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ANALYSIS OF GENETIC VARIATION IN NORMAL FEATHERED, NAKED NECK AND FULANI- ECOTYPE NIGERIAN INDIGENOUS CHICKENS BASED ON HAEMOGLOBIN POLYMORPHISM

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Abstract: Haemoglobin polymorphism was investigated in 50 normal feathered, 33 naked neck and 42 Fulani-ecotype Nigerian indigenous chickens. Haemoglobin typing was carried out using cellulose acetate electrophoresis. Two co-dominant haemoglobin alleles (HbA and HbB) were found in the chickens. From the electrophoretic band patterns, three genotypes (HbAA, HbAB and HbBB) were observed. The frequencies of the A and B genes were 0.68 and 0.32; 0.71 and 0.29; 0.75 and 0.25 for normal feathered, naked neck and Fulani-ecotype chickens respectively. The corresponding genotype frequencies for AA, AB, and BB in the three chicken populations were 0.54, 0.28 and 0.18; 0.58, 0.27and 0.15; 0.62, 0.26 and 0.12 respectively. The gene and genotype frequencies of naked neck and Fulani-ecotype birds were in Hardy-Weinberg equilibrium while those of normal feathered birds deviated significantly from the theoretical proportions. The average amount of heterozygosity at haemoglobin locus was 0.41. The results obtained could serve as a reference point in the genetic improvement of indigenous chickens using biochemical markers.

Key words: Haemoglobin, polymorphism, equilibrium, heterozygosity, indigenous chickens.

Introduction

Livestock populations have evolved unique adaptation to their agricultural production system and agro- ecological environments. The knowledge of their genetic diversity is important as it forms the basis for designing breeding programmes and making rational decisions on sustainable utilization of animal genetic resources *(Mwacharo et al., 2005)*. It also represents a unique resource to respond to the present and future needs of livestock production both in developed

and developing countries. Genetic characterisation through the use of molecular markers is providing new avenues for decision making choices for the conservation and rational management of Animal Genetic Resources (AnGR) *(Ajmone-Marsan et al., 2010; Groeneveld et al., 2010; FAO., 2011)*.

The structures of proteins enable them to act as catalysts which control the rates of all biological reactions, to serve as the carriers of essential substances within the organisms, to serve as regulators of physiological relationships and to serve as building block units for substances, cellular and organic structures *(Das and Deb, 2008)*. The occurrence of two or more discontinuous forms of proteins in a species in such a proportion that rarest of them cannot be maintained merely by recurrent mutation is called protein polymorphism. Protein polymorphisms were the first molecular markers used in livestock *(Hanotte and Jianlin, 2005)*. A number of blood protein systems have been found to exhibit heterogeneity in different species *(Prasad et al., 1983)*. The polymorphic blood traits are useful in studies of relationship, structure of breeds and their evolution. Of these biochemical polymorphic loci, haemoglobin stands out because of its essentiality to mammalian life as it carries and delivers oxygen for cell survival. Vertebrate Haemoglobin, contained in erythrocytes, is a globular protein with a quaternary structure composed of four globin chains (two alpha and two beta) and a prosthetic group named heme bound to each other *(de Souza and Bonilla-Rodriguez, 2007)*. It is the most studied of all proteins. Indeed, the molecular analysis of haemoglobin has been the testing ground for many contemporary ideas and concepts in biology, particularly the understanding of the crystallographic structure and structure-function relationship of proteins, ligand binding, structural transitions between conformers, allosteric interactions and others *(Perutz, 1984; Berenbrink, 2006)*. Haemoglobin types have been associated with some meat quality parameters in rabbits, *(Bezova et al., 2007)*, productive traits in cattle *(Boonprong et al., 2007)* and gastrointestinal helminths in goats and sheep *(Ndamukong, 1995)*.

The Nigerian indigenous chicken populations still provide the basis for the poultry sector *(Yakubu et al., 2009; Yakubu, 2010)*. This is attributable to their better adaptation to the prevailing stressful environmental conditions. In spite of their importance, information is scarce on their genetic variability and genetic relationships using blood protein markers. In poultry species, it is known that haemoglobin types come through heredity with autosomal co-dominant alleles A and B *(Ugur et al., 2006)*. Therefore, the present study aimed at characterising the genetic pool of the native chickens using haemoglobin polymorphism. The results obtained could be useful in supporting decisions on conservation and further use of the chicken populations in crossbreeding programmes designed to create genetic stocks with improved adaptability and productivity.

Materials and Methods

Blood samples were randomly collected from a total of one hundred and twenty five birds comprising fifty normal feathered (17 males and 33 females), thirty three naked neck (18 males and 15 females) and forty two Fulani-ecotype (20 males and 22 females) indigenous chickens. The birds were extensively managed in Nasarawa State, North Central Nigeria.

2.5mls of blood was collected from each bird by jugular venipuncture. The blood was drawn into labelled bijou bottles containing EDTA (Ethylene-diamine-tetra-acetic acid) anticoagulant. Haemoglobin was typed using cellulose acetate electrophoresis as described by *Imumorin et al. (1999)* but with a slight modification.0.5-1ml of whole unsedimented blood was placed into a centrifuge tube and 10-15mls of cold 0.155M NACl was added to wash the red cells. The samples were centrifuged. Cold distilled water was added to the sedimented cells to release the haemoglobin (Hb) by haemolysis. The haemolysates were removed with a transfer pipette.

Cellulose acetate strips (77 x 150mm) were prepared and labelled. They were soaked in Tris EDTA-Boric acid buffer (TEB) at a p^H of 8.6 and blotted slightly with a filter paper to remove excess buffer. Haemolysates were applied with a Shandon Southern Electrophoresis tank with TEB (p^H) as the electrode buffer. A voltage of 250volts and current of 25 milliamperes were applied for two hours to get a good resolution of haemoglobin. The strips were stained with Ponceau red S for 5-10 minutes and progressively destained in 5% and then 12% acetic acid solution. The strips were then dried in the oven for 30 minutes at 60^0 C. The direct gene counting method was used to score Hb bands based on the separation of Hb variants. Haemoglobin typing was carried out at the Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria.

Statistical analysis

Genotype and gene frequencies of Haemoglobin (Hb) alleles were estimated. Gene frequencies were calculated as follows:

$$p = \frac{(2NAA + NAB)}{2N}$$

$$q = \frac{(2NBB + NAB)}{2N}$$

where ,

p= gene frequency of allele A

q= gene frequency of allele B
N= total number of birds sampled
N_{AA}= observed genotype number for AA
N_{AB}= observed genotype number for AB
N_{BB}= observed genotype number for BB

Data on Hb alleles and of genotype frequencies were subjected to chi-square analysis to test for goodness-of-fit for observed and expected frequencies under Hardy-Weinberg equilibrium (HWE). Heterozygosity (H) was estimated as the expected proportion of heterozygotes under HWE.

Results and Discussion

Genotype and gene frequencies of indigenous chickens are presented in Table 1. Two haemoglobin alleles A and B of distinctly different mobilities were observed. From the pattern of the bands, three genotypes (homozygous AA and BB and heterozygous AB) were observed. The genotype frequencies of Hb AA, Hb AB and Hb BB were 0.59,0.29 and 0.12; 0.61,0.17 and 0.22; 0.67,0.20 and 0.13 for cocks and 0.52, 0.27 and 0.21; 0.53, 0.40 and 0.07; 0.59, 0.30 and 0.11 for hens of normal feathered, naked neck and Fulani-ecotype chickens. When the data of both sexes were pooled, the respective genotype frequencies were 0.54, 0.28 and 0.18; 0.58, 0.27 and 0.15; 0.62, 0.26 and 0.12 for normal feathered, naked neck and Fulani-ecotype birds. The distribution of allele A appeared to be higher in the three chicken populations sampled. No abnormal (rare) haemoglobin patterns associated with non- agglutinating erythrocytes were found.

The three haemoglobin types (Hb AA, Hb AB and Hb BB) determined by two co-dominant alleles (Hb A and Hb B) in the present study are consistent with those reported for chuckars and pheasants by *Ugur et al. (2006).* The preponderance of the A allele observed is similar to those obtained by *Salako and Ige (2006)* in indigenous chickens of South-West Nigeria. However, *Okamoto et al. (2003)* reported that in general, Asian native fowl was being fixed in Hb B while Hb A was detected at extremely low frequencies in some chickens.

Chi-square analysis showed no significant differences in the observed and the expected frequencies of haemoglobin types in the naked neck and Fulani-ecotype flocks. This revealed that the gene and genotype frequencies of the two populations were in Hardy-Weinberg proportions as they were not affected by non-random mating, genetic drift, mutation, genetic migration and selection. Similarly, *Maina et al. (2002)* reported that observed and expected genotypes for haptoglobin in Kenyan indigenous chickens were in Hardy-Weinberg equilibrium. In normal feathered birds, the discrepancy between the observed and the expected numbers was significant thereby violating the Hardy-Weinberg frequencies. This was quite

unexpected although the departure fits the theoretical expectation of differences between observed and expected genotype frequencies in a population which is a mixture of subpopulations with different gene frequencies. However, sample size could have been a limiting factor since there are no improved free range native chickens in the country.

Table 1. Distribution of haemoglobin genotypes and gene frequencies in local chicken.

Genetic Group	Sex	No.	Genotype frequency			Gene frequency		
			AA	AB	BB		A	B
Normal feathered	Male	17	10 (0.59)	5 (0.29)	2 (0.12)		0.74	0.26
	Female	33	17 (0.52)	9 (0.27)	7 (0.21)		0.65	0.35
	Total	50	27 (0.54)	14 (0.28)	9 (0.18)		0.68	0.32
Naked neck	Male	18	11 (0.61)	3 (0.17)	4 (0.22)		0.69	0.31
	Female	15	8 (0.53)	6 (0.40)	1 (0.07)		0.73	0.27
	Total	33	19 (0.58)	9 (0.27)	5 (0.15)		0.71	0.29
Fulani-ecotype	Male	15	10 (0.67	3 (0.20)	2 (0.13)		0.77	0.23
	Female	27	16 (0.59)	8 (0.30)	3 (0.11)		0.74	0.26
	Total	42	26 (0.62)	11 (0.26)	5 (0.12)		0.75	0.25

X^2 ($P< 0.05$)= 3.841; Normal feathered= 6.36*, Naked neck= 3.68^{ns}, Fulani-ecotype= 3.81^{ns}
*= Significant
ns: Not significant

Heterogeneity was estimated to be 0.44, 0.41 and 0.38 for normal feathered, naked neck and Fulani-ecotype chickens. However, the average amount of heterozygosity at haemoglobin locus was 0.41. Heterozygosity is a measure of genetic variation or gene diversity. Biological diversity, according to *Maina et al. (2002)*, is exhibited both at the intrapopulation and interpopulation levels. The value of 0.41 in the present study, which could serve as gene diversity index at

haemoglobin locus, is relatively high. This could be a contributing factor to better adaptability of the local fowls to the prevailing tropical conditions.

Table 2. **Haemoglobin heterozygosity within Nigerian indigenous chickens**

Genetic group	Heterozygosity
Normal feathered	0.44
Naked neck	0.41
Fulani-ecotype	0.38
Average	0.41

Subsequent findings should employ the use of neutral markers (microsattelites) in addition to a larger sample size, thereby contributing to the global plan of action on the genetic characterization of indigenous birds.

Conclusion

The haemoglobin system showed variation in the different chicken genetic types of Nigeria investigated. This could serve as a reference point for future studies involving the use of more blood protein markers with a larger sample size; and correlate these polymorphic sites with economic traits including disease resistance. This may pave way for marker-assisted selection in the genetic improvement of indigenous chickens.

Analiza genetske varijacije kod autohtonih nigerijskih rasa živine na osnovu polimorfizma hemoglobina

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Rezime

Polimorfizam hemoglobina je ispitan na uzorku od 50 grla, sa perjem, 33 gološijana i 42 grla živine Furlani-ekotip, nigerijske autohtnone rase živine.

Tipiziranje hemoglobina je urađeno korišćenjem elektroforeze na podlozi celuloza acetat . Dva ko-dominantna haemoglobin alela (HbA i HbB) su utvrđeni kod živine. Na dobijenim elektroforetskim bandovima/trakama, utvrđena su tri (HbAA, HbAB i HbBB) . Frekvencije A i B gena su bile 0.68 i 0.32; 0.71 i 0.29; 0.75 i 0.25 kod živine sa nomralnim perjem, gološijana i Fulani-ekotipa žvine, respektivno. Odgovarajuće frekvencije genotipova za AA, AB, i BB u tri populacije živine su bile 0.54, 0.28 i 0.18; 0.58, 0.27 i 0.15; 0.62, 0.26 i 0.12 respektivno. Frekvencije gena i genotipova kod gološijana i grla furlani-ekotipa su bile u okviru Hardy-Weinberg ekvilibrijuma, dok je kod grla živine sa normalnim perjem utvrđena signifikantna devijacija od teoretskih proporcija. Prosečna heterozigotnost na haemoglobin lokusu je bila 0.41. Dobijeni rezultati mogu poslužiti kao referentna tačka u genetskom poboljšanju autohtonih Trasa živine korišćenjem biološko-hemijskih markera.

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