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Characterization of Calabar normal and frizzle feather chickens based on haemoglobin type and body morphometry

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Target Audience: Animal geneticists, poultry farmers, Genetic Resource conservationists

Abstract

The study was designed to characterize the normal and frizzle feather chicken in Calabar, Nigeria based on their haemoglobin type and body morphometric traits. One hundred and twenty (120) adult chickens comprising 60 normal (NF)and 60 frizzle feather (FF) genotypes (30 per sex) respectively were used for the study. Cellulose Acetate Electrophoresis method was used for determination of haemoglobin type. Linear body measurements such as keel length (KL), body circumference (BC), body length (BL), shank length (SL) and thigh length (TL) were taken. Results of the morphometric measurements indicated a significant difference ($p<0.05$) in TL, KL and BL between the normal and frizzle feather chickens, normal feather being higher in these traits. The values were recorded as follows: Normal and frizzle feather 17.65cm and 16.70cm (thigh length) 19.78 cm and 17.73cm (shank length), 14.83cm and 14.45cm (keel length) and 18.83cm and 20.20cm (body length) respectively. There was however, no significant difference ($p> 0.05$) in body weight and body circumference between the normal and frizzle feathered chickens. Normal feather and frizzle feather Nigerian indigenous chickens in Calabar were found to have haemoglobin (Hb) types A and B, having three genotype forms: AA, AB and BB. Gene frequencies of the population of chicken used in this study were A (0.5625) and B (0.4375). Frequency of Hb AB was predominant (42 %), followed by Hb AA (35%) and Hb BB (22%). Females were predominantly of Hb AA (45%) while males were mostly Hb AB (50%). Normal feather chickens were taller and longer than the frizzle feather birds, though they were not significantly ($p>0.05$) heavier in body weight. Information obtained from this study can be useful in the conservation of genetic resource as well as improvement of these breeds in the study area.

Keywords: Haemoglobin type, gene frequency, genotype frequency, body morphometric traits, chickens

Description of problem

Nigeria is a heavily populated country whose human population is constantly on the increase. This increase has given rise to high demand for the animal products nationwide. Poultry products serve as one of the affordable and available sources of protein for this teaming population. [1] identified chickens as important genetic resources among the avian species in Nigeria. Understanding the distribution of chicken genetic diversity would be expedient in enhancing both conservation and exploitation stratagems for indigenous chicken genetic resources in the country. Nigerian local chickens are described basically using their phenotypic traits. Such information if complemented with findings obtained using molecular markers could be useful in

formulating long term inference or plans for genetic improvement programs for native chickens. Nigerian indigenous chickens are often utilized for several purposes simultaneously [2] and possess superior levels of genetic diversity and traits of potential interest to commercial breeders having unique traits of valuable local adaptations [3].

The genetic differences which exist among animals both at individual and breed levels account for the variability in the reproductive and performance abilities of those animals. Distinguishing this variability could be a foundation for selection for successive genetic improvement of farm animals. Polymorphism can be said to occur in a population when two or more distinctly inherited varieties co-exist in the same individual [4]. Evolutionary relationships in many animal species have been studied using blood protein. It is possible to use polymorphisms that exist among Proteins to map various gene types such as disease-causing genes, economically important trait genes, and for selection of superior breeding animal [5]. Proteins structures permit them to serve as catalysts which control the rate of all biological reactions, to act as the carriers of vital substances within the organisms, to serve as regulators of physiological associations and to work as building block units for substances, cellular and organic structures [4]. Electrophoresis can be applied to study this variation in proteins to decipher genetic diversity within a population's gene pool [6].

Polymorphism has been defined as the occurrence together of two or more varieties in the same population that the rarest of them cannot be maintained by mutation alone [7]. Hemoglobin (Hb) contains four globin chains and one prosthetic group called "haem" bound to each other. Variations in the Hb structure occurs only in the globin portion, as the haem portion is alike in all forms of Hb [8].With structural variances in the globin portion of the Hb, modifications which might result could engender formation of variants that may bestow certain advantages or minimize the potentials of an animal. Three type of Hb have been observed in poultry which are controlled by two autosomal alleles A1 and A2 respectively. Selective advantages in different geographical regions due to different Hb types have been reported. Such advantages include helminthic infestation resistance [9], effect on meat quality [10], productive traits [11] and hair length [12]. The study had the objectives to determine haemoglobin type in the normal and frizzle feather Nigerian indigenous chickens, to obtain their gene and genotypic frequencies and to characterize them using their body morphometry.

Materials and Methods

One hundred and twenty adult chickens, aged between 1and 3 years, comprising 60 normal and 60 frizzle feather, 30 per sex respectively, were purchased from local markets in Akpabuyo local government area of Cross River State and used for the study. 2mls of blood was collected from the vein in the wing of each bird by brachial venipuncture. About 1ml of whole unsedimented blood was placed in a centrifuge tube and 10 - 15mls of cold 0.155m NaCl was added to wash the red cells. The samples were centrifuged. Haemoglobin was released following haemolysis of the sedimented cells by addition of cold distilled water to the cells[13].

Haemoglobin typing was done using cellulose acetate electrophoresis. Cellulose acetate strips were prepared, labeled and soaked in EDTA borate buffer (pH 8.6) and blotted a little with filter paper to remove excess buffer. The haemolysates were placed on the cellulose acetate paper and put in the electrophoresis tank (Shandon southern electrophoresis tank) using forceps. The samples were allowed to air dry for some minutes after electrophoretic separation.

Resulting haemoglobin bands after electrophoresis were scored using direct gene counting method[14].

- A single faster band was designated as the AA homozygote.
- A single slower band was designated as BB homozygote.
- When both bands are present, it was designated as AB heterozygote.

Genotype frequency was calculated according to [13] as follows:

$$\frac{\text{No. of AA}}{\text{Total No.}} \times \frac{100}{1}$$

$$\frac{\text{No. of AB}}{\text{Total No.}} \times \frac{100}{1}$$

$$\frac{\text{No. of BB}}{\text{Total No.}} \times \frac{100}{1}$$

Gene frequencies were calculated as follows using Hardy Weinberg equation as follows:

P = Gene frequency of allele A

Q = Gene frequency of allele B

$$P = \frac{(2N_{AA} + N_{AB})}{2N}$$

$$Q = \frac{(2N_{BB} + N_{AB})}{2N}$$

Where N = Total number of individuals sampled

N_{AA} = Observed genotype number for AA

N_{AB} = Observed genotype number for AB

N_{BB} = Observed genotype number for BB

Data generated were subjected to the T-test experimental design.

Body weight (BW) of individual birds was taken using a weighing scale and recorded in grams while morphometric parameters were taken using a flexible tailor measuring tape and recorded in centimeters. The body parameter measured included keel length (KL), breast circumference (BC), body length (BL) and thigh length (TL).

Results

Haemoglobin type distribution

Table 1 shows the haemoglobin type distribution of the birds. The following Haemoglobin types were identified in the two breeds of local chickens: HbAA, HbBB and HbAB. Out of the 60 individuals sampled in Normal feather breed, 27 exhibited HbAA, 27 were HbAB and 6 HbBB, while 15 out of 60 birds in the frizzle feather breed exhibited HbAA, 24 were of HbAB and 21 were of HbBB. When considered on sex basis, HbAA individuals were 9 males and 18 females in the normal feather breed, and 6 males plus 9 females in the frizzle feather breed. HbAB birds comprised 15 male plus 12 female normal feather and 15 male plus 9 female frizzle feather individuals. In HbBB, 6 males and no females were identified in the normal feather breed while 9 males and 12 female individuals were identified in the frizzle feather breed.

Genotype frequency

The genotype frequencies of the birds are presented in Table 2. The highest genotype frequency was recorded in the normal feather having HbAA (45.0%) and HbAB (45.0%), closely followed by frizzle feather HbAB (40.0%), and the least value of genotype HbBB (10.0%) was equally recorded among the normal feather. The HbAB had the highest value of (42.5%) followed by HbAA (35.0%) when all breeds were pooled together irrespective of sexes, which was different from HbBB (22.5%). Furthermore, when the chickens were separated according to sexes, the result indicated that the male had higher number of HbAB (50.0%) followed by female HbAA (45.0%) and HbAB (35.0%), which shows that HbAA was predominant in female than in male.

Table 1: Haemoglobin type distribution of normal and frizzle feather Nigerian chickens

		Genotype			
Breed of bird	Sex	AA	AB	BB	Total
Normal feather	Male	9	15	6	30
	Female	18	12		30
	Total	27	27	6	60
	Male	6	15	9	30
Frizzle feather	Female	9	9	12	30
	Total	15	24	21	60

Table 2: Effect of breed and sex on genotype frequency of normal and frizzle feather Nigerian chickens

	Genotype			
Breed of birds	AA (%)	AB (%)	BB (%)	Total (%)
Normal feather	45	45	10	100
Frizzle feather	25	40	35	100
Pooled	35	42	22	100
Sex				
Male	25	50	25	100
Female	45	35	20	100

Gene frequency

The effect of breed and sex on gene frequency of normal and frizzle feather Nigeria local chickens is shown in Table 3.

Among the population of chickens, gene A was more predominant (0.6750) than gene B (0.3250) in the normal feather breed. On the other hand, frequency of gene B was slightly higher (0.5500) than A (0.4500) in the frizzle feather breed. When genes were compared across sexes, gene A was higher in female (0.6250) than male (0.5000), while frequency of B was higher in male (0.5000) than in female (0.3750). When the genes of the chickens were pooled, gene frequency A (0.5625) was higher than B (0.4375).

Table 3: Effect of breed and sex on gene frequency of normal frizzle feather Nigerian chickens

Breed of birds	Gene type		
	A	B	Total
Normal feather	0.675	0.325	1
Frizzle feather	0.45	0.55	1
Pooled	0.5625	0.4375	1
Sex			
Male	0.5	0.5	1
Female	0.625	0.375	1

Morphometric parameters

The influence of sex and breed on the morphometric parameters of the birds are presented in Tables 4 and 5 respectively.

Body weight

NF and FF male chickens did not differ significantly ($p>0.05$) in the BW. Values recorded were 1240g and 1190g in NF and FF birds respectively. The female birds however differed significantly ($p<0.01$) with the FF having BW of 1130g, being superior to the NF (1035g). The two breeds did not differ significantly ($p<0.05$) as regards their BW.

Thigh length

Significant ($p<0.01$) differences were recorded between males of the two breeds with respect to their TL. Values recorded were 19.05cm and 17.20cm respectively. The females were however, not significantly different ($p>0.05$), having values of 16.25cm and 16.20cm for the NF and FF birds respectively. Comparing the breeds, NF birds were found to be significantly higher in TL than the FF chickens. Values were at 17.65cm and 16.70cm respectively.

Shank length

Sex affected SL of the birds. The males of the two breeds differed significantly ($p<0.01$) with values of 8.65 cm for NF and 8.30 cm for FF breeds respectively. Contrarily, the females did not differ significantly ($p>0.05$) in their SL. Breed of chicken equally significantly ($p<0.01$) affected the SL of the chickens; NF (19.78cm) being superior to FF (17.73cm).

Keel length

Birds did not differ significantly ($p>0.05$) in their KL irrespective of sex. When compared based on breed however, significant difference was observed, NF (14.82cm) being higher than FF (14.45cm) chickens.

Body Circumference

BC of both sexes and breeds of chickens did not differ significantly ($p>0.05$). Values recorded for the NF birds were 27.30 and 26.00 cm for males and females respectively. The FF chickens had similar average figures of 26.30 and 26.00 cm for males and females respectively.

Body length

Male chickens did not differ significantly ($p>0.05$) in BL but the females differed significantly ($p<0.01$) with the FF (20.10cm) being superior to the NF (18.10cm) chickens. Breed significantly ($p<0.01$) affected BL of the birds; FF chickens (20.20cm) being superior to the NF (18.83cm).

Table 4: Influence of sex on morphometric parameters of normal and frizzle feather Nigerian chickens

		Morphometric parameter					
Breed of bird	Sex	BW(g)	TL(cm)	SL(cm)	KL(cm)	BC(cm)	BL(cm)
Normal feather	Male	1240± 53.79	19.05± 0.24[a]	8.65± 0.16[a]	15.95± 0.31	27.30± 0.58	19.55± 0.47
Frizzle feather	Male	1190± 41.4	17.20± 0.25[b]	8.30± 0.14[b]	15.30± 0.34	26.30± 0.46	20.40± 0.42
Normal feather	Female	1035± 53.8[b]	16.25± 0.18	7.30± 0.21	13.70± 0.28	26.00± 0.5	18.10± 0.14
Frizzle feather	Female	1130± 39.6[a]	16.20± 0.18	7.15± 0.15	13.60± 0.37	26.00± 0.29	20.10± 0.51

Means on the same column with different superscripts are significantly different ($p<0.01$). BW =body weight, TL =thigh length, SL = shank length, KL = keel length, BC = Body circumference, BL = body length

Table 5: Breed influence on morphometric parameters of normal and frizzle feather Nigerian chickens

Breed of bird	Morphometric parameter					
	BW	TL	SL	KL	BC	BL
Normal feather	1137±	17.65±	9.78±	14.83±	26.65±	18.83±
	43.59	0.35[a]	0.35[a]	3.04	2.41	2.84[b]
Frizzle feather	1160±	16.70±	7.73±	14.45±	26.15±	20.20±
	29.98	0.19[b]	0.16[b]	3.13	3.62	3.11[a]
Sig		***	***			***

a, b = Means on the same column with different superscripts are significantly different. *** = ($p<0.01$). BW =body weight (g), TL =thigh length (cm), SL = shank length (cm), KL = keel length (cm), BC = Body circumference (cm), BL = body length (cm)

Discussion

Two haemoglobin variants (A and B) were identified among the chicken populations used in this study. Furthermore, three genotype combinations (Hb AA, HbAB and Hb BB) were established. These findings are in agreement with the reports [14] and [15] in Nigerian native chickens of the Middle Belt and Niger Delta regions of Nigeria respectively. The result confirms the heterogeneous nature of the chicken population in Calabar axis. Earlier study had affirmed that there were strictly no pure breeds of local fowl in Nigeria with regards to haemoglobin locus [13]. Frequency of Hb AB was highest, followed by Hb AA and lastly Hb BB among the population of chickens used. Altitude to an extent, affects Hb type distribution in animals. It was reported [16] that breeds of sheep reared in mountainous regions of Britain tended to have Hb AA, while lowland breeds tend to have Hb AB and Hb BB. Similarly, [17] found Hb A to frequent in sheep living in areas above latitude 40º N of the equator. These reports are in line with the present research findings, having Hb AB more frequent than the homozygous genotypes, as Calabar is in a lowland area of Nigeria. [18] reported that growth rate and egg hatchability were affected by Hb type. The authors found hatchability to be highest in Hb AA, followed by AB and lastly BB. Results from the present study show that females were predominantly of Hb AA (45%) while males were mostly AB (50%). This finding is in agreement with the report of [15] in Nigerian indigenous chickens of the Niger Delta Region. The similarity of both reports is not surprising as a result of the close proximity of the research sites. This research established that sexual differences did not determine the fixing of haemoglobin type in NF and FF Nigerian chickens.

BW values recorded in this study for male chickens (1190g and 1240g) are lower than figure (1360g) reported by [19]. The authors report for female local chicken (1060g) was however within the range of figures recorded in this research (1130g and 1035g). On the other hand, [20] reported BW as high as 2428.1g at 56days of age in naked neck broiler chickens. The disparity between the findings of the present experimental work and the earlier reports could be attributed to breed, environmental differences and variations in the system of management. Free range system of management under which the chickens used for this study were raised, does not encourage optimum body build up, hence the low BW values recorded. The non-significant difference recorded in body weight between the NF and FF could be due to a possible genetic relatedness of their ancestors, since

indiscriminate cross breeding is a feature of the free range system of poultry management.

Male NF chickens exhibited significantly higher TL and SL than the FF birds. These two parameters impact on the height of the chickens. Thigh length figures recorded in this study (19.05cm and 17.20cm) are slightly lower than what [19] observed (21.67cm) in male local chickens in Makurdi metropolis. The authors also recorded a higher value 19.29cm of TL in the female local chickens in this study (16.25cm and 16.20cm). KL recorded in this experiment (14.83cm and 14.45cm) are at variance with the figure (5.63cm) reported by [20]. [21] on the other hand recorded slightly higher KL value of 18.14cm than observed in this study. Results of the present research indicate that the NF chickens were taller and longer than the FF birds, though they were not significantly ($p>0.05$) heavier in weight. The FF chickens can be said to only possess the advantage of ease of heat dissipation during the periods of heat stress, over the NF.

Conclusion and Application

1. The normal and frizzle feather chickens of Calabar, Nigeria possess two haemoglobin variants A and B, with three genotype combinations (Hb AA, HbAB and Hb BB).
2. Frequency of Hb AB was predominant (42 %), followed by Hb AA (35%) and Hb BB (22%). Females were predominantly of Hb AA (45%) while males were mostly Hb AB (50%).
3. Normal feather chickens were taller and longer than the frizzle feather birds, though they were not significantly ($p>0.05$) heavier than the frizzle feather in body weight.
4. Findings can serve as basis for planning genetic improvement programmes and conservation of the genetic resources of the Calabar normal and frizzle feather chickens by geneticists and animal breeders.

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