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HAEMOGLOBIN GENOTYPES IN THE NIGERIAN INDIGENOUS CHICKEN IN THE NIGER DELTA REGION OF NIGERIA

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ABSTRACT
This study assessed the haemoglobin polymorphism in the Nigerian indigenous chicken in the Niger Delta region of Nigeria. Blood samples were collected from 54 local chickens in the markets and homesteads of smallholders farmers which included 15 frizzle feathered, 19 naked neck and 20 normal feathered indigenous. Haemoglobin typing was accomplished using cellulose acetate electrophoresis. Genotype and gene frequencies were calculated using Hardy Weinberg equation. From the results, in the 15 frizzled feathered chickens sampled, 1 had AA genotype, 3 had AB and 11 had BB genotype. However, among the 20 normal feathered chickens sampled, 6 had AA genotype, 8 had AB and 6 had BB genotype. A similar trend for frizzle feathered was seen among the naked neck chicken. The highest genotype among the local breeds of chicken is BB except among the normal feathered chickens where AB is higher. The lowest value is 6.67% found in AA among the frizzle feathered chickens and the highest value of 73.00% was found also among the frizzle feathered chickens. When all breeds were pooled together, BB had the highest value of 50% followed by AB (26.32%) and then AA (20.37%). Frizzle feathered chicken B had a higher gene frequency of 0.83 compared to A (0.17). The frequency for A and B in the normal feathered chicken was 50:50 but in Naked neck B was higher than A as well (0.66 and 0.34 respectively). These results point out that local indigenous fowl of Nigeria in the Niger Delta region are characterized into frizzle feathered, naked neck and normal feathered and they are a mixture of both AA, AB and BB Hb types. The chicken population indigenous to Nigeria was however found to be in Hardy Weinberg's equilibrium.

KEY WORDS: Indigenous chicken, Haemoglobin, Polymorphism, Electrophoresis.

INTRODUCTION

The population of the indigenous chickens of Nigeria is about 103 million (RIM 1992), 85% of which are found in the north and the rest being in the southern part of the country. The indigenous chickens of Nigeria are classified into light and heavy ecotypes on the basis of body weight and body size. The light ecotype represents the chicken type from the Swamp; Rainforest and Derived savannah agro-ecological zones whose mature body weight ranges from 0.68 to 1.5 kg and the heavy ecotype are those of the Guinea savannah, Sahel savannah and some Motane regions whose mature body weight ranges from 0.9 to 2.5 kg (Atteh, 1990). The Nigerian indigenous chickens have many economic traits such as high fertility, adaptation to harsh environment, resistance to disease, broodiness, high sexual maturity, high quality egg shell among others when compared to their exotic counterparts. However, their small size has made consumers prefer the exotic breeds.

In recent times, the introduction of biotechnology in Animal Breeding has changed the trend of research. Today, DNA and blood proteins form the basis of genetic improvement due to polymorphism. Polymorphism is a genetic variant that appears in at least one percent of a population or a herd. Protein polymorphism can be identified by serology (i.e. using antibodies to detect the different versions of a protein) and electrophoresis. Blood groups and blood proteins have been widely used to characterize animal population especially haemoglobin which is an iron – protein compound in red blood cells that gives blood its red colour and transport oxygen, carbon dioxide and nitric oxide. Different haemoglobin types may be selective advantages in different geographical regions (Ndamukong, 1995).Variation in haemoglobin has also been reported to cause depression in percentage egg production in mutant haemoglobin type compared with normal or heterozygous haemoglobin (Lowe and Washburn, 2007).This study was therefore designed to assess the effect of breed and sex on haemoglobin genotypes in indigenous chicken in Niger Delta area of Nigeria.

MATERIALS AND METHODS

Study area

The study was carried out in five selected local government areas in Rivers State. They are Ikwere, Obio/Akpor, Abua/Odua, Khana and Port Harcourt city. Small holders flocks in villages and markets were visited where blood samples were obtained from birds at Ekeoma and Station market in Elele, in Ikwere LGA; Abua Main market, in Abua/Odua LGA; Mile III market in Port Harcourt City; Ogbudiogha market, homes of rearers in Bori, Khana LGA; Akpabu, Apani, and Eneka,in Obio/Akpor LGA. Rivers State is located between latitude 4^0 45' N and longitude 6^0 50' E of the equator.

Research Animals

A total of 54 blood samples were collected from the various markets and homesteads of smallholder farmers in the study area comprising - 15 frizzle feathered, 19 naked neck and 20 normal feathered indigenous chicken genotypes.

Blood collection and preparation

2.5mls of blood was collected from the birds through the vein found at the wings of the local birds. A portion of the vein was blocked to prevent flow back of blood until the required quantity of blood was drawn into labeled sample bottles containing drops of anticoagulant (EDTA). Blood contamination was prevented by using separate syringes and needles for individual birds. About 2mls of blood samples of the following ecotypes (naked neck, frizzle feathered and normal feathered) were placed into different clean test tubes. 5mls of cold normal saline water was added to them (i.e. the different test tubes). The different test tubes containing the samples were centrifuged at 4000 (rpm) for 10 minutes. The supernatant was discarded and the samples were re-washed with 10ml of cold 0.115m NaCl was added to wash the red cells. The supernatant discarded and the different sediments were re-washed using 2ml of cold distil water. The remaining serum and plasma was centrifuged. Cold distilled water was added to the sediment to re-suspend the cells to release the haemoglobin by haemolysis. When the lysate was well separated after standing, it was stored at refrigeration temperature pending electrophoresis.

Electrophoresis

Haemoglobin typing was accomplished using cellulose acetate electrophoresis. Cellulose acetate strips were prepared and labeled. The cellulose acetate strips were soaked in EDTA borate buffer (pH 8.6) and blotted slightly with filter paper to remove excess buffer. The samples (haemolysates) were impregnated or placed on the cellulose acetate paper, with the control and placed on the electrophoresis tank (Shandon southern electrophoresis tank) using forceps (plastic pipette) the tank was powered with the lead closed. The samples were allowed to separate for about 10-15 minutes. After the separation, the cellulose acetate papers were blotted dry using filter paper and then dried in open air for some minutes and then the result were taken.

The direct gene counting method was used to score the resulting haemoglobin bands after electrophoresis.

- A single faster band was designated as the AA homozygote.
- The presence of a single slower band was designated as BB homozygote.
- The presence of both bands was designated as AB heterozygote.

Genotype frequency was calculated as follows:

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

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

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

Estimation of Gene Frequency

Using the expressions gene frequencies was calculated as follows using Hardy Weinberg equation:

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

P = Gene frequency of allele A

Q = Gene frequency of allele B

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

Where N =Total number of individual sampled

N_{AA} = Observed genotype number for AA

N_{AB} = Observed genotype number for AB

N_{BB} = Observed genotype number for BB

RESULTS

Table 1 below shows the distribution of the haemoglobin genotypes of the local chicken used for this study according to breed and sex. Of the 15 frizzled feathered chickens sampled, 1 had AA genotype, 3 had AB and 11 had BB genotype. However, among the 20 normal feathered chickens sampled, 6 had AA genotype, 8 had AB and 6 had BB genotype. A similar trend for frizzle feathered was seen among the naked neck chicken.

TABLE 1: Distribution of haemoglobin types between breed and sex of Nigerian indigenous strains

Genotype	Sex	AA	AB	BB	Total
Frizzle feathered	Female	1	2	3	6
	Male	-	1	8	9
	Total	1	3	11	15
Normal feathered	Female	6	8	2	16
	Male	-	-	4	4
	Total	6	8	6	20
Naked neck	Female	4	3	4	11
	Male	-	2	6	8
	Total	4	5	10	19

The effects of breeds on genotype frequency among the various local breeds of chicken are shown in Table 2. The highest genotype frequency was recorded in the frizzled feathered chicken BB(73.00%), closely followed by necked neck(52.63%) and the least value of genotype AA(6.67%) was equally recorded among the frizzled

feathered chickens. When all breeds were pooled together irrespective of sexes, genotype BB still had the highest value (50%) followed by AB (26.32%) which was different from AA (20.37%).However, when the chickens were separated according to sexes, the results indicated that males had a higher number of BB (85.75%) followed by females having AB (39.40%) and AA (33.33%) genotypes. which also showed that the genotype AA was predominant in the females than in the males.

TABLE 2: Effect of breed on Genotype frequency of indigenous chicken strains

Genotype	AA (%)	AB (%)	BB (%)	Total (%)
Frizzle feathered	6.67	20.00	73.00	100.00
Normal feathered	30.00	40.00	30.00	100.00
Naked neck	21.05	26.32	52.63	100.00
Pooled (All together)	20.37	26.32	50.00	100.00
Sex				
Female	33.33	39.40	27.27	100.00
Male	0.00	14.29	85.71	100.00

Table 3 below shows he effect of breed and sex on gene frequency of three Nigerian indigenous chickens among the population of chickens used for the study frizzled feathered chicken had a higher gene frequency B (0.83) compared to A (0.17) and necked neck followed the same trend B (0.66). A (0.34), but the gene frequency for A and B in the normal feathered chicken were 50:50. When the gene frequencies of the local birds were pooled together, B was higher (0.65) than A (0.53). When gene frequencies was compared across sexes, gene frequency A (0.35), was higher in females while gene frequency B (0.93) was higher in males.

TABLE 3: Effect of breed and sex on gene frequency of three Nigerian indigenous chicken

Genotype	A	B	Total
Frizzle feathered	0.17	0.83	1
Normal feathered	0.50	0.50	1
Naked neck	0.34	0.66	1
Pooled (All together)	0.35	0.65	1
Sex			
Female	0.53	0.47	1
Male	0.07	0.93	1

DISCUSSION

There were remarkable variation in the local fowl as three hemoglobin variants AA, AB and BB alleles were identified with corresponding detectable genotype HbAA, HbAB and HbBB. This showed that the population of these indigenous fowl is heterogeneous and thus, suggest that there are no pure breeds of local fowl in Niger Delta Region of Nigeria in the strict sense of genetic homozygosity with regards to the hemoglobin locus. This is in agreement with observation made by Ibe (1990) that chicken flocks in Nigeria consist of two categories improved and unimproved and further explained unimproved to be pure indigenous fowl. The improved are those derived as a result of uncontrolled random mating between indigenous and improved commercial type with no visible destruction. Sonaiya (1990) also reported that there are contentions that there are no more pure local chicken that all are crossed to various degrees. This is because migrant genes became diffused and lost in the population since no selection in a specific direction was practiced.

The variation in the values observed for both the genotype and gene frequencies can be explained by Hardy-Weinberg equilibrium. Most definitely, the population's genotype frequency can change from generation to generation, unless the population is in Hardy-Weinberg equilibrium (Ober *et al*., 1997). For instance, one generation may have a certain genotype frequency, but then mutations, non-random mating and other factors could easily change the genotype frequency. If the population is in H-W equilibrium, then certain conditions are specified (an ideal situation that is rarely if ever seen in real life) that maintain genotype frequency from generation to generation.

The absence of some genotypes in some sexes as seen in this study which could be an indication that the population is not in H-W equilibrium, more so the population size is also relatively small. For instance, take a population of two individuals, one is BB and the other is bb and then they mate and produce offspring, all of whom are Bb. So, the genotype frequency changed drastically between the two generations (from all homozygotes to no homozygotes/only heterozygotes), but the allele frequency is still 1:1 (Ober *et al*., 1997). More so, the values for genotype frequencies and gene frequencies recorded in this study are within the ranged observed by Salako and Ige (2006) in their study that assessed the haemoglobin polymorphism in the Nigerian indigenous chicken in the South Western region of the country. Dans and Deb, (2008) explained that Haemoglobin is the principle molecule for transport of carbon dioxide in blood, which is a conjugated protein and consists of the protein globins

and prosthetic group haemoglobin. The report of Washburn *et al*. (1971) showed that chicken of the homozygous haemoglobin, genotypes were approximately 20% less susceptible to Marek's disease. The higher percentage of homozygous BB reported in this study for the naked neck and frizzle feathered chicken could be an added advantage in conferring resistance to diseases for these rare genes. Though growth study was not conducted in this study, it has earlier been reported that haemoglobin polymorphism affects the growth rate and hatchability (Dimri *et al*., 1981). Hatchability was reported to be highest in AA followed by AB and BB. However in this study females were seem to have more of the AA genotype than males with the gene frequency of AA (33.33%), AB (39.40) and BB (27.27) respectively whereas the males were predominantly BB. This study however showed that sexual dimorphism is not an important factor in the inheritance of haemoglobin.

CONCLUSION

In study, this also pointed out that local indigenous fowl of Nigeria in the Niger Delta region are characterized into frizzle feathered, naked neck and normal feathered and they are a mixture of both AA, AB and BB Hb types. It appears generally, that sex is not important in the inheritance of haemoglobin.

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