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ORIGINAL RESEARCH

Haematological studies on frizzled and naked neck genotypes of Nigerian native chickens

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Abstract Variation in haematological parameters of Nigerian native chickens was studied using 60 clinically normal frizzle-feathered, naked-neck, and normal-feathered native chickens. These included red blood cell count, haemoglobin, packed cell volume, white blood cell count, mean corpuscular volume, mean corpuscular haemoglobin concentration, serum glucose, urea, cholesterol, albumin, globulin and creatinine. Normal-feathered birds had higher ($p<0.05$) mean values compared to frizzled and native neck genotypes except for albumin, red blood and white blood cells, and mean cell haemoglobin concentration. Males generally had higher mean values than their female counterparts across all genotypes. Correlation coefficients among the parameters were significant ($p<0.001$) with r values ranging from 0.26 between red blood cell and mean corpuscular haemoglobin to 0.92 between red blood cell and cholesterol. Sufficient genetic variation therefore exists for haematological parameters among Nigerian native chickens that may represent indicator traits for further study. However, the application of molecular tools will provide better understanding and application of these differences.

Keywords Frizzle-feathered · Naked neck · Haematology · Native chickens · Nigeria

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Introduction

Research on indigenous chickens in Nigeria has increased recently especially on comparative studies of their growth and reproduction (Adebambo 2004; Yakubu et al. 2009; Peters et al. 2010). However, selection in local breeds has been targeted more at adaptation to harsh environments and resistance to diseases rather than enhanced production (Minga et al. 2004). Trail and D'leteren (1992) had earlier reported that tropical breeds were well adapted to tropical conditions and if well-exploited and conserved, are well able to exhibit their potentials since their properties are genetically acquired and nurtured by nature. Information about haematological parameters and blood biochemical indices are very essential in diagnosing various pathological and metabolic disorders in chickens. Haematological changes are routinely used to determine the health status of the body and to determine stresses due to environment, nutritional and pathological factors.

In spite of previously unfavourable reports about these indigenous birds such as poor growth rate, poor body conformation, small egg size (Ibe 1993; Nwagu and Nwosu 1994), and poor reproductive efficiency of both sexes (Egbunike and Nkanga 1999; Gbadamosi and Egbunike 1999), recent efforts have highlighted the need to focus research on their genotypes with the goal of exploiting their genetic potential (Horst 1989; Hoffmann 2005; Peters et al. 2005, 2007 and 2008b).

The Nigerian native chickens (NNC), like most indigenous chickens found around the world, are well adapted to their local environmental and climatic conditions (FAO 2010). They have been reported by Ponsuksili et al. (1996) and Wimmers et al. (2000) to contain a highly conserved genetic system, with high levels of heterozygosity, which may provide biological material for the design of genetic stocks with improved adaptability and productivity. The adaptive potential of the native chickens have been attributed to the possession of major genes of frizzling (*Ff*) and naked neck (Na^{-}), both of which have been implicated in heat tolerance (Horst 1983; Yunis and Cahaner 1999). The naked neck gene reduces feather coverage in chickens by 20% and 40% in heterozygous (*Nana*) and homozygous (*NaNa*) states, respectively (Deeb and Cahaner 2001). Garces et al. (2001) reported that *Ff* chickens showed a reduced density of feather coverage, which provides some heat tolerance to egg-type chickens. The NNC represent valuable resources for local poultry development because their extensive genetic diversity allows for rearing of these chickens under varied environmental conditions, providing a range of products and functions (Yakubu et al. 2009).

The potential of the native chicken has not been fully exploited since there are still growing reports about existing or potential levels of productivity of the local breeds managed under extensive and intensive systems (Mathur et al. 1989; Peters et al. 2002; 2005, 2007, 2008a, and 2008b). Even though several reports on performance of the local chickens in Nigeria have been reported (Ebozoje and Ikeobi 1995; Ikeobi et al. 1996, Adebambo et al. 1999), there are no studies on characterization of NNC based on haematological parameters. Since assessment of variation in haematological parameters in NNC would further help our understanding of diversity, this study was aimed to uncover variation in these parameters of Nigerian native chickens.

Materials and methods

Experimental site and birds

Research was conducted at the Teaching and Research Farm, University of Agriculture, Abeokuta (UNAAB), (latitude 7°10N and longitude 3° 2E) in Southwestern Nigeria. Ambient temperatures range from 28°C in December to 36°C in February with relative humidity of 82% in an area intermediate between tropical rainforest and the derived savannah (Ilori et al. 2010).

Experimental birds

Sixty native chickens comprising 20 each from frizzle-feathered, naked-neck and normal-feathered birds were used for the study. This research was approved by the Institutional Animal Use and Care Committee of the University of Agriculture, Abeokuta, Ogun State, Nigeria.

Egg collection

Eggs were collected from birds maintained at the Poultry Breeding Unit of UNAAB. Females birds were artificially inseminated with 0.1 mL of fresh semen, and fertile eggs were collected once every morning. All eggs collected were first maintained at a temperature of 10–14°C and 75–85% relative humidity for a few days prior to incubation. Eggs were screened and only those without cracks and deformed shapes were sent to the hatchery for incubation.

Management of the birds

Chicks from each genetic group were differentiated and individually identified by wing tagging (sexes combined). The chicks were also vaccinated and medicated routinely. The chicks were transferred to a previously disinfected brooder house where a standard management procedure was strictly adhered to as described in Peters et al. (2005). Brooding was done for 4 weeks during which Neoceryl was administered to reduce stress.

Feeds and feeding

The chicks were fed on a commercial starter ration that provided 20% crude protein and 2,600 kcal/kg ME from 0 to 8 weeks of age. Thereafter, they were fed with commercial growers ration that supplied 17% crude protein and 2850 kcal/kg ME from 8 weeks of age to the end of the experiment. Feed and water were provided ad libitum throughout the experimental period.

Haematological and biochemical parameters

Blood samples from the three genetic groups were randomly collected at 20 weeks of age from 20 males and females. About 3 ml of blood was collected from the right basilica vein of the wing from each selected bird, divided into two parts; one part consisted of 1 ml in EDTA as an

Table 1 Least square means for haematological and biochemical parameters as affected by genotype

Parameters	*N*	Genotypes		
		Normal	Frizzled	Naked neck
Packed cell volume (%)	20	35.60±0.38a	33.85±0.95b	34.65±1.27ab
Haemoglobin (%)	20	11.98±0.12a	11.42±0.31b	11.55±0.41b
Red blood cell (mμ/mm^3)	20	3.92±0.07	3.79±0.11	3.91±0.13
White blood cell (no/mm^3)	20	5,560.00±37.28	5,590.33±36.92	5,660.00±52.52
Glucose (mg/dl)	20	66.75±0.68a	64.25±1.81b	65.05±2.57ab
Albumin (mg/dl)	20	33.30±0.41	32.85±0.90	33.30±0.10
Globulin (mg/dl)	20	22.55±0.25a	21.50±0.60b	22.30±1.13ab
Urea (mg/dl)	20	31.90±0.30a	30.20±0.87b	31.10±1.13ab
Creatinine (mg/dl)	20	1.48±0.02a	1.39±0.04b	1.39±0.05b
Cholesterol (mg/dl)	20	163.80±1.84a	156.60±4.73b	160.30±5.57ab
Mean cell volume (fl)	20	91.15±1.32	89.38±0.95	88.70±1.45
Mean cell haemoglobin (pg)	20	30.67±0.36a	30.14±0.26ab	29.56±0.43b
Mean cell Hb conc (gm %)	20	33.69±0.28	33.75±0.27	33.41±0.46

Means within the row having different lowercase letters are significantly different ($P<0.05$)

anticoagulant, and the rest were allowed to clot to obtain sera (Samour et al. 2010). The anti-coagulated blood was used to determine red blood cell (RBC) count and white blood cell (WBC) count using a manual haemocytometer. Packed cell volume (PCV) was determined using the haematocrit centrifuge method and haemoglobin (Hb) concentration by the cyanmethaemoglobin method. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculations from the RBC, Hb and PCV values as described by Jain (1986). Sera were separated from the clotted blood following centrifugation from which serum metabolites (serum glucose, urea, cholesterol, albumin, globulin and creatinine) were determined by spectrophotometry. Values of all these parameters were analysed using the routine laboratory procedures of Dacie and Lewis (1991).

Data analysis

All parameters were analysed with the Statistical Analysis System (SAS 1999) using a two-way analysis of variance. The model below accommodated the effects of genotype, sex and interaction between genotype and sex.

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

Where:

$Y_{ijk=}$ observed value of the measurable traits of j^{th} sex in the i^{th} genotype.

Table 2 Least square means for haematological and biochemical parameters as affected by sex

Parameters	Sex			
	N	Male	*N*	Female
Packed cell volume (%)	30	38.07±0.41a	30	31.33±0.51b
Haemoglobin (%)	30	12.73±0.13a	30	10.56±0.17b
Red blood cell (mμ/mm^3)	30	4.26±0.05a	30	3.49±0.05b
White blood cell (no/mm^3)	30	5633.33±38.46	30	5573.33±31.42
Glucose (mg/dl)	30	71.87±0.77a	30	58.83±1.03b
Albumin (mg/dl)	30	36.27±0.38a	30	30.03±0.39b
Globulin (mg/dl)	30	24.27±0.30a	30	19.97±0.32b
Urea (mg/dl)	30	33.90±0.41a	30	28.23±0.49b
Creatinine (mg/dl)	30	1.54±0.02a	30	1.29±0.02b
Cholesterol (mg/dl)	30	176.23±1.71a	30	144.23±2.20b
Mean cell volume (fl)	30	89.48±0.82	30	90.01±1.22
Mean cell haemoglobin (MCH) (pg)	30	29.92±0.21	30	30.33±0.36
Mean cell haemoglobin concentration (gm %)	30	33.51±0.36	30	33.73±0.18

Means within the row having different lowercase letters are significantly different ($P<0.05$)

μ overall mean
G_i effect of i^{th} genotype (i=1,2,3)
S_j effect of j^{th} sex (j=1, 2)
e_{ijk} random residual error.

Duncan's multiple range of test was used to separate means that differed significantly. Pearson correlation (r) was used to ascertain relationships between measurable traits.

Results

The least square means and standard errors of means for haematological parameters and biochemical indices as influenced by major genes and sex are presented in Tables 1 and 2, respectively. Significant differences ($p<0.05$) among chicken genotypes were observed for PCV, globulin, urea, creatinine and cholesterol. Haemoglobin (Hb) was highly significantly ($p<0.01$) different among chicken genotypes, with highest values recorded in normal-feathered chickens (11.98±0.12%) followed by the naked neck birds (11.55± 0.41%) and finally the frizzled birds (11.42±0.31%). There were no differences in the other haematological parameters and biochemical indices ($p>0.05$).

Sex differences were highly significant ($p<0.01$) for Hb, with males having mean values of 12.73±0.13% compared to females (10.56±0.17%). In addition, PCV, RBC, glucose, albumin, globulin, urea, creatinine, and cholesterol exhibited sex differences ($p<0.01$) but not MCV, MCH and MCHC ($p>0.05$). Significantly higher values for these parameters were obtained for the male chickens compared to the female ones.

Least square means for interactions between genotype and sex are presented in Tables 3 and 4. Parameters such as PCV, WBC, MCV, MCH and MCHC were not significantly ($p>0.05$) influenced by the interaction effect of genotype and sex. However, Hb, RBC, glucose, albumin, globulin, urea, creatinine, and cholesterol had very highly significant genotype and sex interaction effects ($p<0.001$), with the highest red blood cell value of 4.46±0.08 mμ/mm^3 recorded for the naked-neck native cocks followed by frizzle-feathered males (4.20±0.08 mμ/mm^3), normal-feathered males (4.12± 0.03 mμ/mm^3), normal-feathered females (3.72±0.10 mμ/ mm^3) and the least values of 3.38±0.06 mμ/mm^3 and 3.36± 0.05 mμ/mm^3 were recorded for frizzle-feathered females and naked-neck females, respectively.

The correlation coefficients among the studied parameters are presented in Table 5 and showed that correlation between some parameters studied were weak and non-significant ($p<0.05$) while some parameters showed negative relationships. Very highly significant ($P<0.001$) and strong associations were observed between most parameters

Table 3 Least square means for haematological and biochemical parameters as influenced by interactive effect of genotype and sex

Genotype	Sex	Parameters							
		PCV	Hb	RBC	WBC	Glucose	Albumin	Globulin	Urea
Normal	Male	36.7±0.33	12.32±0.14b	4.12±0.03b	5,560±49.89	68.30±0.75c	34.7±0.21b	23.1±0.23b	32.6±0.31bc
	Female	34.50±0.48	11.65±0.14^c	3.72±0.10c	5,560±58.12	65.20±0.93d	31.9±0.93c	22.0±0.37c	31.2±0.42c
Frizzled	Male	37.7±0.54	12.7±0.16ab	4.20±0.08b	5,580±51.64	71.80±0.76b	36.5±0.40a	23.9±0.38b	33.6±0.56b
	Female	30.0±0.52	10.13±0.17d	3.38±0.06d	5,600±51.64	56.70±0.75e	29.2±0.55d	19.1±0.28d	26.8±0.55d
Naked neck	Male	39.80±0.80	13.18±0.28a	4.46±0.08a	5,760±77.75	75.50±1.34a	37.6±0.85a	25.8±0.51a	35.5±0.85a
	Female	29.50±0.56	9.91±0.19d	3.36±0.05d	5,560±58.12	54.60±1.33e	29.0±0.56d	18.8±0.32d	26.7±0.58d

Means within the same row having different lowercase letters are significantly different ($P<0.05$)
PCV packed cell volume, *Hb* haemoglobin, *RBC* red blood cell, *WBC* white blood cell

Table 4 Least square means for haematological and biochemical parameters as influenced by interaction between genotype and sex

Genotype	Sex	Parameters				
		Creatinine	Cholesterol	MCV	MCH	MCHC
Normal	Male	1.51±0.03ab	169.6±1.42b	89.11±0.87	29.91±0.34	33.58±0.38
	Female	1.45±0.02b	158.0±2.21c	93.20±2.39	31.43±0.55	33.80±0.43
Frizzled	Male	1.54±0.03a	176.0±2.31ab	89.94±1.41	30.28±0.28	33.72±0.45
	Female	1.24±0.12c	137.2±2.32d	88.83±1.34	30.00±0.45	33.78±0.34
Naked neck	Male	1.58±0.02a	183.1±3.27a	89.40±1.94	29.57±0.47	33.23±0.94
	Female	1.19±0.03c	137.5±2.21d	87.99±2.25	29.55±0.74	33.59±0.12

Means within the same row having different lowercase letters are significantly different ($P<0.05$)

MCV mean corpuscular haemoglobin, *MCH* mean corpuscular haemoglobin, *MCHC* mean corpuscular haemoglobin concentration

with r values ranging from 0.92 between Hb and cholesterol (Chol), Hb and glucose (Glu), PCV and Glu to 0.77 between albumin (Alb) and creatinine (Cret). However, correlation between PCV and white blood cell WBC (r=0.16), PCV and MCV (r=0.26) and r=0.26 between RBC and MCH were low but still significant ($P<0.05$).

Discussion

Variation in haematological parameters among chicken genotypes with the exception of MCV agree with Harper and Lowe (1998), but the values for other parameters in this study were higher than previously reported for exotic chickens in Nigeria (Iheukwumere and Herbert 2003; Talebi et al. 2005). But the present results are in agreement with Sturkie (1986), Uko and Ataja (1996), Mushi et al. (1999) and Iheukwumere et al. (2006). Reports from these authors indicated variability in haematological profiles among different breeds. While haematograms of these strains remained almost the same, there are differences in leukograms between the exotic strains and the indigenous chickens as equally reported by Islam et al. (2004). The high value of WBC obtained from this investigation may simply demonstrate the immunological status of the local chicken.

On the other hand, low WBC values in exotic strains may explain the higher level of susceptibility to avian disease-causing agents compared with indigenous chickens that are relatively tolerant to many poultry diseases (Campbell and Coles 1986). These authors reported that since the WBC in avian species serves a phagocytic role as in mammals, they are mainly responsible for defence of the body against infections. Also, Shaniko (2003) reported that leukocyte counts as well as heterophiles and lymphocytes ratio were used as indicators of stress responses and sensitive bio-makers that are crucial to immune functions. Previous reports stated that PCV, Hb and MCH are major indices for evaluating circulating avian erythrocytes, and are very significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals (Awodi et al. 2005; Chineke et al. 2006). The higher values for PCV, Hb, and MCH values in this investigation in normal feathering birds compared to naked neck and frizzled birds probably reflect inherent genetic differences in agreement with the findings of Agaie and Uko (1998). They reported variation in erythrocyte values due to season and species. In other reports, Oladele et al. (2001), Iheukwumere et al. (2002) and Adejumo (2004), attributed low values of PCV and Hb to poor nutrition especially protein deficiency whereas Ikhimioya et al. (2002) attributed low erythrocyte values to system of management. These reasons cannot be advanced for variations found in our studies where all birds were exposed to common environment for feeding and management. Therefore, the only logical factor implicated is chicken genotype. Chineke et al. (2006) reported that high PCV reading indicated either an increase in number of circulating RBC or reduction in circulating plasma volume. Reports by Brackenbury et al. (1981a, b) and Donkoh (1989) earlier showed that variation in erythrocytes can be attributed to increased body temperature from increase ambient temperature, respiration, respiratory water loss and oxygen consumption of birds, which in turn increase oxygen intake and partial pressure of oxygen in the blood. 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 that are associated with naked-neck and frizzle-feathered birds in this study. This may be the most likely mechanism by which the expression of naked neck and frizzle genotypes produce thermoregulatory effects. The lower mean values of PCV, Hb and MCH were still within normal ranges as reported by Wayne and Huxtable (1988) and Campbell (1994). These authors reported normal avian PCV as ranging from 35% to 50%, and a PCV less than 35% may

Table 5 Pearson correlations among haematological and biochemical parameters

	PCV	Hb	RBC	WBC	Glu	Alb	Glo	Urea	Creat	Chol	MCV	MCH	MCHC
PCV	1.00												
Hb	0.91***	1.00											
RBC	0.88***	0.15	1.00										
WBC	0.16*	0.15	0.17	1.00									
Glu	0.92***	0.92	0.88***	0.27	1.00								
Alb	0.85***	0.86	0.84***	0.25	0.83***	1.00							
Glo	0.89***	0.86	0.90***	0.23	0.89***	0.80***	1.00						
Urea	0.87***	0.88	0.87***	0.20	0.85***	0.81***	0.91***	1.00					
Creat	0.90***	0.92***	0.84***	0.10	0.89***	0.77***	0.85***	0.85***	1.00				
Chol	0.89***	0.92***	0.92***	0.22	0.89***	0.87***	0.91***	0.86***	0.90***	1.00			
MCV	0.26*	0.03	0.23	−0.14	0.10	0.04	−0.02	0.01	0.14	−0.005	1.00		
MCH	0.40	0.16	0.26*	−0.05	0.05	0.02	−0.02	−0.02	0.13	−0.02	0.68***	1.00	
MCHC	−0.27	0.16	0.84	−0.05	−0.04	−0.02	−0.11	−0.02	−0.02	−0.06	−0.54	0.25	1.00

PCV packed cell volume, *Hb* haemoglobin, *WBC* white blood cell, *RBC* red blood cell, *Glu* glucose, *Alb* albumin, *Glo* globulin, *Creat* creatinine, *Chol* cholesterol, *MCV* mean cell volume, *MCH* mean cell haemoglobin, *MCHC* mean cell haemoglobin concentration

*$P<0.05$, **$P<0.01$, ***$P<0.001$

be detrimental to the individual animal. Results of other biochemical parameters monitored in this study (serum glucose, serum globulin, serum urea, creatinine, and cholesterol) indicated that the normal-feathered birds had higher mean values than naked and frizzle-feathered genotypes, respectively. These significant differences which may be attributed to strain differences are consistent with Agaie and Uko (1998), Islam et al. (2004) and Chineke et al. (2006), strengthening the argument for inherent genetic differences.

Significant variation between sexes in this study was consistent with the reports of Islam et al. (2004). Our results showed that males generally had significantly higher mean values in PCV, Hb, RBC, glucose, albumin, globulin, creatinine, and cholesterol than their female counterparts. The higher mean values for haematological and biochemical parameters in males compared to females may also be attributed to physiological status of the birds. Kral and Suchy (2000) attributed high mean values of erythrocytes in male birds as a characteristic of gonadal and spermiogenetic development which occurs during the period of sexual maturation and at the onset of reproductive activity in breeding cocks. In a related report, Sturkie (1986) and Oladele et al. (2001) reported that matured males generally had higher erythrocyte values than females and reported that androgen stimulates erythropoeisis and increases the number of circulating erythrocytes and consequently, PCV and Hb in birds. There was a non-significant effect of the sex of bird recorded for MCV, MCH, and MCHC; and since these parameters derived are likely to be more sensitive to sample size, although female birds had slightly higher values than the males. This is further supported by significant interaction effects between genotype and sex on Hb, RBC, Glu, Alb, Glo, urea, creatinine, and Chol. These results were expected because genotype and sex individually had significant effects on most of these parameters.

The correlation coefficients involving MCV, MCH and MCHC with any other parameter were not significant. However, strong and positive correlations among other parameters such as PCV and Glu, PCV and Alb, PCV and Glo, Hb and Glu, Hb and urea, RBC and Glo, RBC and Glu, RBC and Chol, and RBC and urea clearly demonstrate inter-relationships among these parameters. These relationships further strengthen the roles that PCV, Hb and RBC play in better understanding of normal physiology, pathology and total health monitoring of birds (Maxwell and Burns 1986; Talebi et al. (2005). These authors reported that the levels of PCV and Hb were major indices in evaluating circulating avian erythrocytes and were very significant in the diagnosis of anaemia. However, the non-significant correlation obtained between WBC and other parameters may indicate less dependence on other parameters and diminished role in the immune functions of the native chickens.

In summary, while this study may indicate that sufficient genetic variation exists among NNC as indicator traits for further study in the context of selection and improvement programme, there remains a need to apply molecular tools to characterise these genetic groups for better resolution of these differences and long-term utilisation.

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