PRELIMINARY ANALYSIS OF ALLOZYME-BASED GENETIC DIVERSITY OF DIFFERENT POULTRY CHICKEN TYPES IN SOUTHWESTERN NIGERIA

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ABSTRACT

Genetic diversity of poultry chickens in southern Nigeria was assessed using 80 poultry chickens with four allozymes markers. The different poultry chicken types are Layers, Broilers and Indigenous poultry chicken sampled from farms, market places and rural homesteads. The mean gene diversity ranged from 0.22 in indigenous chicken from Abeokuta North to 0.45 in broilers from Ijebu Imushin. Deviations from Hardy–Weinberg equilibrium (HWE) were not statistically significant ($p < 0.05$) except at the Carbonic anhydrase locus. Observed and expected heterozygote ranged from 0.20 to 0.70 with a mean of 0.44 and 0.15 to 0.5 with a mean of 0.35. Average F statistic estimate across all loci revealed F_{IT}, F_{IS} and F_{ST} to be -0.11, -0.16 and 0.03 respectively. All the loci were polymorphic in all the populations sampled. The measure of genetic distance between pairs of chicken types indicated that the lowest distance was between layers and broilers (0.05) and the highest distance was between layers and indigenous chicken (0.10), respectively. The estimated dendrogram clustered these chicken types into twelve sub-populations and two major genetic groups. The study suggests that chicken types populations in southwestern Nigeria may be collapsed from three chicken types into two distinct genetic groups, possibly due to extensive cross-breeding and gene flow between them, which are symptomatic of uncontrolled crossing across much of the country. The populations studied were out bred in nature with small genetic differentiations among the various populations.

Keywords: Genetic diversity; chicken type; allozymes; southwestern Nigeria; dendrogram; heterozygosity.

INTRODUCTION

Domestic chickens fulfill various roles ranging from food and entertainment to religion and ornamentation. Existing poultry varieties comprise a wide range of breeds and strains that have evolved in the process of domestication and systemic breeding programmes. Since domestication, chickens have been distributed to various countries, continents and cultures. The use of chicken for food has been limited to a few specialized commercial breeds and a vast range of non-commercial chicken breeds. The Nigerian indigenous chicken is a dual-purpose bird that is used both for meat and egg production in the rural and peri-urban areas of the country; they are found in large numbers distributed across different agroecological categories under a traditional family based scavenging management system Ajayi, (2010). Most of the birds are kept in small flocks under a scavenging system and the feed resources for the birds are household refuse, homestead pickings, crop residues, herbage, seeds, green grasses, earthworms, insects and small amount of supplemented feeds offered by the flock owner. They are well adapted to the adverse climatic conditions of the tropical environment and low management inputs. They contain a highly conserved genetic system with high levels of heterozygosity (Wimmers *et al.,* 2000). These indicate that they are highly important farm animals, kept for good source of animal protein, for income and socio-cultural roles. Ebozoje and Ikeobi (1995) reported the adaptive potentials of the Nigerian indigenous chicken to varied ecological conditions, stresses and diseases. There have been some efforts at characterizing the Nigerian indigenous chickens. These efforts include classification based on ecotypes (Sonaiya and Olori, 1990), plumage and shank colour (Ikeobi *et al.,* 1996), possession of the major genes of feather distribution and feather structure (Peters *et al.,* 2002, 2005, 2007, 2008a, and 2008b). Wekhe (1992) earlier reported that Nigerian indigenous chickens are more resistant to infectious disease agents

than their exotic counterparts. These chicken population estimated at about 140 million (FAO, 2006) is currently underutilized in the development of acceptable improved breeds. There is a need to expand the narrow genetic base in which the world's poultry breeding company currently operates by including local chicken resources that has been widely reported to be well adapted to the local conditions. In addition to the phenotypic characterization that has been done and reported above, there is a need to perform molecular characterization for information with regard to phylogeny, diversity and relatedness. Protein polymorphisms have been used as marker systems to estimate genetic variation within and between chicken populations (Awobajo, et al., 2020) and while microsatellites and other DNA markers are more polymorphic and informative than protein markers in diversity studies, there is a need to use protein markers to do a preliminary screening on genetic diversity of Nigerian local chickens. This investigation therefore sought to find the genetic diversity, as a preliminary assessment, among Nigerian indigenous chickens broiler and layers reared intensively using blood protein polymorphisms by estimating genetic similarity. The main objective of this research is to examine genetic diversity in different chicken types using allozymes in southwestern Nigeria, **w**hile the specific objectives are; to evaluate alleles and genotypes and their frequencies. To evaluate Hardy-Weinberg's equilibrium for the four population and to estimate heterozygosity of allele at various protein loci and testing Hardy-Weinberg's equilibrium.

MATERIALS AND METHODS

A total of 80 samples made up of 25 layers, 25 broilers and 30 indigenous chickens were sampled across Ogun State in southwestern Nigeria but only 72 were typed. About 10 ml of whole blood was collected by the wing vein venipuncture, using needle and syringe into heparinized vacutainer tubes and stored at 4 °C and transported to the laboratory. Red blood cell was prepared from the erythrocyte fraction of heparinized blood by centrifuging at 2500-3000 rpm for 10 mins at 4 ^{O}C. The RBCs were lysed with a fourth fold volume of distilled H_2O to release heamoglobin according to RIKEN, (2006). The supernatant was used. Cellulose acetate plates were soaked in the same buffer as the electrode buffer for at least 20 minutes. Samples were applied on to the plate using the applicator and once loaded; plates are rested on the wicks in the tank. Since the current runs from the cathode to the anode electrode (negative to positive), the loaded zone on the plate was positioned at the cathodal end of the tank for the majority of the enzyme systems which migrate anodally. For those systems which migrate cathodally e.g carbonic anhydrase, the loading zone was placed on the anodal end, RIKEN, (2006). When the gel run was complete, the plates were removed from the tank and placed in an empty Petri dish. They were stained with Ponceau stain before they dry out, RIKEN, (2006). After 20 minutes the plate was sufficiently stained, it was destained several times until clear and sharp bands appear, the bands were scored visually based on their migratory pattern as described by RIKEN, (2006) and direct counting was used for calculating gene frequencies.

Table 1: Electrophoresis conditions

Locus	System	Time	PH	Voltage	Stain
Haemoglobin	Tris EDTA borate	40	8.4	350	Ponceau stain
Transferin	Tris glycine	45	5.6	150	Ponceau stain
Carbonic Anhydrase	EDTA Sodium Acetate	45	5.6	200	Ponceau stain
Albumin	Tris Citrate	30	7.6	180	Ponceau stain

Statistical analysis

Polymorphism information content (PIC) for each microsatellite marker was calculated using CERVUS software (Marshall, 1998). Population statistics were estimated using Tools for Population Genetic Analyses (TFPGA) software version 1.3 (Miller, 1997). The analyses included allele frequencies, expected heterozygosity (H_E), observed heterozygosity (H_O) and Hardy–Weinberg equilibrium (HWE). For the analysis of genetic differentiation between populations, Wright's fixation indices were computed by bootstrapping with a 95% confidence interval based on 1 000 replicates. Additionally, F-statistics covering F_{IS}, consanguinity or loss in heterozygosity within population; F_{ST}, measure of differentiation

among populations, and F_{IT} global loss in heterozygosity and exact test of Hardy–Weinberg proportion for multiple alleles (Guo and Thompson, 1992) were estimated using the Markov Chain procedure (10 batches, 1 000 iterations, 1 000 de-memorization steps). Both genetic distance (DA) estimated according to the method of Nei (1978) and the UnPaired Group Method of Algorithm (UPGMA) for dendrogram construction were carried out using Tools for Population Genetic Analyses TFPGA version 1.3 (Miller, 1997).

RESULTS AND DISCUSSIONS

Allozymes markers used in this study are similar to Awobajo et al., (2016) for diversity in Nigerian West African Dwarf goats. All loci studied were polymorphic as indicated by the average PIC of 100%. Since Takezaki and Nei (1996) suggested that microsatellite loci for genetic diversity studies should have more than four alleles to reduce the standard error estimates of genetic distances, the total numbers of alleles per locus and high PIC values suggest that these markers are informative for genetic diversity in different chicken types in Nigerian and that different Nigerian chicken types possess a wide genetic base that allows for adaptation to a wide variety of ecological environments. Allelic richness in Nigerian chickens reported in this study is similar to what was reported by Ajayi, et al. 2013. Gene diversity indicated by H_E in Table 2 had a range of 0.16 for haemoglobin to 0.50 for carbonic anhydrase with an average of 0.35, this current estimates fall within the recommended average heterozygosity between 0.3 and 0.8 in a population (Takezaki and Nei, 1996), for markers to be useful for measuring genetic variation. Toro and Maki-Tanila (2007) suggested that the high genetic diversity observed within population groups could arise from overlapping generations and population mixtures from different geographical locations, with natural selection favouring heterozygosity or subdivision.

Table 2. Heterozygosity and F-statistics

Locus	H_E	H_O	F_{IT}	F_{ST}	F_{IS}
Haemoglobin	0.16	0.20	-0.06	0.19	-0.28
Transferin	0.37	0.44	0.13	-0.06	-0.16
Carbonic Anhydrase	0.50	0.70	-0.62	-0.01	-0.64
Albumin	0.36	0.42	0.15	0.11	-0.04
Mean over all loci	0.35	0.44	-0.11	0.03	-0.16
Jack knifing (all loci)			-0.11±0.23	0.03± 0.05	-0.32±0.18
Bootstrapping (95% CI)			0.14	0.13	-0.07
Bootstrapping (95% CI)			-0.46	-0.03	-0.56

H_E, expected heterozygosity; H_O, observed heterozygosity; F_{IT}, amount of inbreeding like effect within entire population; F_{ST}, amount of variations due to differentiation between subpopulations; F_{IS}, amount of inbreeding like effect among individuals within subpopulations; CI, confidence interval.

The effect of these factors according to Agha et al. (2008) is more pronounced when the effective population size is very large, which is supported by the poor infrastructure on ground presently for livestock improvement and lack of proper breeding policy in Nigeria. However, all the loci had H_O higher than their H_E indicating departure from random mating which suggest that they are heterozygous in these populations and may indicate an outbred populations or may be linked to other loci affecting morphological, productive or adaptive traits undergoing selection (Dixit et al., 2008; Bruno-de-Sousa et al., 2011) . Observed negative values for F_{IT} and F_{IS} in all the loci suggests excess heterozygosity resulting from mating of unrelated individuals which may be a consequence of the emergent population structure of Nigerian chicken populations, not previously uncovered by protein polymorphisms used

which has also been reported in other studies on other livestock (Barker et al., 1997; Luikart et al., 1999; Agha et al., 2008; Rout et al., 2008; Dixit et al., 2009).. Additional factors include population subdivision owing to genetic drift, null alleles and selection against heterozygotes or inbreeding (Hoarau et al., 2005). The F_{ST} values ranged from -0.06 for transferrin to 0.19 for haemoglobin with a mean of 0.03 this value is low indicate small genetic differentiation according to (Weir and Cockerham, 2014). Low F_{ST} indicates some measure of gene flow between the sampled populations. According to Laval et al. (2000), migration may exert a greater effect than mutation or drift on the reduction in genetic differentiation between populations.

Population structure and genetic diversity.

Average gene diversity within chicken types is described locus by locus in Table 3. Gene diversity ranged between 0.17 for layers from Sagamu on transferin and broilers from Sagamu and Ijebu Imushin on haemoglobin and tranferrin respectively to 0.69 for broilers from Abeokuta North on tranferrin.

Table 3 Measure of gene diversity in studied populations.

Population	No	Chicken Type	Haemoglobin	Transferin	Carbonic Anhydrase	Albumin
ABKN	7	Layers	0.00	0.54	0.53	0.00
AOO	6	Layers	0.00	0.53	0.55	0.53
Sagamu	6	Layers	0.30	0.17	0.53	0.49
IJM	6	Layers	0.41	0.41	0.53	0.30
ABKN	7	Broilers	0.00	0.69	0.54	0.36
AOO	6	Broilers	0.00	0.53	0.49	0.41
Sagamu	6	Broilers	0.17	0.55	0.55	0.49
IJM	6	Broilers	0.53	0.17	0.55	0.55
ABKN	5	Indigenous	0.00	0.00	0.53	0.36
AOO	5	Indigenous	0.00	0.20	0.36	0.56
Sagamu	5	Indigenous	0.20	0.47	0.36	0.47
IJM	5	Indigenous	0.36	0.20	0.53	0.20

ABKN --→ Abeokuta North, AOO ---→ Ado Odo Otta, , IJM ---→ Ijebu Imushin.

Table 4 shows observed and expected heterozygosity among different chicken types by location. The H_E range in this study was higher than 0.54 reported by Muema *et al.* (2009), 0.51 reported by Adebambo *et al.* (2011) in Nigerian goats, and is within 0.61–0.78 in Indian goats (Rout *et al.,* 2008; Dixit *et al.,* 2010). Observed and expected heterozygosity ranged between 0.30 to 0.67 and between 0.22 to 0.45 for indigenous chicken from Abeokuta North and broilers from Ijebu Imushin respectively. The values of H_O and H_E indicate a departure from random mating in all the population and that mates are less related leading an heterozygote populations, (Dixit et al., 2008; Bruno-de-Sousa et al., 2011).

Table 4. Average genetic diversity among breeds by location

Population	Chicken Type	Number	H_O	H_E
ABKN	Layers	07	0.46	0.27
	Broilers	07	0.50	0.40
	Indigenous	05	0.30	0.22
AOO	Layers	06	0.42	0.40
	Broilers	06	0.42	0.36
	Indigenous	05	0.40	0.28
Sagamu	Layers	06	0.42	0.37

	Broilers	06	0.54	0.44
	Indigenous	05	0.35	0.37
IJM	Layers	06	0.54	0.41
	Broilers	06	0.67	0.45
	Indigenous	05	0.40	0.32

ABKN --→ Abeokuta North, AOO ---→ Ado Odo Otta, IJM ---→ Ijebu Imushin. No., number of individual goats sampled; H_O, observed heterozygosity; H_E, expected heterozygosity.

Genetic distance matrix of the three different chicken types (Tables 5) showed that indigenous chickens shared the highest (94.26%) similarity with broilers and the greatest diversity (11.26%) is between indigenous chickens and layers populations.

Table 5. Genetic distance matrix showing genetic identity and diversity by chicken type.

chicken type	Layers	Broilers	Indigenous chicken
Layers	_____	0.0591	0.1126
Broilers	0.9362	_____	0.0659
Indigenous chicken	0.8935	0.9426	_____

Nei's (1972) genetic distance matrix. Genetic identity is entered below the diagonal, genetic diversity is entered above the diagonal.

Genetic distance matrix of the four populations of different chicken types (Tables 6) showed that population from Ado Odo Otta shared about 96.16% similarities with population from Sagamu. The greatest diversity (7.73%) is between population from Sagamu and Ijebu Imushin. Results from table 7 showed that two sub-populations of broilers from Ado Odo Otta and Sagamu are more related (98.25%) than all others, while genetic diversity was greatest between populations of broilers from Abeokuta North and indigenous chicken from Ado Odo Otta (37.92%). Genetic distance between broilers and layers chicken type was the closest, while the genetic distance between indigenous chicken and broiler was the farthest.

Table 6. Genetic distance matrix showing genetic identity and diversity by chicken populations.

Population	ABKN	AOO	Sagamu	IJM
ABKN	_____	0.0606	0.0745	0.0415
AOO	0.9256	_____	0.0391	0.0626
Sagamu	0.9393	0.9616	_____	0.0773
IJM	0.9593	0.9282	0.9412	_____

Nei (1972) genetic distance matrix. Genetic identity is below the diagonal, genetic diversity is above the diagonal.
ABKN --→ Abeokuta North, AOO ---→ Ado Odo Otta, SGM --→ Sagamu, IJM ---→ Ijebu Imushin.

Lower genetic distance observed in this study (between different chicken types and between different sampled populations) may indicate a higher level of cross-breeding among chickens in Ogun State of Nigeria concomitant with higher population of humans and by extension higher population density of reared chickens.

Table 7. Genetic distance matrix showing genetic identity and diversity by chicken type and location.

	ABKLAY	ABKBRO	ABKIND	AOOLAY	AOOBRO	AOOIND	SAGLAY	SAGBRO	SAGIND	IJBLAY	IJBBRO	IJBIND
ABKLAY		0.0830	0.1738	0.0479	0.2104	0.3056	0.0854	0.2290	0.0963	0.0183	0.1116	0.0744
ABKBRO	0.9725		0.2788	0.1152	0.1406	0.3792	0.0965	0.1584	0.1265	0.0949	0.2258	0.2466
ABKIND	0.9068	0.8904		0.1189	0.1531	0.0242	0.2627	0.2237	0.1019	0.2409	0.0626	0.0527
AOOLAY	0.9365	0.9645	0.9259		0.1092	0.1783	0.0305	0.0955	0.0500	0.0483	0.0789	0.0683
AOOBRO	0.7984	0.8611	0.8400	0.9473		0.1250	0.1052	0.0176	0.0312	0.1798	0.1056	0.1784
AOOIND	0.8462	0.8598	0.9553	0.9321	0.9379		0.3166	0.1885	0.1196	0.3571	0.0925	0.1151
SAGLAY	0.8912	0.9117	0.6997	0.8873	0.8282	0.7286		0.0641	0.0704	0.0457	0.1510	0.1670
SAGBRO	0.8366	0.8998	0.8355	0.9693	0.9825	0.9001	0.8825		0.0541	0.1743	0.1495	0.2251
SAGIND	0.9340	0.9241	0.9529	0.9513	0.9089	0.9699	0.8367	0.8966		0.0770	0.0361	0.0656
IJBLAY	0.9487	0.9393	0.7859	0.9031	0.7996	0.7690	0.9760	0.8581	0.8879		0.1161	0.0979
IJBBRO	0.7815	0.7979	0.9095	0.8812	0.8535	0.9080	0.6844	0.8688	0.8912	0.7567		0.0279
IJBIND	0.9283	0.8944	0.9819	0.9082	0.7953	0.9182	0.7367	0.8103	0.9532	0.8405	0.9204	

Nei's (1972) genetic distance matrix. Genetic identity is entered below the diagonal, genetic diversity is entered above the diagonal.

ABKLAY= Abeokuta Layers, ABKBRO= Abeokuta Broilers, ABKIND= Abeokuta indigenous chicken, AOOLAY= Ado Odo Otta Layers, AOOBRO= Ado Odo Otta Broilers, AOOIND= Ado Odo Otta indigenous chicken, SAGLAY= Sagamu Layers, SAGBRO= Sagamu Briolers, SAGIND= Sagamu indigenous chicken, IJBLAY= Ijebu Imushin Layers, IJBBRO= Ijebu Imushin Broilers, IJBIND= Ijebu Imushin indigenous chicken.

Regardless of location, the indigenous chicken stood out clearly as a chicken while broilers and layers showed a measure of close relationship (Figures 1).

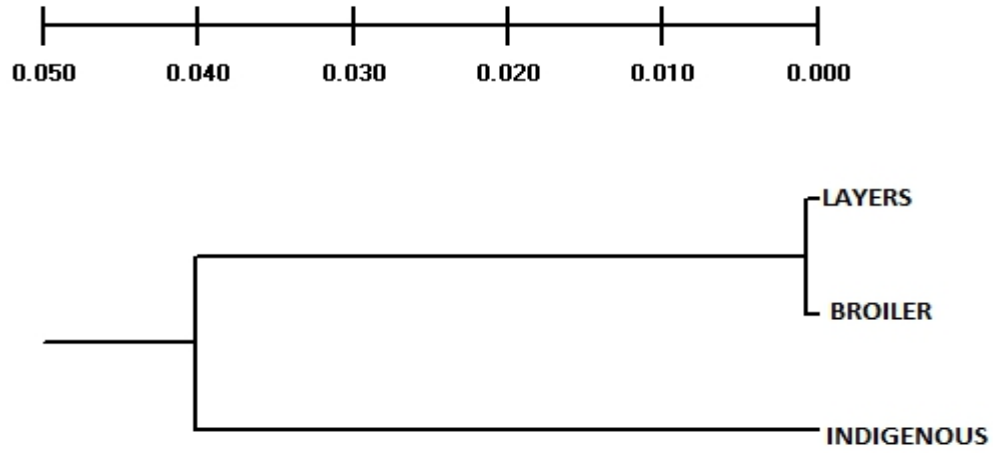

Figure 1: The Dendrogram of the three chicken types reared in Ogun State.

The chicken populations branched in to two with Ado Odo Otta and Sagamu populations clustering together on one side while Abeokuta north and Ijebu Imushin populations clustered together on the other side (Figures 2).

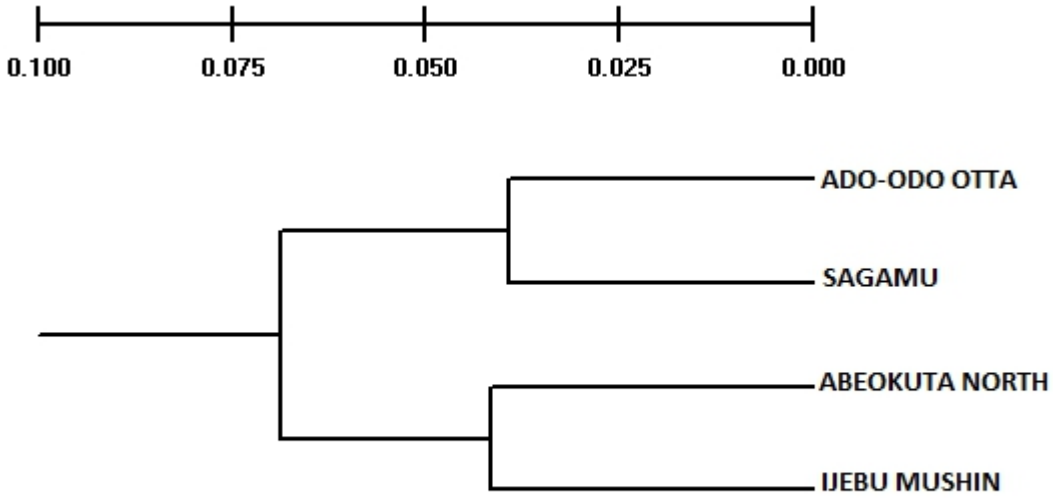

Figure 2: Genetic relationship of chicken from four different populations

In Figures 3, the chicken population branched into two with all the chicken types from four populations clustering together except for broilers from Abeokuta north and layers from Abeokuta north, Sagamu and Ijebu Imushin forming another cluster.

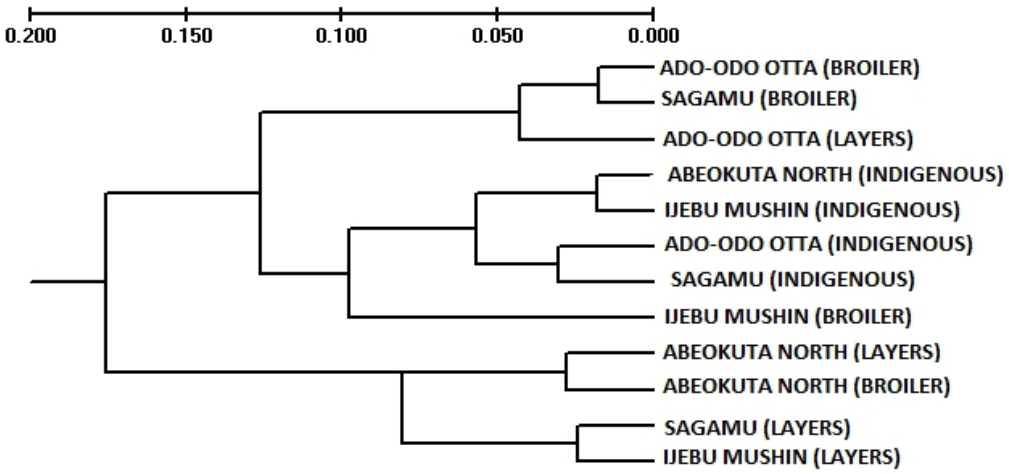

Figure 1: Genetic relationship of 3 chicken types from 4 different populations

CONCLUSION

This study revealed great genetic diversity among the different poultry chicken typed reared in Ogun State based on the value of their expected gene diversity on the population studied which give room for improving the chicken types reared in Ogun State. Information provided in this study is important to enable the development of appropriate breeding and policies strategies to improve the indigenous chicken populations and serve as reference for larger-scale diversity studies using advanced technology like microsatellite analysis. It also serves as a guide in defining objectives for designing future investigations of the genetic integrity and developing conservation strategies for chicken species.

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