

Influence of Sex and Genotype on the Haematological Profile of FUNAAB Alpha Broiler Line, Pullet Line and Noiler Chickens Reared Intensively in Abeokuta, Nigeria

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Abstract

Analysis of normal haematological parameters of chickens is essential in diagnosing the various pathological and metabolic disorders and can be used as a tool to assess the health status of an individual or a flock. The aim of this study was to analyse the haematological indices of FUNAAB Alpha broiler line, FUNAAB Alpha pullet line and Noiler chicken genotypes reared intensively in Abeokuta. The study made use of a total of three hundred, 100 birds each of the three genotypes with equal sex. The birds were fed ad libitum and the research lasted for 12 weeks. Blood samples were collected and were analysed for the following haematological indices: Packed cell volume (PCV%), Haemoglobin (g/dL), Red blood cell count ($m\mu/mm^3$), Monocytes (%), Lymphocyte's concentration (%), Basinophils concentration (%), Eosinophil concentration (%) and Heterophil concentration (%). Results obtained from haematological analysis of the blood were statistically analysed using the General Linear Model Procedure of the SAS. Significant difference among treatment means were compared using Least Significant Difference (LSD). High counts of lymphocytes (66.94 %), eosinophils (1.21 %) and basophils (1.33 %) were found in FUNAAB Alpha broiler line while FUNAAB Alpha pullet line recorded the highest for heterophils (32.46 %) count. The male chickens had the best count for basophils and heterophils, females had a better lymphocytes while both sex had the same value for eosinophil. The general assessment of the haematological indices showed that FUNAAB Alpha broiler line had better indices when compared with FUNAAB Alpha pullet line and Noiler genotypes. Findings of this study can be used as reference to assess normal health status of FUNAAB Alpha broiler line, Pullet line and Noiler chickens. It can also guide the development of performance improvement strategies.

Keywords: Birds, blood, concentration, genotype, Haemoglobin, Health, sex

Running title: Haematological Indices on three genotypes of chickens

Influence du sexe et du génotype sur le profil hématologique de la lignée de poulets à griller FUNAAB Alpha, de la lignée de poulettes FUNAAB Alpha et des poulets Noiler élevées intensivement à Abeokuta, Nigéria



Résumé

L'analyse des paramètres hématologiques normaux des poulet est essentielle pour diagnostiquer divers troubles pathologiques et métaboliques et peut être utilisée comme outil pour évaluer l'état de santé d'un individu ou d'un troupeau. L'objectif de cette étude était d'analyser les indices hématologiques de la lignée de poulets de chair FUNAAB Alpha, de la lignée de poulettes FUNAAB Alpha et des génotypes de poulet Noiler élevés intensivement à Abeokuta. L'étude a utilisé un total de trois cents oiseaux, soit 100 oiseaux de chaque génotype avec un sexe équivalent. Les oiseaux étaient nourris ad libitum et la recherche a duré 12 semaines. Des échantillons de sang ont été prélevés et analysés pour les indices hématologiques suivants : volume de globules rouges (PCV %), hémoglobine (g/dL), nombre de globules rouges ($m\mu/mm^3$), monocytes (%), concentration de lymphocytes (%), concentration de basophiles (%), concentration d'éosinophiles (%) et concentration d'hétérophiles (%). Les résultats obtenus de l'analyse hématologique du sang ont été analysés statistiquement en utilisant la procédure

du modèle linéaire général du SAS. Les différences significatives entre les moyennes des traitements ont été comparées à l'aide de la différence significative minimale (LSD). Des nombres élevés de lymphocytes (66,94 %), d'éosinophiles (1,21 %) et de basophiles (1,33 %) ont été trouvés dans la lignée de poulets de chair FUNAAB Alpha, tandis que la lignée de poulettes FUNAAB Alpha a enregistré le nombre le plus élevé d'hétérophiles (32,46 %). Les poules mâles avaient les meilleurs comptes de basophiles et d'hétérophiles, tandis que les femelles avaient de meilleurs lymphocytes, les deux sexes ayant la même valeur pour les éosinophiles. L'évaluation générale des indices hématologiques a montré que la lignée de poulets de chair FUNAAB Alpha avait de meilleurs indices par rapport à la lignée de poulettes FUNAAB Alpha et aux génotypes Noiler. Les résultats de cette étude peuvent être utilisés comme référence pour évaluer l'état de santé normal de la lignée de poulets de chair FUNAAB Alpha, de la lignée de poulettes et des poules Noiler. Ils peuvent également guider le développement de stratégies d'amélioration des performances.

Mots-clés : Oiseaux, sang, concentration, génotype, hémoglobine, santé, sexe

Introduction

In Nigeria, chickens are the most widely distributed of all livestock species with a population of about 180 million (FAOSTAT, 2017). Being the most extensively distributed of the poultry, the domestic chicken provides humans with a stable source of animal protein in form of meat and eggs, also the demand for certified products such as meat and eggs has emerged (FAO, 2007). Nigerian indigenous chicken production is vital in the livelihood of many house-holds in the country, especially the resource poor rural farmers providing nutrition for the family (good source of protein), a small cash flow reserve for times of celebrations or need and in some areas contribute to religious ceremonies and recreation (Emuron *et al.*, 2010). The strong nature of the local chicken as manifested in their resistance to certain diseases and their ability to thrive well under harsh condition had helped in their adaptation in the tropical environment which is characterised by heat stress (Adedeji *et al.*, 2008).

Blood act as a pathological reflector of the status of exposed animals to toxicant and other conditions (Olafedehan *et al.*, 2010). Animals with relatively good blood composition are likely to show good performance (Isaac *et al.*, 2013). Laboratory tests on the blood are vital tools that help detect any deviation from normal in the animal or human body (Ogunbajo *et al.*, 2009). The examination of blood gives the opportunity to investigate the presence of several metabolites and other constituents in the

body of animals and it plays a vital role in the physiological, nutrition and pathological status of an organism (Aderemi, 2004; Doyle, 2006). Haematology results have been used in the diagnosis of many diseases as well as investigation of the extent of damage to blood (Togun *et al.*, 2007; Merck Manual, 2012).

Haematological studies are also of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment (Ovuru and Ekweozor, 2004) and so could be useful in the selection of animals that are genetically resistant to certain diseases and environmental conditions (Mmereole, 2008; Isaac *et al.*, 2013). Analysis of normal haematological parameters of chickens is therefore essential in diagnosing the various pathological and metabolic disorders and can be used as a tool to assess the health status of an individual or a flock. Changes in the haematological parameters are often used to determine various status of the body and stresses due to environmental, nutritional and/or pathological factors. According to Islam *et al.* (2004) haematological values of poultry are influenced by age, sex, breed, climate, geographical location, season, day length, time of day, nutritional status, life habit of species, present status of individual and their factors.

There is scanty information on the haematological values of the Nigerian local chicken. The little information available were derived from those scavenging on the free range whose management in terms of feeding,

housing and health/medication were neither standardized nor documented. This study was carried out to investigate packed cell volume (PCV), haemoglobin, red blood cell (RBC), white blood cell (WBC) and concentrations of basophil, eosinophil, heterophil and lymphocyte and to find out the effect of genotype and sex on haematological indices of three chicken genotypes. This will add to the existing literature on haematological studies as well as provide basis for further researches on FUNAAB Alpha broiler line, FUNAAB Alpha pullet line and Noiler chicken genotypes.

Materials and Methods

Description of experimental site

The study was carried out at the Poultry Breeding Unit of the Sustainable Livelihoods and Development Networks for Africa (SLIDEN AFRICA), along Federal University of Agriculture, Abeokuta (FUNAAB), Agbede village, off Alabata road, Ogun State, Nigeria. The area lies in the South Western part of Nigeria (latitude 7°14'N and longitude 3°27'E) and has a prevailing tropical climate with a mean annual rainfall of about 1037mm. The mean ambient temperature ranges from 28°C in December to 36°C in February with a yearly average humidity of about 82%. The vegetation represents an interphase between the tropical rainforest and derived Savannah (Latlong, 2019).

Experimental Birds

A total of 300 birds was used for this research comprising 100 each of FUNAAB Alpha broiler line, FUNAAB Alpha pullet line and Noiler. FUNAAB Alpha chicks were purchased from the Programme of Emerging Agricultural Research Leaders (PEARL) Hatchery unit of FUNAAB, while Noiler was procured from AMO Farms Sieberer Hatchery, Awe Oyo, Oyo state.

Management of experimental birds

The birds were raised intensively using the deep litter management system. Prior to the arrival of the birds, the pen house was thoroughly washed, disinfected and rested for a week to allow the smell of the disinfectant to wear out. Wood shavings were spread on the floor to help

in the absorption of moisture from their droppings and also serve as an insulator. The chicks were brooded for two weeks and subjected to the same management practices for 12 weeks. For proper record keeping, all birds were wing tagged by way of identification.

Feeding of experimental birds

Commercial feed was provided to the birds *ad libitum*. Chick starter mash containing 21% crude protein and 2840kcal ME/kg metabolizable energy was fed to the birds for the first four week, then grower mash containing 16% crude protein and 2642 kcal ME/kg metabolizable energy was fed to the birds for the remaining eight weeks. Clean water was provided for the birds *ad libitum*.

Medication and vaccination

Appropriate medications and vaccinations were given to the birds as at when due to ensure that the birds develop adequate immunity against infections and diseases, such as Newcastle diseases and Infectious bursal diseases. On arrival of the chicks, glucose was administered orally to the chicks to ease the stress of transportation. Medication and vaccination schedule was strictly adhered to and bio-security measures were practiced to prevent occurrence and spread of diseases.

Blood Collection

A total of 150 blood samples were collected from the birds, which comprise 50 randomly selected samples from each chicken genotype. Two millilitre of blood was collected from each bird using a sterilized syringe with needle through the brachial venial puncture into a 5ml ethylene di-amine-tetra-acetic acid (EDTA) to prevent blood clotting. Each bottle was labeled according to the chicken's genotype, sex and tag number. The blood samples were transferred to the laboratory for haematological analysis.

Haematological Parameters

Blood samples collected from the chickens were analysed for the following haematological indices: Packed cell volume (PCV%), Red blood cell count ($\mu\text{m}/\text{mm}^3$), Monocytes (%), Haemoglobin (g/dL), Lymphocyte's

concentration (%), Basinophils concentration (%), Eosinophil concentration (%) and Heterophil concentration (%) as described by Lamberg and Rothstein (1977).

Statistical Analysis

Results obtained from haematological analysis of the blood samples were statistically analysed. Data generated were subjected to analysis of variance using the General Linear Model Procedure of the SAS (2002) to determine the effects of genotype, sex and their interaction on the haematological parameters. Significant difference among treatment means were compared using Least Significant Difference (LSD). A probability of ($P < 0.05$) was considered to be statistically significant.

The following linear model was employed:

$$Y_{ijk} = \mu + G_i + S_j + (GS)_{ij} + e_{ijk}$$

Y_{ijk} = Observation made on the dependent variable

μ = Overall estimate of the population mean.

G_i = Fixed effect of i^{th} genotype (i = FUNAAB Alpha Broiler line,

FUNAAB Alpha Pullet line and Noiler) on the haematological parameters.

S_j = Fixed effect of j^{th} sex of chickens (j =Male, Female) on the haematological parameters.

$(GS)_{ij}$ = Fixed effect of interaction between the genotype and sex on the haematological parameters.

e_{ijk} = Random residual error.

Results

Effect of genotype on haematological parameters of three chicken genotypes at 12 weeks.

The least square means for the haematological indices of the three chicken genotypes (FUNAAB Alpha broiler line, FUNAAB Alpha pullet line and Noiler) at 12 weeks are presented in table 1. The results revealed that there were no significant ($P > 0.05$) differences among the chicken genotypes for all the haematological indices.

Table 1. Effect of genotype on haematological parameters at 12 weeks

GENOTYPE/BLOOD INDICES	FB	FP	N
PCV (%)	29.5±0.90	29.0±0.82	29.6±0.82
HB (g/dL)	10.30±0.31	9.81±0.28	10.14±0.28
RBC (10 ¹² /L)	2.77±0.10	2.57±0.09	2.58±0.09
MONO (%)	1.47±0.15	1.34±0.13	1.37±0.13
BAS (%)	1.33±0.09	1.13±0.09	1.20±0.09
EOS (%)	1.21±0.07	1.07±0.06	1.09±0.06
LYM (%)	66.94±0.73	65.86±0.65	66.77±0.66
HET (%)	30.23±0.79	32.46±0.71	30.83±0.72

FB=FUNAAB Alpha broiler line, FP= FUNAAB Alpha pullet line, N=Noiler

PCV= Packed cell volume, HB=Hemoglobin, RBC= Red blood cell, MONO= Monocytes, BAS = Basophil, EOS = Eosinophil, LYM =Lymphocyte, HET =Heterophil

Effect of sex on haematological parameters at 12 weeks.

The effect of sex on haematological parameters are presented in Table 2. The values obtained for the sex effects are insignificant ($p > 0.05$) for most of haematological parameters (red blood

cell, monocytes, basophils, eosinophils, lymphocytes and heterophils) considered at 12 weeks of age. The males had significantly ($p < 0.05$) superior indices for both PVC and hemoglobin.

Table 2. Effect of sex on haematological parameters at 12 weeks

Influence of Sex and Genotype on the Haematological Profile of FUNAAB Alpha Broiler Line, Pullet Line and Noiler Chickens Reared Intensively in Abeokuta, Nigeria

GENOTYPE/BLOOD INDICES	Male	Female
PCV (%)	30.6±0.63 ^a	28.2±0.75 ^b
HB (g/dL)	10.40±0.21 ^a	9.76±0.26 ^b
RBC (10 ¹² /L)	2.66±0.07	2.62±0.09
MONO (%)	1.50±0.10	1.29±0.12
BAS (%)	1.23±0.07	1.21±0.08
EOS (%)	1.12±0.05	1.12±0.05
LYM (%)	66.33±0.51	66.72±0.61
HET (%)	31.30±0.55	31.04±0.66

^{a, b} Means on the same row for each parameter with different superscripts are significantly different.

PCV= Packed cell volume, HB=Hemoglobin, RBC= Red blood cell, MONO= Monocytes, BAS = Basophil, EOS = Eosinophil, LYM =Lymphocyte, HET =Heterophil

Effect of interaction between genotype and sex on haematological parameters at week 12

The result of interaction between genotype and sex on haematological parameters for the three

chicken genotypes at 12 weeks are presented in Table 3. There were no significant ($P>0.05$) differences in the least squares means obtained for all the haematological indices at week 12

Table 3. **Effect of interaction between genotype and sex on haematological parameters at week 12**

INTERACTION/ BLOOD INDICES	FB*M	FB*F	FP*M	FP*F	N*M	N*F
PCV (%)	30.6±1.12	28.5±1.42	29.9±1.20	28.13±1.12	31.2±0.94	28.0±1.35
HB (g/dL)	10.41±0.38	10.18±0.47	10.20±0.41	9.41±0.38	10.6±0.32	9.67±0.46
RBC (10 ¹² /L)	2.74±0.13	2.79±0.16	2.59±0.13	2.56±0.13	2.65±0.11	2.51±0.15
MONO (%)	1.44±0.18	1.50±0.23	1.50±0.19	1.19±0.18	1.57±0.15	1.18±0.22
BAS (%)	1.56±0.12	1.10±0.15	1.00±0.13	1.25±0.12	1.13±0.10	1.27±0.14
EOS (%)	1.13±0.08	1.30±0.10	1.07±0.09	1.06±0.08	1.17±0.07	1.00±0.60
LYM (%)	65.69±0.90	68.20±1.14	66.21±0.96	65.50±0.90	67.09±0.75	66.45±1.09
HET (%)	31.25±1.00	29.20±1.23	32.36±1.04	32.57±1.00	30.30±0.81	31.36±1.18

FB*M=FUNAAB Alpha broiler line male, FB*F=FUNAAB Alpha broiler line female, FP*M= FUNAAB Alpha pullet line male, FP*F= FUNAAB Alpha pullet line female, N*M =Noiler male, N*F =Noiler female

PCV= Packed cell volume, HB=Hemoglobin, RBC= Red blood cell, MONO= Monocytes, BAS = Basophil, EOS = Eosinophil, LYM =Lymphocyte, HET =Heterophil

Discussion

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood and the use of these results in the diagnosis and monitoring of disease (Merck Manual, 2012). Its study is useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood (Togun *et al.*, 2007). Khan and Zafar, (2005) also opined that haematological indices are good indicators of the physiological status of animals.

The normal range of the haematological indices of commercial chickens has been reported to be; RBC: $2.5-3.5 \times 10^6 \mu\text{L}$, PCV: 22-35 %, WBC: $12-30 \times 10^3 \mu\text{L}$ (Bounous and Stedman, 2000), hemoglobin 6.5-13.0g/mL (Abdi-Hachsoo *et al.*, 2011), BAS: 1-3%, EOS: 0-2%, LYM: 50-62% and HET: 18-35% (Nemi, 1993). The values obtained in this study fell within the normal physiological range which indicates that the birds were healthy with normal immune system, normal metabolic rate and not anemic, it also indicates that the birds could withstand respiratory stress and can fight against bacterial and fungal infections.

Haemoglobin is the iron-containing oxygen-transporting protein in the red blood cells. The deficiency of haemoglobin in the red blood cells decreases blood oxygen-carrying capacity leading to symptoms of anemia while higher red blood cell value indicates higher oxygen carrying capacity of the blood and greater ability to resist disease infection. The function of RBC is to transport oxygen from the lungs to tissues and remove carbon dioxide from the tissues to the lung in the body via haemoglobin. Haemoglobin transport oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals (Isaac *et al.*, 2013).

The PCV is involved in the transport of oxygen and absorbed nutrients. PCV or Hematocrit value refers to percentage of whole blood comprises RBC and therefore is crude marker of red cell number (Forhead *et al.*, 2002).

High PCV value indicate dehydration or abnormal increase in red blood cell production. Low PCV indicates red blood cell loss which in turn leads to vitamin B⁶ or B¹² deficiency. This could occur based on variety of reasons like cell destruction, blood loss and failure of bone marrow production. High value of red blood cell count indicate increase in oxygen carrying cells in the bloodstream, it results from a condition that limits oxygen supply.

The RBC range of chickens is affected by sex and diet of birds (Kaminski *et al.*, 2014). Effect of sex on haematological indices of this study indicated that male chickens had more counts than the female chickens. According to Campbell (2012), this is expected since the male hormone, testosterone, is known to stimulate erythropoiesis in male animals while the female hormone, oestrogen inhibits erythropoiesis.

The lymphocytes were the most numerous and frequent WBC type in this study, followed by heterophils, basophils and eosinophils. Bounous *et al.* (2000) observed the same trend and described the lymphocytes as the most numerous WBC in chickens and turkeys. The lymphocytes, which is the agranulocytes of WBC, was within the normal range from 47.2 to 85.0% for a healthy chicken (Riddell, 2011). In comparison with other studies for haematological indices on chickens, Islam *et al.* (2004) reported similar lymphocytes values for local chicken of Bangladesh. Higher Lymphocytes and Heterophils count in this study are consistent with the findings of Riddell (2011) who also observed higher lymphocytes and heterophils in chicken. This is in contrast to the findings of Kaminski *et al.* (2014) who observed higher WBC count and lowered lymphocyte counts in Nigerian fowls.

Heterophils and eosinophils that are granulocytes of WBC were within normal range for a healthy chicken as reported by Pampori and Iqbal, (2007) and Riddell (2011). However, the heterophil count was higher and the eosinophil was lower in this study than the values reported for local chicken of Bangladesh (Islam *et al.*, 2004). The basophils are

responsible for the elaboration of histamines and heparin in circulating blood while eosinophil functions in phagocytosis. The result of this study showed a non-significant difference in all haematological indices investigated which is in line with Lala *et al.* (2016) for broiler chickens. This is in contrast with Abdulazeez *et al.* (2016) who reported significant differences in all the treatments for the haematological indices investigated. The values obtained for lymphocyte were similar to that of Lala *et al.* (2016) but contrasting for basophil.

On the effect of interaction, FUNAAB Alpha broiler line males had Heterophil count value similar to broiler chickens supplemented with humic acid as reported by Lala *et al.* (2016) while other genotypes were higher for heterophil count. The values obtained in this study for eosinophil and lymphocyte were not significant and this is consistent with Imaseun and ijeh (2017).

Basophils release enzymes to improve blood flow and prevent blood clots. Bush (1991) pointed out that low percentages of white blood cells of both eosinophil and basophil in chickens could cause poor immunity against diseases. Lala *et al.* (2017) recorded values less than 1.0 which is lesser when compared with the values obtained in this study and the disparity could be attributed to differences in genotypes used for the research, as Lala *et al.* (2017) made use of broiler chickens, while this study was carried out with improved indigenous chickens, hence, the possibility of a higher basophil count in this study. The eosinophil counts recorded in this study fell within the range of 0-2%, as reported by Nanbol *et al.* (2016) which is an indication of healthy immune system.

Haematological studies are of ecological and physiological interest. It knowledge helps to understand the relationship of blood characteristics to the environment (Ovuru and Ekweozor, 2004) and could serve as a useful tool in the selection of animals that are genetically resistant to certain diseases and

environmental conditions (Mmereole, 2008; Isaac *et al.*, 2013).

Conclusions

The Packed cell volume, Hemoglobin, Red blood cell, Monocytes, Basophil, Eosinophil, Lymphocyte, Heterophil were within the normal range reported in literature and can be used to assess physiological and health status of chickens. However, sex had effect on two of the haematological indices studied (packed cell volume and hemoglobin) with the least squares means in favour of male chickens.

Recommendations

The haematological indices obtained from this study can be used as normal reference to assess health status of FUNAAB Alpha broiler line, Pullet line and Noiler chickens. Further studies could be carried out with higher sample size.

Authors contributions

A. J. Sanda conceptualized, supervised the research and developed the manuscript; P. O. Adegoke, M. A. Ajibola and A. A. Soliu conducted the research; M. O. Sanda and A. I. Iyanda carried out the statistical analysis and proof read the manuscript.

Human and animal rights consent

All the protocols for this research were approved by the Animal Care Use Committee of the Federal University of Agriculture Abeokuta, Nigeria.

Conflict of interest

The authors declare that they do not have any conflict of interest regarding any financial, personal or other relationships with other people or organization related to the material discussed in the manuscript.

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