality of products through heterosis or morphological and physiological changes.

In addition, indigenous chickens' usefulness in the expansion of animal breeding programs cannot be overemphasized. Neglect of the indigenous strains and the increasing use of exotic strains poses a major threat to the future of indigenous chickens and can lead to their eventual disappearance which has its disadvantages.

A number of factors such as nutrition, growth rate, sex, breed and age do affect the performances of chickens. Among all these factors, according to Sola-Ojo *et al.* (2012), sex has greatest impact on the possibility of genetically improving performance. While Studies of blood indices play important roles in the physiological, pathological and nutritional status of an organism; it also provides the opportunity to evaluate the presence of several metabolites and other constituents in the body of animals (NseAbasi *et al.*, 2014). Changes in the constituent compounds of blood when compared to normal values could serve as a reflector of the metabolic stage of an animal as well as quality of feed. These can then be used to determine systemic relationships and physiological adaptations in the body of animals to environmental, nutritional and or pathological factors (Saulawa *et al.*, 2012). Therefore, this study is aimed at determining the effect of sex and strain on haematological and serum biochemical indices of tropical indigenous chickens (FUNAAB Alpha and Yoruba ecotype).

### **Materials and methods**

The study was carried out at the Poultry Unit of the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, Abeokuta (FUNAAB). The farm is located in the derived savanna region with an average temperature of 38°C and a relative humidity of 82%. It is in the region 70 m above sea level of latitude 7° 53'N and longitude 3°20'E. It receives a

mean precipitation of 1037mm per annum (Google Earth 2018).

One hundred and twenty day-old chicks, comprising thirty (30) chicks each of FUNAAB alpha males, FUNAAB alpha females, Yoruba ecotype males and Yoruba ecotype females, assigned to four treatments consisting of three replicates of ten (10) birds each, were sourced from the University's hatchery. The birds were intensively managed in a deep litter system. Sexing was done at the end of the chick phase (8 weeks), when the distinguishing characteristics, especially comb was obvious. The experimental layout was a 2 x 2 factorial arrangement. Commercial (Corn soymeal based) grower mash (Protein 16%, Energy -2458 KCal/Kg) was given *ad libitum* throughout the period of the experiment which lasted 12 weeks. Clean water was supplied in a thoroughly cleaned drinker to the birds every day. At 20th week of the experiment, blood was collected from the wing vein of two selected birds per replicate with the aid of needle and syringe for haematological and serum biochemical analysis. A 5 ml sample of blood from each chicken was collected and transferred into a set of bottles with anti-coagulant for haematological analysis and without anti-coagulant for serum biochemical analysis. The values of Haemoglobin (Hb), Packed cell volume (PCV), Red blood cells (RBC) and White Blood Cells (WBC) were determined. The Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) were calculated. Differential counts, that is neutrophils and lymphocyte, were analysed following the description of Jains (1986). Standard chemical procedures were used to determine Total serum protein (Gornall *et al.*, 1949), serum enzymes (AST and ALT), (Reitman and Frankel, 1957) and urea nitrogen (Chawla, 1999).

*Effect of strain and sex on haematological and serum biochemical indices of tropical indigenous chickens*

The data collected were subjected to Analysis of Variance (ANOVA) in a 2 x 2 factorial arrangement using SAS 9.0 (SAS Institute, Cary, NC). Significant ( $p < 0.05$ ) means among variables were separated using Duncan Multiple Range Test of the software.

The model is given below

$$Y_{ijk} = \mu + S_i + G_j + (SG)_{ij} + e_{ijk}$$

Where  $Y_{ijk}$  is observed values on the variable of interest

$\mu$  is population means

$S_i$  is fixed effect of  $i$ th sex (male and female)

$G_j$  is fixed effect of  $j$ th strain (FUNAAB alpha and Yoruba ecotype)

$SG_{ij}$  is fixed effect of interaction between  $i$ th sex and  $j$ th strain

$e_{ijk}$  is random residual errors

**Results**

The main effect of strain and sex on the haematological indices of the Nigerian indigenous chickens is presented in Table 1. The strain effect did not show significant ( $p > 0.05$ ) difference on the measured parameters except for the white blood count, mean corpuscular volume and the mean corpuscular haemoglobin. The white blood count was significantly ( $p < 0.05$ ) higher in FUNAAB alpha than in the Yoruba ecotype. The mean corpuscular volume and mean corpuscular haemoglobin values of the Yoruba ecotype were significantly ( $p < 0.05$ ) higher compared with FUNAAB Alpha. Chicken of both sexes showed similar ( $p > 0.05$ ) values of haematological characteristic.

**Table 1: The main effects of strain and sex on the haematological characteristics of Nigerian indigenous chickens**

Parameters	Strain		SEM	Sex		SEM
	FUNAAB alpha	Yoruba ecotype		Male	Female	
Packed Cell Volume (%)	32.3	33.3	1.00	33.42	32.25	1.02
Haemoglobin (g/dl)	10.84	11.12	0.33	11.18	10.78	0.34
Red Blood Count ( $\times 10^2/L$ )	4.10	4.09	0.12	4.19	4.00	0.11
White Blood Count (g/100 ml)	13.55 <sup>a</sup>	10.10 <sup>b</sup>	1.12	13.18	10.48	1.17
Heterophil (%)	22.75	23.00	3.40	20.33	25.42	3.31
Lymphocyte (%)	73.25	72.91	3.17	75.00	71.17	3.11
Heterophil, lymphocyte ratio	0.35	0.35	0.08	0.31	0.39	0.07
Monocytes (%)	1.83	1.92	0.25	2.25	1.50	0.24
Basophil (%)	0.50	0.58	39.59	0.67	0.42	0.41
Eosinophil (%)	1.67	1.50	17.06	1.67	1.50	0.59
Mean Corpuscular Volume (fl)	78.76 <sup>b</sup>	81.18 <sup>a</sup>	13.38	79.43	80.51	0.24
Mean Corpuscular Hemoglobin Conc. (%)	33.53	33.16	0.21	33.26	33.43	0.22
Mean Corpuscular Hemoglobin (pg)	26.42 <sup>b</sup>	27.18 <sup>a</sup>	1.00	26.68	26.93	1.02

<sup>a,b</sup>: Means in the same row with different superscripts by factor differ significantly ( $P < 0.05$ ).

The interactive effect of strain and sex on the haematological indices of the Nigerian indigenous chickens is presented in Table 2. The white blood count recorded the least ( $p < 0.05$ ) value on the Yoruba ecotype females (7.78 **g/100 ml**) while all the males and FUNAAB Alpha female sexes recorded

similar ( $p > 0.05$ ) values. The FUNAAB Alpha females recorded significantly ( $p < 0.05$ ) higher heterophil while FUNAAB Alpha males had the least ( $p < 0.05$ ) value. Both sexes of the Yoruba ecotype are indifferent. The Yoruba ecotype males and females recorded the highest ( $p < 0.05$ )

values for Mean corpuscular volume (MCV) While for Mean corpuscular haemoglobin (MCH), both sexes of Yoruba ecotype and FUNAAB Alpha females had the highest ( $p < 0.05$ ) values. the lowest values. FUNAAB Alpha males recorded significantly ( $p < 0.05$ ) lower values in both MCV and MCH.

The main effect of strain and sex on the Serum biochemical indices of the Nigerian

indigenous chickens is presented in Table 3. Glucose and phosphorus are significantly ( $p < 0.05$ ) higher in FUNAAB Alpha. Significantly ( $p < 0.05$ ) lower values of glucose and phosphorus were recorded for the Yoruba ecotype. AST, ALT and calcium were higher ( $p < 0.05$ ) in the Yoruba ecotype. The effect of sex showed that significantly higher ( $p < 0.05$ ) values were recorded in the female sex for glucose, total protein, albumin, globulin and uric acids.

**Table 2: The interactive effects of breed and sex on the haematological characteristics of Nigerian indigenous chickens**

Parameters	FUNAAB Alpha		Yoruba ecotype		SEM
	Male	Female	Male	Female	
Packed Cell Volume (%)	32.00	32.67	34.83	31.83	1.39
Hemoglobin (g/dl)	10.73	10.95	11.63	10.61	0.46
Red Blood Count ( $\times 10^2/L$ )	4.09	4.12	4.29	3.89	0.16
White Blood Count (g/100 ml)	13.93 <sup>a</sup>	13.17 <sup>a</sup>	12.42 <sup>a</sup>	7.78 <sup>b</sup>	1.40
Heterophil (%)	15.50 <sup>b</sup>	30.00 <sup>a</sup>	25.17 <sup>ab</sup>	20.83 <sup>ab</sup>	4.09
Lymphocyte (%)	79.33	67.17	70.67	75.17	3.96
Heterophil, lymphocyte ratio	0.20	0.50	0.41	0.28	0.09
Monocytes (%)	2.67	1.00	1.83	2.00	0.34
Basophil (%)	0.50	0.50	0.83	0.33	0.60
Eosinophil (%)	2.00	1.33	1.33	1.67	0.64
Mean Corpuscular Volume (fl)	78.33 <sup>c</sup>	79.18 <sup>bc</sup>	80.52 <sup>ab</sup>	81.83 <sup>a</sup>	0.31
Mean Corpuscular Hemoglobin Conc. (%)	33.55	33.51	32.97	33.35	0.26
Mean Corpuscular Hemoglobin (pg)	26.28 <sup>b</sup>	26.55 <sup>ab</sup>	27.07 <sup>ab</sup>	27.30 <sup>a</sup>	1.39

<sup>a,b,c</sup>: Means in the same row with different superscripts differ significantly ( $P < 0.05$ ).

**Table 3. The main effects of breed and sex on the serological characteristics of chickens**

Parameters	Strain			Sex		
	FUNAAB alpha	Yoruba ecotype	SEM	Male	Female	SEM
Glucose (mg/dl)	193.24 <sup>a</sup>	163.30 <sup>b</sup>	7.43	160.00 <sup>b</sup>	196.53 <sup>a</sup>	6.75
Total Protein (g/dl)	63.55	63.75	2.01	59.63 <sup>b</sup>	68.05 <sup>a</sup>	1.50
Albumin (g/dl)	36.73	38.21	1.45	35.22 <sup>b</sup>	39.72 <sup>a</sup>	1.29
Globulin (g/dl)	27.57	25.55	0.92	24.46 <sup>b</sup>	28.66 <sup>a</sup>	0.78
AST (iu/l)	106.56 <sup>b</sup>	124.57 <sup>a</sup>	5.63	123.49	107.63	5.93
ALT (iu/l)	12.62 <sup>b</sup>	15.73 <sup>a</sup>	0.98	14.08	14.26	1.09
Cholesterol (mg/dl)	153.86	193.43	8.68	171.37	176.37	11.02
Bilirubin	0.67	0.68	0.03	0.68	0.66	0.03
Calcium	7.27 <sup>b</sup>	9.40 <sup>a</sup>	0.21	8.28	8.40	0.38
Phosphorus (mg/dl)	4.61 <sup>a</sup>	3.60 <sup>b</sup>	0.15	4.08	4.13	0.21
Uric (mg/dl)	3.50	3.93	0.28	3.00 <sup>b</sup>	4.42 <sup>a</sup>	0.19

<sup>a,b</sup>: Means in the same row with different superscripts by factor differ significantly ( $P < 0.05$ ) AST- Aspartate amino transferase , ALT - Alanine amino transferase

The interactive effect of strain and sex on the Serum biochemical indices of the Nigerian indigenous chickens is presented in Table 4. Glucose is significantly ( $p<0.05$ ) high in FUNAAB Alpha females than in all other groups. Yoruba ecotype males had the least glucose value. The Total protein and Uric values for the females of both strains were significantly ( $p<0.05$ ) higher than for the males. Globulin is significantly ( $p<0.05$ ) higher in both sexes of FUNAAB alpha and Yoruba ecotype female, while

Yoruba ecotype males had the least value. Aspartate amino transferase (AST) was high ( $p<0.05$ ) in the Yoruba ecotype males. Calcium values, both in males and females of the Yoruba ecotype chickens were significantly ( $p<0.05$ ) higher when compared to the FUNAAB Alpha males and females. Phosphorus was significantly ( $p<0.05$ ) higher in FUNAAB Alpha males and females while both sexes of Yoruba ecotype recorded significantly ( $p<0.05$ ) lower values.

**Table 4: The interactive effects of breed and sex on the serological characteristics of chickens**

Parameters	FUNAAB Alpha		Yoruba ecotype		SEM
	Male	Female	Male	Female	
Glucose (mg/dl)	178.13 <sup>b</sup>	208.35 <sup>a</sup>	141.88 <sup>c</sup>	184.72 <sup>b</sup>	7.14
Total Protein (g/dl)	60.58 <sup>bc</sup>	67.10 <sup>ab</sup>	58.68 <sup>c</sup>	68.83 <sup>a</sup>	2.15
Albumin (g/dl)	34.27	39.18	36.17	40.25	1.88
Globulin (g/dl)	26.40 <sup>a</sup>	28.73 <sup>a</sup>	22.52 <sup>b</sup>	28.58 <sup>a</sup>	0.93
AST (iu/l)	109.98 <sup>b</sup>	103.13 <sup>b</sup>	137.00 <sup>a</sup>	112.13 <sup>b</sup>	7.28
ALT (iu/l)	12.12	13.12	16.05	15.40	1.44
Cholesterol(mg/dl)	142.25	164.18	197.48	188.56	12.20
Bilirubin	0.67	0.67	0.70	0.65	0.04
Calcium	7.34 <sup>b</sup>	7.20 <sup>b</sup>	9.22 <sup>a</sup>	9.60 <sup>a</sup>	0.29
Phosphorus (mg/dl)	4.60 <sup>a</sup>	4.62 <sup>a</sup>	3.55 <sup>b</sup>	3.65 <sup>b</sup>	0.22
Uric (mg/dl)	2.72 <sup>b</sup>	4.28 <sup>a</sup>	3.30 <sup>b</sup>	4.55 <sup>a</sup>	0.26

<sup>a,b,c</sup>: Means in the same row with different superscripts differ significantly ( $P<0.05$ ). AST - Aspartate amino transferase, ALT - Alanine amino transferase

## Discussion

Haematological characteristics help to evaluate the level of inflammation, anaemia, infection, blood clotting disorders, haemophilia and lots more. In this study, the similarities in the Packed Cell Volume (PCV), haemoglobin, lymphocyte, heterophil-lymphocyte ratio, monocyte, eosinophil and Mean Corpuscular Haemoglobin Concentrations (MCHC) in the blood of both FUNAAB Alpha and Yoruba ecotype strains were within the normal range as reported by Jain (1993). The values obtained for Red Blood Count (RBC) in both FUNAAB Alpha and Yoruba ecotype were slightly higher than the normal range ( $2.5-3.5 \times 10^2/L$ ) recorded

by Jain (1993) and general reference interval (GRI) for RBC ( $2.5 - 3.9 \times 10^2/L$ ) reported by Harrison and Lightfoot (2005). The values of Basophil, Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) were however lower compared to the normal range reported by Jain (1993) and the range recorded by Wikivet (2012) for Thai indigenous chicken. There was a significant increase in white blood count of FUNAAB Alpha strain compared with the Yoruba ecotype. This could be as a result of the weight difference amongst the two strains used for this study. Adeyemo *et al.* (2018) recounted that weight, age, sex, diet type, strain and climate are factors which cause variation in

the haematological parameters of chicken. The MCV and MCH values of Yoruba ecotype were higher than that of the FUNAAB alpha considering the main effect result. The values recorded for both strains fell below the normal range by Jain (1993) and the GRI by Harrison and Lightfoot (2005). The difference may be as a result of the climatic variation. Bahman *et al.* (2011) noted that haematological and biochemical parameters in indigenous chicken vary from across the regions of the world. The interaction effect of strain and sex on the haematological parameters reveals that the Yoruba ecotype males have significantly higher levels of white blood count than their female counterpart. This result is similar to the report by Simaraks *et al.* (2004) that Hb and PCV of male chickens were higher than that of female. The result may have been influenced by increased androgen activity at about the period of sexual maturity in the male chicken. The FUNAAB Alpha males white blood count increase over their female counterpart is however not significant. FUNAAB Alpha females, which is the improved indigenous, exhibited a higher heterophil percentage than their male counterpart. The result was similar to the report by Simaraks *et al.* (2004) where the percentages of lymphocytes in females was higher than in male chickens. FUNAAB Alpha chickens had a significantly higher glucose content than the Yoruba ecotype. The values for both strains of indigenous chickens fell below the normal range (197-299mg/dl) that was reported for poultry serum (Clinical Diagnostic Division, 1990). In this study the serum calcium levels in indigenous birds were different between the sexes and this disagreed with the report of indifference among sexes by Meluzzi *et al.* (1992) and Simaraks *et al.* (2004). The value obtained for calcium in Yoruba ecotype fell within the reference range (8.1-12mg/dl) by Clinical Diagnostic Division

(1990) and the range (9-12 mg/dl) obtained by Simaraks *et al.* (2004) for Thai indigenous chicken while that of FUNAAB Alpha fell slightly below these ranges. Serum Phosphorus in this study however, was higher in both sexes of FUNAAB alpha than Yoruba ecotype hens and cocks. The female chickens of both strains recorded higher serum glucose, total protein, albumin, globulin and uric acid than their male counterpart. It has been reported that serum glucose of female chicken was lower than that of male chickens (Sturkie, 1965; Homswat *et al.*, 1999). However, in the present study, serum glucose levels of female indigenous chickens were significantly higher than their male counterparts. This may be due to the physiological state of the birds. The females are at point of lay when blood samples were collected and at this point a lot of organ changes may cause serum glucose rise in females. Total protein of the females of both FUNAAB Alpha and Yoruba ecotype were higher than in males. This result was similar to the report by Simaraks *et al.* (2004) who studied the haematological, electrolyte and serum biochemical values of indigenous chickens. The higher value of globulin in female birds of both strains with the males of FUNAAB Alpha strain could be attributed to an estrogen-induced increase in globulins which occurs just at the onset of egg laying. The proteins are the yolk precursors (vitellogenin and lipoproteins), which when synthesized in the liver are transported through the plasma to the ovary where they will be incorporated in the oocytes according to Ritchie *et al.* (1994). Cholesterol of these indigenous birds were not significantly different between strain and sexes, similar to the report by Bahman *et al.* (2011) (male =  $167.60 \pm 35.68$  mg/dl; female =  $152.60 \pm 28.11$  mg/dl) and Simaraks *et al.* (2004) (male =  $101.7 \pm 19.4$  mg/dl; female =  $103.1 \pm 41.0$  mg/dl). The serum cholesterol of the indigenous

chickens fell within the reference range (129 – 297mg/dl) by Clinical Diagnostic Division (1990) and that recorded by Simaraks *et al.* (2004) (72 – 133mg/dl) for indigenous chicken. The study concluded that strain of Nigerian indigenous chickens influenced white blood count, MCV, MCH, glucose, liver enzymes (AST and ALT), calcium and phosphorus, while sex had significant effect on white blood count, heterophil, glucose, total protein and uric acid.

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