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HAEMATOLOGICAL PROFILE OF BIRDS PRODUCED FROM DIALLEL CROSSING OF THREE GENOTYPES OF NIGERIAN INDIGENOUS CHICKENS

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ABSTRACT

The research was designed to evaluate the variations in haematological parameters of Nigerian indigenous chickens as affected by genetic group and sex with the aim of investigating the possibility of using haematological parameters as a candidate marker in selection and improvement programs. A total of 601 chicks were hatched from a diallel crossing between three genotypes (Normal, Frizzle and Naked neck) of Nigerian indigenous chickens. A mating ratio of one male to five females was employed. At 20 weeks of age, a total of 90 blood samples were randomly collected from all the nine genetic groups. The result revealed that genetic groups significantly ($P<0.05$) affected PCV, Hb, HET, LYMP and MCH, but did not affect ($P>0.05$) WBC, pH, EOSI, MCV and MCHC. On the other hand, sex and group × sex interaction did not affect ($P>0.05$) all the haematological parameters measured. However, we observed significant genetic variations in all haematological parameters of pure and crossbred Nigerian indigenous chicken, except LYMP and pH. The result, therefore, indicates that these haematological parameters could be used in selection and improvement program for fitness and productivity in Nigerian indigenous chickens.

Keywords: Haematology, Nigerian indigenous chicken, diallel crossing.

INTRODUCTION

The Nigerian indigenous chickens have been described as small bodied, slow growing, poor feed converters and poor meat animals (Ajayi, 2010). Despite these drawbacks, the indigenous chickens play a vital role in the socio-economic life of the rural and semi-urban populace. High temperature is one of the critical stress factors in the tropical environment, which could lead to thermal stress. The naked neck and frizzle birds have been found to be thermal stress tolerant as compared to their normally feathered counterparts (Nwachukwu *et al.*, 2006). Genetic progress can be attained by selection and crossbreeding (Adebambo *et al.*, 2011). Thus, diallel crossing, a form of crossbreeding, which is a set of possible combinations between several genotypes and general populations and analysis of data from such crosses (Hayman, 1954), is a possible tool that can be employed for the improvement of these indigenous chickens. Haematological values are widely used to determine the systemic relationship and physiological and pathological adaption in animals (Shah *et al.*, 2007). Also, it could be used to develop marker-assisted technology which can be incorporated into the traditional animal breeding methodology to fast track

improvement and also to improve the efficiency of selection in breeding programs (Adedibu *et al.,* 2014). The information on haematological parameters of various genotypes of Nigerian indigenous chickens have been reported (Ladokun *et al.,* 2008; Ibrahim *et al.,* 2012; Isidahomen and Njidda, 2012) but information on various crosses of the genotypes are scanty. The study was therefore designed to evaluate the haematology of birds produced from a full diallel crossing between three genotypes of Nigerian indigenous chickens in Northern guinea savannah.

MATERIALS AND METHODS

This study was conducted at the Department of Animal Science Teaching and Research Farm, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria. The geographical location of this area is in the Northern Guinea Savannah of Nigeria which lies between latitude 11° 09' 06" N and longitude 7° 38' 35" E (Ovimaps, 2012).

A total of 601 chicks were hatched from all possible mating (Table 1) between the three genotypes of Nigerian indigenous chickens (normal, frizzle and naked neck). The foundation stock, from which the experimental birds were randomly purchased, comprised of 25 hens and five cocks each of the three genotypes. The foundation stock was fed breeder mash (2520 kcal/kg ME and 18% crude protein). The experimental birds were fed *ad libitum* with chick mash (2650 kcal/kg ME and 21% crude protein) from 0 to 8 weeks of age and grower mash (2400 kcal/kg ME and 16% crude protein) from 8 to 20 weeks of age. The birds were raised on deep litter and males were separated from the hens at 12 weeks of age. The birds were vaccinated against Newcastle, Infectious Bursal and fowl-pox diseases. Anti-coccidial medication, deworming and delousing were also carried out.

Table 1: Mating scheme of the foundation stock for the production of F_1 generation

Male		Female	Progeny
Pure cross			
Normal feathered	×	Normal feathered	N
Frizzle feathered	×	Frizzle feathered	F
Naked neck	×	Naked neck	Na
Main Cross			
Normal feathered	×	Frizzle feathered	NF
Frizzle feathered	×	Naked neck	FNa
Naked neck	×	Normal feathered	NaN
Reciprocal cross			
Frizzle feathered	×	Normal feathered	FN
Naked neck	×	Frizzle feathered	NaF

Normal feathered	×	Naked neck	NNa

Blood samples were collected at random into Ethylene Diamine Tetra Acetic acid (EDTA) bottles from 90 birds comprising of 10 randomly selected birds (five males and five females) from each genetic group. Samples were immediately taken to the laboratory for analysis in the Department of Veterinary Physiology, Ahmadu Bello University, Zaria, Nigeria. The collected blood samples were analysed for haemoglobin concentration (Hb), differential leukocyte count, packed cell volume (PCV) using the microhaematocrit method and total leukocyte count using haemocytometer method (Schalm *et al.*, 1975). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the PCV, Hb and red blood cell (RBC) values according to Jain (1993). Blood samples were collected at 20 weeks of age.

The general linear model procedure of Statistical Analysis System program (SAS Institute Inc., 2011) was used to test the effects of the genetic groups and sex. Significant means were separated using Tukey's studentized range (HSD) test. The following model was used:

$$y_{ijk} = \mu + G_i + S_j + GS_{ij} + e_{ijk}$$

Where: y_{ijk} = the observation on the bird of the j^{th} sex in the i^{th} genetic group

μ = overall mean

G_i = effect of the ith genetic group

S_j = effect of the j^{th} sex

GS_{ij} = interaction between genetic group and sex

e_{ijk} = random error

RESULTS AND DISCUSSION

Haematology of pure and crossbred Nigerian indigenous chickens

The FN, FNa, N and NaN genetic group recorded a significantly ($P < 0.05$) higher PCV values than those of F, Na, NaF and NF (Table 2). The range of PCV obtained in this study agrees with the reference value of 22.00 – 35.00% (Jain, 1993), and the range of 22.00 - 37.00% reported by Simaraks *et al.* (2004). The range was, however, not comparable to the reports of other authors (Ladokun *et al.*, 2008; Isidahomen and Njidda, 2012) who reported values ranging from 32.60 to 48.80%. The discrepancy in PCV values may be due to differences in environment and method of trait determination.

For Hb, the highest mean value was obtained in the FN group and was only statistically ($P < 0.05$) different from NaF (Table 2). The range obtained for Hb in this study was within the reference values of 7.00 – 13.00 g/dl (Jain, 1993) but lower than the range of 11.60 to 13.68 g/dl obtained by Ibrahim *et al.* (2012). This discrepancy in Hb values could be due to the source of experimental birds, where such birds might have undergone previous selection and improvement, unlike birds that were purchased fresh from the market as a base population which was the case in this study.

Table 2: Means and Coefficient of Variation (CV %) of haematological parameters across the various genetic groups of Nigerian indigenous chickens

Parameters	F	FN	FNa	N	Na	NaF	NaN	NF	NNa	SEM	CV %	LOS
NO (90)	10	10	10	10	10	10	10	10	10			
PCV (%)	26.70^{b}	30.56^{a}	30.90^{a}	31.33^{a}	25.80^{b}	24.20^{bc}	31.33^{a}	26.70^{b}	29.11^{ab}	1.03	15.19	*
Hb (g/dl)	8.66^{ab}	10.14^{a}	9.34^{ab}	8.87^{ab}	8.56^{ab}	8.03^{b}	10.41^{a}	8.86^{ab}	9.69^{a}	0.67	19.81	*
WBC ($x10^{3}$/ml)	1.72	1.68	1.48	2.28	2.07	2.12	2.73	1.86	1.72	0.50	51.54	NS
HET (%)	10.90^{a}	10.00^{ab}	12.00^{a}	8.30^{ab}	8.90^{ab}	8.30^{ab}	7.44^{b}	8.60^{ab}	10.00^{ab}	0.94	37.53	*
LYMP (%)	88.40^{ab}	88.22^{ab}	86.80^{b}	91.10^{a}	90.00^{a}	90.80^{a}	91.44^{a}	89.60^{ab}	89.44^{ab}	0.94	3.91	*
pH	7.71	8.03	7.95	7.89	7.61	8.00	7.97	7.89	8.03	0.31	4.85	NS
EOSI (%)	2.33	3.00	2.80	2.00	2.75	1.50	2.50	2.6	1.67	0.57	55.14	NS
MCV (fl)	60.28	60.05	60.10	60.11	60.27	60.00	60.13	60.40	60.10	0.32	60.17	NS
MCH (pg)	23.97^{b}	19.94^{c}	19.97^{c}	28.03^{a}	24.44^{b}	19.89^{c}	19.98^{c}	20.04^{c}	20.00^{c}	1.49	40.34	*
MCHC (%)	33.18	33.20	33.21	33.22	33.18	33.15	33.23	33.18	33.28	0.17	33.20	NS

[abc] Means with different superscripts on the same row are significantly different (P<0.05); PCV = Packed Cell Volume; Hb = Haemoglobin; WBC = White blood Cell; HET = Heterophil; LYMP = Lymphocyte; EOSI = Eosinophils; MCV = Mean Corpuscular Volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; F= Frizzled; FN= Frizzled-Normal; FNa= Frizzled-Naked; N= Normal; Na= Naked neck; NaF= Naked-Frizzled; NaN= Naked-Normal; NF= Normal-Frizzled and NNa= Normal-Naked; SEM= standard error of means; CV= Coefficient of Variation; LOS= level of significance; *= (P<0.05); NS= (P>0.05); NO=number of observation

The N group recorded a significantly (P< 0.05) higher mean value of MCH than the values recorded by birds of other genetic groups (Table 2). The MCH range obtained in this study was within the range (22.97 – 27.72 pg) reported in Fayoumi chicken breed (Islam *et al.,* 2004) but lower than the reference range (33.00 – 47.00 pg) (Jain, 1993). The difference in values of MCH may be as a result of the difference in age of experimental birds as MCH value decreases gradually with advancement in age (Islam *et al.,* 2004). The observed differences across the genetic groups and higher PCV, Hb and MCH in N than F and Na genotypes agree with previous studies (Chineke *et al.,* 2006; Peters *et al.,* 2011). Variation in erythrocytes is attributed to increased body temperature, due to increased ambient temperature, respiration, respiratory water loss and oxygen consumption of birds, which increases oxygen intake and its partial pressure in the blood (Brackenbury *et al.,* 1981a; Brackenbury *et al.,* 1981b; Donkoh, 1989). Increase in partial pressure decreases the production of red blood cells and consequently reduces the mean value of circulating erythrocytes. These mechanisms may partly explain the lower values of PCV, Hb and MCH observed in F, Na and NaF birds in this study. This may be the most likely mechanism by which the expression of naked neck and frizzle genotypes produce thermoregulatory effects.

HET count shows that FNa group had the highest mean and it only significantly (P < 0.05) differed from NaN genetic group. FN recorded the highest value for LYMP count which was only significantly (P < 0.05) higher than the values obtained by FNa genetic group (Table 2). Heterophil (HET) counts fell considerably below the reference value of 15.00 - 40.00% (Jain 1993) and the reported value of 22.00 to 25.40% for Thai indigenous chickens (Simaraks *et al.,* 2004). Furthermore, reported reference value for lymphocyte count was between 45 - 70% (Jain, 1993) and 54 - 73% (Simaraks *et al.,* 2004). Observed values in this study did not agree with these findings and were far higher than reported ranges. These discrepancies may be as a result of HET being the most abundant WBC in the peripheral blood of most species of birds, whereas in some avian species lymphocytes are the predominant cell type in the differential count (Schmidt *et al.,* 2009). The indigenous chickens in our study had lymphocytes as the most abundant WBC in the peripheral blood, and our results agree with those of Wakenell (2010) who reported that lymphocytes are the predominant WBC in the peripheral blood of chickens and turkeys. Eosinophil ranges were comparable to the reference range of 1.50 - 6.00% (Jain, 1993). HET and LYMP counts are bio-markers for stress and immune functions (OpenStax College, 2013). Thus, the differences observed across genetic group may indicate the different genetic potential for disease resistance.

Eosinophil (EOSI), White Blood Cell (WBC), pH, MCV and MCHC were not affected (P > 0.05) by genetic groups (Table 2). The coefficient of variation for these parameters ranged from 3.91% (LYMP) to 60.17% (MCV) (Table 2; P < 0.05). The MCV values obtained in this study were lower compared to ranges of 75.98 – 95.27fl (Ladokun *et al.,* 2008) and 134.02 - 141.91fl (Isidahomen and Njidda, 2012). MCHC values obtained were consistent with other reports (Ladokun *et al.,* 2008; Isidahomen and Njidda, 2012) and were also within the reference range of 26.00 – 35.00% (Jain, 1993) but were higher than the values in another study (Simaraks *et al.,* 2004). The lack of significant difference in these traits among groups was consistent with a previous report (Peters *et al.,* 2011) but contrasted with the observation in Nigerian indigenous chicken (Isidahomen and Njidda, 2012). Furthermore, MCV and MCHC are bio-markers for detecting anaemia (Coles, 2007). Therefore, the insignificant difference in MCV and MCHC across genetic group may be an indication of equal genetic potential against anaemia or that the birds

were not suffering from anaemia. The coefficient of variation for haematological parameters was highest at MCV indicating that MCV varied more across genetic groups.

Effect of sex on haematology of Nigerian indigenous chickens

Table 3 shows the least square means (± standard error) and coefficient of variation for haematological parameters as affected by sex. Sex did not affect (P > 0.05) all the studied haematological parameters. This result is consistent with those of Schmidt *et al.* (2007) who reported that sex did not affect RBC, WBC and Hb. Similarly, sex did not affect PCV, Hb, WBC, RBC, MCH and MCHC in yellow-legged gulls (Antonio Garcia *et al.*, 2010) and helmeted guinea fowl (Adedibu *et al.*, 2014). However, other studies in indigenous chickens (Peters *et al.*, 2011; Abdi-Hachesoo *et al.*, 2013) and helmeted guinea fowl (Okoro *et al.*, 2011) found that sex affected haematological parameters. These authors attributed higher PCV, RBC and Hb in males than females to the increased courting of the females by the males during their breeding season which was when they bled. Reproductive hormones, such as androgen and oestrogen, depress erythropoiesis (Oke *et al.*, 2003), whereas androgen and thyroxin stimulate erythropoiesis (Herbert *et al.*, 1989). Thus, the insignificant effect of sex on haematological parameters in this study may be as a result of non-mating and separation of males from the females.

Table 3: Least Square Means (±Standard Error) and Coefficient of Variation (CV %) of haematological parameters of male and female Nigerian indigenous chickens

Parameters	Male	Female	SEM	CV (%)	LOS
PCV (%)	28.22±0.67	27.80±0.63	1.03	15.19	NS
Hb (g/dl)	9.18±0.29	9.19±0.27	0.67	19.81	NS
WBC ($x10^3$/ml)	1.92±0.16	1.93±0.15	0.50	51.54	NS
HET (%)	10.10±0.56	8.90±0.53	0.94	37.53	NS
LYMP (%)	89.09±0.56	89.72±0.53	0.94	3.91	NS
pH	7.94±0.06	7.85±0.06	0.31	4.85	NS
EOSI (%)	2.34±0.37	2.33±0.31	0.57	55.14	NS
MCV (fl)	60.19±0.07	60.14±0.06	0.32	60.17	NS
MCH (pg)	22.94±1.39	20.81±1.30	1.49	40.34	NS
MCHC (%)	33.20±0.02	33.21±0.02	0.17	33.2	NS

PCV = Packed Cell Volume; Hb = Haemoglobin; WBC = White blood Cell; HET = Heterophil; LYMP = Lymphocyte; EOSI = Eosinophils; MCV = Mean Corpuscular Volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; SEM= standard error of means; CV= Coefficient of Variation; LOS= level of significance; NS= (P>0.05)

Effect of sex and genetic group on haematology of Nigerian indigenous chickens

Tables 4a and b present the least square means (± standard error) for haematological parameters as affected by the interaction between genetic group and sex. There was also no significant (P > 0.05) genetic group × sex interaction on at all haematological parameters studied. The result of no

significant interaction between genetic group and sex on haematological parameters in Nigerian indigenous chicken indicates that these parameters were not dependent on the interaction between genotype and sex. This result is in line with a previous report of no significant effect of genotype × sex interaction on all haematological parameters except Hb and RBC (Peters *et al.,* 2011).

Table 4a: Least Square Means (± Standard Error) for haematological parameters as affected by genetic group × sex interaction of Nigerian indigenous chickens

Groups	Sex	NO	PCV (%)	Hb (g/dl)	WBC ($x10^3$/ml)	HET (%)	LYMP (%)
F	FM	5	27.60±1.90	8.76±0.81	1.80±0.45	11.80±1.58	88.00±1.56
F	M	5	25.80±1.90	8.56±0.81	1.64±0.45	10.00±1.58	88.80±1.56
FN	FM	5	30.00±1.73	9.95±0.74	1.85±0.41	8.67±1.44	89.33±1.43
FN	M	5	31.67±2.45	10.53±1.05	1.35±0.58	12.67±2.03	86.00±2.02
FNa	FM	5	28.00±1.90	9.30±0.81	1.68±0.45	9.20±1.58	89.20±1.56
FNa	M	5	33.80±1.90	9.39±0.81	1.29±0.45	14.80±1.58	84.40±1.56
N	FM	5	27.60±1.90	9.18±0.81	2.55±0.45	7.40±1.58	92.00±1.56
N	M	5	25.80±1.90	8.56±0.81	2.02±0.45	9.20±1.58	90.20±1.56
NF	FM	5	26.67±1.73	8.85±0.74	1.95±0.41	7.33±1.44	90.50±1.43
NF	M	5	26.75±2.12	8.88±0.91	1.72±0.51	10.50±1.76	88.25±1.75
NNa	FM	5	29.40±1.90	9.78±0.81	1.65±0.45	10.60±1.58	89.00±1.56
NNa	M	5	28.75±2.12	9.58±0.91	1.80±0.51	9.25±1.76	90.00±1.75
Na	FM	5	27.60±1.90	9.16±0.81	2.16±0.45	8.60±1.58	89.20±1.56
Na	M	5	24.00±1.90	7.96±0.91	1.98±0.45	9.20±1.58	90.80±1.56
NaF	FM	5	23.17±1.73	7.68±0.74	2.01±0.41	8.50±1.44	90.67±1.43
NaF	M	5	25.75±2.12	8.55±0.91	2.29±0.51	8.00±1.76	91.00±1.75
NaN	FM	5	30.00±2.45	10.00±1.05	1.72±0.58	8.00±2.03	89.67±2.01
NaN	M	5	32.00±1.73	10.62±0.74	3.23±0.41	7.17±1.44	92.33±1.43
SEM			2.26	1.47	1.10	2.10	2.10
LOS			NS	NS	NS	NS	NS

M= Male; FM= Female; PCV= Packed Cell Volume; Hb= Haemoglobin; WBC= White Blood Cell; HET= Heterophil; LYMP= Lymphocyte; F= Frizzled; FN= Frizzled-Normal; FNa= Frizzled-Naked; N= Normal; Na= Naked neck; NaF= Naked-Frizzled; NaN= Naked-Normal; NF= Normal-Frizzled and NNa= Normal-Naked; SEM= standard error of means; FN= Frizzled-Normal; FNa= Frizzled-Naked; N= Normal; Na= Naked neck; NaF= Naked-Frizzled; NaN= Naked-Normal; NF= Normal-Frizzled and NNa= Normal-Naked; SEM= standard error of means; LOS= level of significance; NS= (P>0.05); NO= number of observations

Table 4b: Least Square Means (± Standard Error) for haematological parameters as affected by genetic group × sex interaction of Nigerian indigenous chickens

Groups	Sex	NO	pH	EOSI (%)	MCV (fl)	MCH (pg)	MCHC (%)
F	FM	5	7.40± 0.17	1.00± na	60.53±0.18	20.09±3.95	33.18±0.05
F	M	5	8.02±0.17	3.00±1.41	60.03±0.18	27.87±3.95	33.18±0.05
FN	FM	5	8.02±0.16	3.67±2.08	60.20±0.17	19.97±3.61	33.17±0.05
FN	M	5	8.07±0.22	2.00±0.00	59.76±0.18	19.88±5.10	33.27±0.05
FNa	FM	5	7.92±0.17	2.67±1.15	60.00±0.18	19.95±3.95	33.20±0.06
FNa	M	5	7.98±0.17	3.00±1.41	60.20±0.18	19.99±3.95	33.21±0.05
N	FM	5	7.92±0.17	1.50±0.71	59.99±0.18	27.95±3.95	33.26±0.05
N	M	5	7.86±0.17	3.00±na	60.24±0.18	28.11±3.95	33.17±0.05
NF	FM	5	7.98±0.17	2.60±1.82	60.36±0.17	20.04±3.61	33.19±0.04
NF	M	5	7.73±0.19	3.00±na	60.45±0.20	20.06±4.42	33.17±0.05
NNa	FM	5	7.98±0.17	2.00±na	59.97±0.20	19.94±4.42	33.28±0.05
NNa	M	5	8.10±0.19	1.50±0.71	60.29±0.24	20.07±5.10	33.29±0.06
Na	FM	5	7.52±0.17	2.75±0.96	60.29±0.23	20.00±3.95	33.17±0.05
Na	M	5	7.70±0.17	na	60.25±0.20	30.00±4.42	33.20±0.05
NaF	FM	5	7.97±0.16	1.25±0.50	59.81±0.18	19.80±3.95	33.10±0.05
NaF	M	5	8.05±0.19	2.00±0.00	60.24±0.20	20.01±4.42	33.21±0.05
NaN	FM	5	7.93±0.22	3.50±2.12	60.00±0.24	20.00±5.10	33.33±0.06
NaN	M	5	7.98±0.16	1.50±0.71	60.20±0.17	19.97±3.61	33.18±0.04
SEM			0.68	0.84	0.69	3.20	0.37
LOS			NS	NS	NS	NS	NS

M= Male; FM= Female; PCV= Packed Cell Volume; Hb= Haemoglobin; WBC= White Blood Cell; HET= Heterophil; LYMP= Lymphocyte; F= Frizzled; FN= Frizzled-Normal; FNa= Frizzled-Naked; N= Normal; Na= Naked neck; NaF= Naked-Frizzled; NaN= Naked-Normal; NF= Normal-Frizzled and NNa= Normal-Naked; FN= Frizzled-Normal; FNa= Frizzled-Naked; N= Normal; Na= Naked neck; NaF= Naked-Frizzled; NaN= Naked-Normal; NF= Normal-Frizzled and NNa= Normal-Naked; SEM= standard error of means; LOS= level of significance; NS= (P>0.05); NO= number of observations

CONCLUSION

Thus, enormous genetic variations exist in some haematological parameters of pure and crossbred Nigerian indigenous chicken. These observed variations may be incorporated into breeding programs for genetic improvement of Nigerian indigenous chickens for improved productivity and fitness traits.

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