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BIOCHEMICAL CHARACTERIZATION OF THE NIGERIAN INDIGENOUS GUINEA FOWL (Numida meleagris)

Abel Olusegun Oguntunji[1]*, Kolawole Luke Ayorinde[2], Taiwo Olayemi Aremu[1]

[1]Department of Animal Science and Fisheries Management,
Bowen University, P.M.B. 284, Iwo, Osun State, Nigeria.
[2]Department of Animal Production,
University of Ilorin, Ilorin, Kwara State, Nigeria.
*Corresponding author: abelmendel@yahoo.co.in
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Abstract: Blood protein polymorphism has been extensively used for characterization and estimation of genetic diversity in farm animals. A study on biochemical characterization and estimation of genetic diversity of Nigerian indigenous guinea fowls *(Numida meleagris)* was conducted using four blood proteins {Haemoglobin (Hb), Carbonic anhydrase (CA), Transferrin (Tf) and Albumin (Alb)}. Cellulose acetate electrophoresis indicated that all the protein markers were polymorphic; expressing two co-dominant genes and two genotypes at their respective locus. Heterozygouse genotypes were prevalent at Hb, Tf and Alb loci while homozygoutes were more frequent for CA. Allelic constitution was similar (A and B) for Hb, Tf and Alb while F and S were typed at CA locus. Gene A had higher frequency of occurrence at Tf and Alb loci while gene F and B was prevalent at CA and Hb locus, respectively. Average estimated genetic diversity (heterozygosity) across the genetic systems was 0.40 and moderate. Prevalence of genes F, A and B at their respective locus is suggestive of their relevancies to the survival and adaptability of the studied population to its natural habitat.

Key words: Protein polymorphism, electrophoresis, genetic diversity, heterozygosity, polymorphic.

Introduction

Efficient utilization, improvement and conservation of a species or breed are practically impossible in the absence of certain relevant background information of its unique attributes. Characterization of genetic resources of farm animals encompasses all activities associated with the identification, quantitative and qualitative description, and documentation of breed populations as well as its natural habitats and production systems to which they are or are not adapted

(Gizaw et al., 2011). Delgado et al. (2001) identified characterization of a livestock breed as the first approach to sustainable use of animal genetic resources. Contributing to the importance of characterization of farm animals, *Halima (2007)* posited that genetic characterization of the domestic animals is an integral component of the Food and Agriculture Organization's (FAO) global strategy for the management of farm animal genetic resources while *Gholizadeh et al. (2008)* stated that genetic characterization of populations/breeds allows the evaluation of genetic variability, a fundamental element in planning breeding strategies and genetic conservation plans.

Phenotypic diversity, morphological characters and indices which are easily assessed, having low cost and are easily measured have been widely used by researchers to characterize, discriminate and assess inherent diversities in farm animals. Nevertheless, in order to reduce the influence of environment on systematic information, biologists have borrowed approaches from protein chemists which mainly involve analysis of deoxyribonucleic acid (DNA) or of primary product (protein) from which codes are expressed so as to provide information on biological characters, status of individuals and populations within and among taxonomic units *(Hammed et al., 2011)*. Genetic characterization based on molecular assessment has been reported as the most common method to evaluate genetic diversity between and within livestock breeds, but requires high technology and is costly *(Wimmers et al., 2000; Romanov and Weigend, 2001; Hillel et al., 2003).*

Alternatively, the study of genetically-controlled biochemical polymorphisms of blood proteins has been used by researchers to characterize livestock breeds and populations and for the evaluation of genetic diversity existing in farm animals *(Oguntunji and Ayorinde, 2015; Akinyemi et al., 2014; Ige et al., 2013; Nyamsamba et al., 2003)*. In addition, the analysis of genetic markers based on protein variants detected by electrophoretic method has been a tool for studying genetic differentiation among populations, phylogenetic studies and has become equally important in biosystematics and evolutionary studies *(Nyamsamba et al., 2003). Egena and Alao (2014)* showed that biochemical study becomes imperative because of its importance in the improvement of farm animals, and the fact that some polymorphic alleles may be connected or linked with traits of economic importance due to pleiotropic effect, or general heterozygosity; and also possibly through complex interaction of additive and non-additive genes.

Guinea fowl is one of the indigenous poultry and also an integral part of rural poultry in Nigeria. A recent report indicated that it is the second most widely domesticated poultry after chicken *(NBS, 2012)* and is found mainly in northern Nigeria, while the few found in southern region were introduced through inter-

regional trade. This *galliforme* is also abundant in the wild in their natural habitat in grassland savanna of northern Nigeria.

In spite of the abundance and popularity of guinea fowl in northern Nigeria, its sizeable contribution to animal protein production and absence of known taboos and superstitions against its rearing, consumption and marketing; this bird has not received attention it deserves by researchers most especially in regions where it contributes to internal animal production and consumption. The neglect suffered is exemplified in sparse literature on this bird; and studies on its genetic attributes are limited. Furthermore, to the best knowledge of the authors, researches geared towards characterization using blood proteins or molecular markers and estimation of genetic diversity within and between Nigerian indigenous guinea fowl varieties are practically non-existent. The dearth of information on genetic resources present in the indigenous farm animals in developing countries has led to their under-utilization, replacement and dilution through crossbreeding and has underscored the significance of the characterization and conservation of the indigenous species *(Yakubu and Ugbo, 2013).*

In view of the foregoing, it is evident that characterization and conservation of diverse genetic attributes of the indigenous species are imperative and long overdue in order to maintain genetic biodiversity of the indigenous animals, enhance food security of the teeming population and boost economic empowerment in developing countries *(Oguntunji, 2013).* The present study was therefore conducted to characterize and estimate genetic diversity in Nigerian indigenous guinea fowls based on four blood proteins.

Materials and methods

Experimental animals

Thirty (30) adult pearl variety of guinea fowl were used for this study. The sample was randomly drawn from a large random mating population of guinea fowl of north-western part of Nigeria. They were reared primarily under a traditional free range system; whereby birds scavenged for their feed and water with little or no supplementation. Only apparently healthy birds were used for this study.

Electrophoretic procedures

The blood samples (3-5ml) were collected from the birds through venipuncture of the jugular veins into heparinsed tubes. The blood samples were then refrigerated in the ice packs and transferred to the Animal Breeding and Genetics Laboratory in the Department of Animal Science, University of Ibadan, Ibadan, Oyo State,

Nigeria for electrophoretic analysis. The electrophoretic procedures used were as described by (*Akinyemi et al., 2014; Akinyemi and Salako, 2012*).

Data analyses

The allelic frequency was estimated by simple gene counting method since all the observed variants were controlled by co-dominant alleles. Agreement of the observed genotype frequency with Hardy-Weinberg equilibrium (HWE) was tested with the chi-square test (X2).

Three genetic diversity parameters namely: Heterozygosity (H), effective number of allele (ne) and percentage polymorphic (%P) were used to assess genetic variability in the sample used in this study.
The unbiased estimate of mean heterozygosity *(Nei, 1978)* was estimated as:

Heterozygosity (H) = 1-Σx2í

Where:

X = the gene frequency of the íth allele in a locus

í= the number of tested loci

The effective number of allele (ne) per locus was estimated as:

1/1-H

Where:

H = heterozygosity *(Nyamsamba et al., 2003).*

Polymorphicity (%): a locus is polymorphic if the segregating genes are more than one and frequency of the rarest gene is at least 0.01% (Sanjalj et al., 2000).

The within population inbreeding coefficient (F) was estimated as 1- Ho/He *(Jean-Clauder, 2015).*

Where:

Ho = observed heterozygosity

He = expected heterozygosity

Results and discussion

Genotype and gene frequencies of blood proteins of adult Nigerian indigenous guinea fowls

Two iso-enzymatic variants controlled by a pair of allelic autosomal genes are detected at all the loci investigated (Table 1). Besides, the genotypic frequencies of Hb, Tf and Alb significantly ($P < 0.05$) deviated from the Hardy Weinberg equilibrium.

Table 1. Gene and genotype frequency distributions of blood proteins of adult Nigerian guinea fowl.

Locus	Genotypes	Observed	Expected	X2	Gene Frequency
Hb	AA	0	3.69		A: 0.35
	AB	21	13.65	8.71*	
	BB	9	12.68		B: 0.65
CA	FF	28	25.95		F: 0.93
	FS	0	3.91	6.35	
	SS	2	0.15		S: 0.07
Tf	AA	2	8.43		A: 0.53
	AB	28	14.95	22.90*	
	BB	0	6.63		B: 0.47
Alb	AA	2	8.43		A: 0.53
	AB	28	14.95	22.90*	
	BB	0	6.63		B: 0.47

*X2 significant at $P < 0.05$

Non-agreement of genotype frequencies with HWE is an indication that the loci under investigation have not been subjected to any of the systematic (selection, migration and mutation) and dispersive forces (genetic drift and inbreeding) *(Ramamoorthi et al., 2009).*

As far as authors are aware, precedent studies on biochemical characterization and estimation of genetic diversity of guinea fowl using blood protein systems are scarce. Unavailability of such study makes it impossible to compare the results. Nevertheless, the results were compared with the available reports on other indigenous poultry.

Haemoglobin

The two genotypes observed at the Hb locus in this study is lower than the three (HbAA, HbAB and HbBB) reported for Nigerian local chickens *(Yakubu and*

Aya, 2012), Muscovy ducks *(Oguntunji and Ayorinde, 2015),* chucker *(Alectoris chucker)* and pheasant *(Phasianus colchicus) (Ugur et al., 2006)*. Contrary to the prevalence of the heterozygouse genotype at this locus in guinea fowl, higher frequency of homozygous genotype HBBB has been reported for two ecotypes of Nigerian local chickens (Ige et al., 2013) while homozygouse HBAA was reported for Muscovy ducks *(Oguntunji and Ayorinde, 2015)* and three varieties of Nigerian indigenous chicken *(Yakubu and Aya, 2012).*
The higher frequency of gene Hb-B in guinea fowl agrees with the previous reports on mallard ducks *(Akinyemi et al., 2014)*, two Nigerian ecotypes of chicken *(Ige et al., 2013)* and four Chinese native breeds of chicken *(Okamoto et al., 2003).* Conversely, related studies on different indigenous poultry in Nigeria reported higher frequency of gene Hb-A *(Oguntunji and Ayorinde, 2015; Akinyemi et al., 2014; Yakubu and Aya, 2012; Salako and Ige, 2006)*. The non-agreement in gene and genotype frequencies of Hb in the population under study with some previous reports on indigenous poultry could probably be attributed to sample size or species differences in relevance of different genes and genotypes to their adaptability to their natural environments.

Schiliform and Folaranmi (1978) reported that different Hb allele types have selective advantage in different geographical areas. Higher frequency of Hb-B has been reported for breeds of sheep reared in drier northern part of Nigeria *(Akinyemi and Salako, 2012)* and sahelian countries of West Africa *(Missohou et al., 1999)*. The higher frequency of Hb-B in arid and semi-desert breeds of sheep in Nigeria and elsewhere lends credence to the assertion of *Schiliform and Folaranmi (1978)* that Hb-B confers adaptive advantage on carriers in arid region. Recent report of higher frequency (0.70) of Hb-B in Mallard duck *(Akinyemi et al., 2014)* commonly reared in drier savanna agro-ecological zones in Nigeria corroborates further this assertion. In view of the foregoing, it is postulated that higher frequency of Hb-B in the sampled population compared with high frequency of HB-A in other indigenous poultry species which are not limited to a particular geographical area is not just coincidental, but possibly an indicator of adaptive physiological modification in guinea fowl to survive in drier savanna region where they are mostly found in Nigeria. However, this assertion is subject to confirmation in future studies since the present sample size was not sufficient to draw conclusion.

Carbonic anhydrase

Comparison of the number of genes and genotypes reported in this study with previous reports on other indigenous poultry revealed disparity. Contrary to the two genotypes (CAFF and CASS) observed in this study, three CA variants of different compositions were reported for Muscovy ducks (CAFF, CASS and

CAMM) *(Oguntunji and Ayorinde, 2015)* and Nigerian local chickens (CAFF, CASS and CAFS) *(Ige et al., 2013).* However, in agreement with the report of this study, *Oguntunji and Ayorinde (2015)* reported higher frequency of CAFF in Muscovy ducks while *Ige et al. (2013)* documented prevalence of CASS in *Nigerian local chickens.*

The two genes segregating at the CA locus have their analogues in the earlier reports on Nigerian local chickens *(Ige et al., 2013)* and ducks *(Muscovy and Mallard) (Akinyemi et al., 2014). Conversely, Oguntunji and Ayorinde (2015)* reported three alleles (CA-F, CA-S and CA-M) at the same locus for Muscovy ducks. Similarly, the frequency obtained for CA-F in the present study is comparable to 0.913 reported for Muscovy duck *(Oguntunji and Ayorinde, 2015)* but higher than 0.675 to 0.763 reported for two Nigerian ecotypes of chicken *(Ige et al., 2013)* and 0.567 to 0.675 reported for Muscovy and Mallard ducks *(Akinyemi et al., 2014).* The striking aspect at this locus was that the prevalent genotype and gene were almost fixed and there was no heterozygote individual. The extremely higher frequency of CA-F is a pointer to its relevance to the yet-to-be-known physiological advantage it confers on this bird in its natural habitat.

Transferrin

Comparisons of the gene and genotype frequencies reported in this study are different from the reports of the related studies on indigenous poultry. In contrast to the two genotypes reported in this study; *Oguntunji and Ayorinde (2015)* reported six genotypes for Nigerian Muscovy ducks. In addition, the prevalence of heterozygouse genotype TfAB was contrary to TfBB and TfAC reported for Japanese indigenous fowls *(Tanabe et al., 2000)* and Nigerian local chickens *(Ige and Salako, 2014),* respectively.

The two co-dominant alleles segregating at this locus agrees with the two reported for Muscovy and Mallard ducks in Nigeria *(Akinyemi et al., 2014)* and three indigenous breeds of ducks in Indonesia *(Johari et al., 2013)* but lower compared to three and four reported for Chinese breeds of chicken *(Okamoto et al., 2003)* and Nigerian Muscovy ducks *(Oguntunji and Ayorinde, 2015),* respectively. The higher frequency of gene Tf-A agrees with the reports of *Johari et al. (2013)* on Mojosari breed of duck and *Akinyemi et al. (2014)* on Muscovy and Mallard ducks but contrary to the higher frequency of Tf-B reported for some Asian native breeds of chicken *(Okamoto et al., 2003; Tanabe et al., 2000),* two Indonesian local ducks *(Johari et al., 2013)* and Muscovy ducks in Nigeria *(Oguntunji and Ayorinde, 2015).*

Albumin

The two genotypes typed at this locus is lower compared to the three, four and seven observed in Japanese native fowl *(Tanabe, 2000)*, indigenous breeds of chicken in China *(Okamoto et al., 2003)* and Muscovy ducks in Nigeria *(Oguntunji and Ayorinde, 2015)*, respectively. The higher frequency of AlbCC and AlbBB in Muscovy ducks *(Oguntunji and Ayorinde, 2015)* and Asian indigenous chickens *(Tanabe et al., 2000)*, respectively were contrary to the higher frequency of AlbAB in this study.

The two co-dominant genes expressed at this locus was in agreement with the reports of *Akinyemi et al. (2014)* on Muscovy ducks and three breeds of Indonesian local duck *(Johari et al., 2013)* but at variance with the three reported for Asian indigenous breeds of chicken (Okamoto et al., 2003; Tanabe et al., 2000) and Mallard ducks *(Akinyemi et al., 2014)* and four reported for Muscovy ducks *(Oguntunji and Ayorinde, 2015)*. The higher frequency of gene Alb-A is consistent with the report of *Akinyemi et al. (2014)* on mallard duck.

Estimation of genetic diversity and local inbreeding co-efficient

Estimates of genetic diversity (H, ne, P) were presented in Table 2. The local inbreeding co-efficient (F) was -0.312.

Table 2: Estimates of genetic variability in Guinea fowl

Locus	Heterozygosity (He)	Effective number of allele (ne)	Percentage polymorphism (% P)
Hb	0.46	1.85	100
CA	0.13	1.15	100
Tf	0.50	2.00	100
Alb	0.50	2.00	100
Mean	0.40	1.75	100

Heterozygosity (H)

The average H reported for the loci under investigation was moderate and was comparable to 0.419, 0.41 and 0.450 reported for indigenous helmeted guinea fowl in Ghana using microsatellite markers *(Botchway, 2013)*, three varieties of Nigerian local chickens *(Yakubu and Aya, 2012)* and Nigerian Muscovy ducks *(Oguntunji and Ayorinde, 2015)*, respectively using blood protein markers.

The average estimated H obtained for the blood proteins in the present study was within the range (0.30 – 0.80) suggested for a marker to be useful in appraising genetic variation in a population *(Takenazi and Nei, 1996)*. The moderate heterozygosity in the population under study lends credence to the widely

believed assumption that gene pools of the locally adapted animals are rich and are reserviours of rare genes *(Oguntunji and Ayorinde, 2015).*

The lowest estimated H (0.130) reported for guinea fowl at CA locus could be linked to the absence of heterozygouse genotypes at this locus. Though two alleles were segregating at CA locus as observed for other three loci, however, its genotypic distribution revealed absence of heterozygosity, hence low H.

One possible underlying factor for the moderate heterozygosity in the present study is the low number of alleles segregating at the investigated loci. Though most blood protein genotypes were heterozygotes; they were controlled by just two alleles. A higher number of alleles segregating at different loci in a population are good indicator of genetic diversity and how rich the genome of the population is. In agreement with this assertion, syntheses of studies have shown that higher value of average heterozygosity within a breed could be attributed to the large number of allele detected at the tested loci (Kalinowski, 2002; Akinyemi et al. 2014). In addition, the small sample size could be another contributing factor to the moderate heterozygosity in the studied population. Small sample size limits the number of individuals covered and the number of genotypes and genes expressed at the different investigated loci of a population.

Effective number of allele (ne)

The mean 1.742 obtained in this study was comparable to 1.821 reported for Nigerian Muscovy ducks *(Oguntunji and Ayorinde, 2015)* using blood proteins but lower compared with the average value of 2.04 and 3.80 reported for indigenous guinea fowls in Ghana *(Botchway, 2013)* and four populations of guinea fowl in West Africa *(Kayang et al., 2010),* respectively using microsatellite markers. The higher ne in the referenced populations could be attributed to the fact that microsatellite markers used in those studies revealed higher number of alleles at different loci. Since this genetic variability parameter indicates the allelic richness of a population and its value is a function of number of alleles; consequently in most cases, higher number of allele segregating at different loci will generate higher ne and vice versa.

Percentage polymorphic loci (% P)

The complete polymorphism of the studied loci is consistent with the results of some previous studies on indigenous poultry using blood protein markers *(Oguntunji and Ayorinde, 2015; Akinyemi et al. 2014; Yakubu and Aya, 2012; Salako and Ige, 2006).* Polymorphicity in the studied population indicates absence of selection with respect to the investigated loci and reinforced the widely reported

diversity in the genome of indigenous livestock species in developing countries *(Oguntunji and Ayorinde, 2015).*

Local inbreeding coefficient (F)

This is a genetic index indicating the degree or level of inbreeding in a population. The F values calculated indicated further the potential reduction in heterozygosity due to non-random mating and may serve as an indication of inbreeding within the population *(Hartl, 1998)*. The negative F value reported in this study implies absence of inbreeding among members of the population and excess of heterozygoutes.

Furthermore, the reported low F might partially be attributed to the prevailing management system from which the sample used for this study was drawn. The extensive management system adopted by the farmers allows animals to scavenge, intermingle with animals from different genetic backgrounds and also encourage exchange of genetic materials; hence low inbreeding.

Conclusion

It is evident that majority of the genotypes typed was heterozygoutes at Hb, Tf and Alb loci. Since the base population from which the sample used for this study was random mating and had not undergone mild or intense selection for any trait; the prevalence of certain genes at their respective locus is suggestive of their importance to the adaptability and survival of this galliforme in its natural harsh tropical environment.

The reports of this study are limited due to the sample size and financial constraints. Future studies involving different breeds/varieties of guinea fowl, more sample size and application of microsatellite markers are recommended for further characterization and elucidation of the innate genetic diversity of the studied population.

Biohemijska karakterizacija nigerijske autohtone vrste biserke *(numida meleagris)*

Abel Olusegun Oguntunji, Kolawole Luke Ayorinde, Taiwo Olayemi Aremu

Rezime

Polimorfizam proteina u krvi se intenzivno koristi za karakterizaciju i procenu genetičke raznovrsnosti domaćih životinja. Ispitivanje biohemijske karakterizacije i procena genetske raznolikosti nigerijske autohtone biserke (Numida meleagris) je sprovedena pomoću četiri krvna proteina {hemoglobina (Hb), ugljene anhidraze (CA), transferina (Tf) i albumina (Alb)}. Celuloza acetat elektroforeza ukazuje da su svi proteinski markeri polimorfni, izražavajući dva ko-dominantna gena i dva genotipa na svojim respektivnim lokusima. Heterozigotni genotipovi su bili dominantni na HB, Tf i Alb lokusima, dok su homozigoni bili češći na CA. Alelna struktura je bila sličan (A i B) za HB, Tf i Alb, dok su F i S tipizirani na CA lokusu. Gen A ima veću frekvenciju pojavljivanja na Tf i Alb lokusima, dok su geni F i B bio pretežno na CA i Hb lokusima, respektivno. Prosečna ocenjena genetska raznolikost (heterozigotnost) preko genetskih sistema bila je 0,40 i umerena. Prevalencija gena F, A i B na svojim respektivnim lokusima ukazuje na njihovu relevantnost za opstanak i prilagodljivost ispitivane populacije u svom prirodnom staništu.

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