Genetic diversity of chicken types reared in Ogun State using carbonic anhydrase polymorphism

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ABSTRACT

This study was carried out to examine the genetic diversity of different chicken types reared in Ogun State using Carbonic anhydrase. A total of 80 birds comprising 25 boilers, 25 layers and 30 indigenous chickens were purposely selected from Abeokuta North, Ado Odo Otta, Sagamu and Ijebu Imushin locations. Blood samples of 5 mL were drawn from the wing vein of each bird via 2ml hypodermal needle into anticoagulant treated (heparinized) vials. Carbonic anhydrase polymorphism was investigated using cellulose acetate paper electrophoresis. Based on the estimate allele frequencies the population was characterized by their genetic distance Two alleles were reported for each of the population (F and S in carbonic anhydrase). The results obtained showed that, the estimate of heterozygosity was 0.80, 0.92 and 0.60 for layers, boilers and indigenous chicken population respectively. The closest genetic distance relationship was found between the boilers and layers chicken populations (D = 0.0008), as compared to the genetic distance relationship of the indigenous chickens and broilers (D = 0.0459). The relationships among the chicken population were consolidated by the dendrogram that emanated from their genetic distance. The result obtain will be useful as an initial guide in defining objectives for future investigations of genetic integrity and developing conservation strategies for chicken species.

Keywords: Carbonic anhydrase; Chicken; Electrophoresis; Genetic diversity; Heterozygosity; Polymorphism.

INTRODUCTION

The livestock sector is vital to the socio-economic development of Nigeria. It contributes about 9-10% of agricultural GDP (FAO, 2006). Livestock represents an important source of high-quality animal protein, providing about 36.5% of the total protein intake of the country.

Nigeria's chicken population is estimated at about 150 million (UNDP, 2006) of which 25, 15 and 60% are kept under commercial, semi-commercial, and backyards production system respectively. In case of backyard conditions, genetic diversities in the genome of indigenous chickens in developing world including Nigeria, are reservoirs of rare but valuable genes for production, adaptation and resistance to endemic diseases. Diversity is the product of interaction between environmental and genetic effects leading to the differentiation of the morphological, physiological and productive traits. Genetic diversity provides the raw materials for breed improvement, and adaptation to changing circumstances (Ceriotti et al., 2003). In recent years, DNA-based technologies are methods of choice for genetic characterization of livestock including poultry resource (Arora et al., 2011); but challenged by its high cost, lack of infrastructural facilities and shortage of trained experts. Luckily enough advances in the biotechnology opened up molecular level routine electrophoresis techniques employed for the detection of polymorphism at protein and enzyme loci and serological and immunogenetic procedures for the measurement of variation (Salako et al., 2007). Protein/allozyme polymorphs remain tremendously useful, in developing countries, due to their utility, cost, genetic information accessed and simplicity in data interpretation (Rege and Okeyo, 2006).

Blood protein markers have been widely used in characterization and estimation of genetic diversity within and among breeds and species (Akinyemi and Salako, 2012; Yakubu and Aya, 2012). There has been loss of indigenous animal genetic resources attributed to the indiscriminate introduction of exotic genetic resources, before the proper characterization, utilization and conservation of indigenous genetic resources, (FAO, 1999). This being the cases, the current study was undertaken with the objective of examining genetic diversity in different Chicken types reared in Ogun State in southwestern Nigeria using Carbonic anhydrase polymorphism.

MATERIALS AND METHODS

Description of the Study Area

The sample area includes Ijebu Imushin, Sagamu, Abeokuta North and Ado Odo Otta representing the four provinces of (Ijebu, Remo, Egba and Yewa respectively) Ogun State. The sampling areas were randomly selected using grid method on the map of Ogun State Nigeria.

Blood sample collection and processing

A total of 80 birds comprising of 25, 25 and 30 broilers, layers and indigenous chickens respectively, were randomly selected. Whole blood sample of 5ml was collected from the wing vein into heparinized bottle, stored at 4°C and transported to the animal science genetic laboratory. The blood samples were separated into plasma and red cells by centrifugation at 4°C for 10 minutes at 3500 rpm. Red cells were washed three times in normal saline solution and lysed with cold distilled water. Hemolysates were stored in a refrigerator at -20°C until the electrophoretic studies were carried out. Carbonic anhydrase (CA) were typed, using cellulose acetate electrophoresis as described by RIKEN (2006). Typing of CA was done using red blood cells in 4 volume dH_2O. Prepared and labeled cellulose acetate strips were soaked in EDTA Sodium acetate buffer at P^H 5.6 and blotted lightly with filter paper to remove excess buffer. Hemolysates were applied and electrophoresis carried out with EDTA Sodium acetate buffer at P^H 5.6 as electrode buffer at 200v for 35 minutes. The strips were stained for 2 hours in a dark place using ponceau S while destaining was done using 1% acetic acid solution.

Genetic Analysis

All computations were performed using Popgen (Yeh et al., 1999) and Tools for population genetic analysis (TFPGA), as suggested by Miller, (1997). The population genetic analysis performed included genetic distance (Nei's Original 1972), allele frequency, observed and expected heterozygosity, Hardy-Weinberg Equilibrium coefficient (Wright's F_{IS} and F_{IT}, F_{ST} estimates) and drawing Unpaired Group Method of Algorithm (UPGMA) dendrograms.

RESULTS AND DISCUSSIONS

The results of the Allele and Genotype frequencies are presented in Table 1 and 2. According to Table 1, the highest and lowest frequency of 0.80 and 0.2 was observed in CA^F and CA^S from Ado Odo Otta and Sagamu population respectively with a mean frequency range of 0.35 – 0.65 in CA^S and CA^F from Ado Odo Otta chicken population. This result aligns with that of Ige et al., (2013), who reported the predominance of CA^F in Yoruba and Fulani ecotype chicken. The three chicken types considered in the current study were polymorphic at the CA locus.

As indicated in Table 2, the genotype frequency ranges from 0.14 for CA^{FF} in (Abeokuta North) layers to 1.00 for CA^{FS} in (Abeokuta North, Sagamu and Ijebu Imushin) broiler birds. CA^{FF} genotype does not exist in broiler from Abeokuta North, Sagamu and Ijebu Imushin. Similarly, CA^{SS} genotype for all the chicken

type does not exist in Abeokuta North, Sagamu and Ijebu Imushin and in broilers and indigenous chicken from Ado Odo Otta. Average genotype frequency ranged from 0.1 to 0.90 for CA^{FF} and CA^{FS} respectively from Abeokuta North. However, Oguntunji and Ayorinde (2015) reported higher frequency of CA^{FF} in Muscovy duck. The result of this study is contrary to that of Ige *et al*., (2013), who reported that CA^{SS} was the most prevalent in Nigerian local chickens.

Table 1: Allele frequency of the Experimental Chickens studied.

Population	Allele	Sample size	Allele Frequency (Type)			Average allele frequency
			Layer	Broiler	Indigenous	
Abeokuta North	CA^{F}	19	0.57	0.50	0.60	0.56
	CA^{S}		0.43	0.50	0.40	0.44
Ado-Odo Otta	CA^{F}	17	0.50	0.67	0.80	0.65
	CA^{S}		0.50	0.33	0.20	0.35
Sagamu	CA^{F}	17	0.59	0.50	0.80	0.61
	CA^{S}		0.41	0.50	0.20	0.39
Ijebu Imushin	CA^{F}	17	0.59	0.50	0.60	0.56
	CA^{S}		0.41	0.50	0.40	0.44
Average	CA^{F}	70	0.56	0.54	0.70	0.60
	CA^{S}		0.44	0.46	0.30	0.40

Table 2: Genotype frequency of the Experimental Chickens Studied.

Population	Genotype	Sample size	Genotype Frequency/Type			Average Genotype frequency
			Layer	Broiler	Indigenous	
Abeokuta North	CA^{FF}	19	0.14	0.00	0.20	0.10
	CA^{FS}		0.86	1.00	0.80	0.90
	CA^{SS}		0.00	0.00	0.00	0.00
Ado-odo otta	CA^{FF}	17	0.17	0.33	0.60	0.35
	CA^{FS}		0.66	0.67	0.40	0.59
	CA^{SS}		0.17	0.00	0.00	0.06
Sagamu	CA^{FF}	17	0.17	0.00	0.60	0.23
	CA^{FS}		0.83	1.00	0.40	0.77
	CA^{SS}		0.00	0.00	0.00	0.00
Ijebu Imushin	CA^{FF}	17	0.17	0.00	0.20	0.11
	CA^{FS}		0.83	1.00	0.80	0.89
	CA^{SS}		0.00	1.00	1.00	1.00
Average	CA^{FF}	70	0.16	0.08	0.40	0.20
	CA^{FS}		0.80	0.92	0.60	0.79
	CA^{SS}		0.04	1.000	1.00	1.00

The results of Hardy-Weinberg Equilibrium exact test of the experimental chickens are presented in Table 3. There was no significant difference between all the chickens studied except that of the broilers in Hardy-Weinberg Equilibrium exact test ($P>0.05$). There was significant difference ($p<0.05$) among broilers population selected from Abeokuta North. This result implied the existence of Non-random mating within the chicken population of Abeokuta North, (Falconer, 1989).

Table 3: Test for Hardy-Weinberg Equilibrium of the Chickens Studied.

Population	Allele	Sample size	Hardy-Weinberg Equilibrium /(Type)			Average
			Layer	Broiler	Indigenous	
Abeokuta North	CA^{F}	19	0.1608^{NS}	0.0373*	0.4286^{NS}	0.0008**
	CA^{S}					*
Ado-odo otta	CA^{F}	17	1.0000^{NS}	0.5152^{NS}	1.0000^{NS}	0.5914^{NS}
	CA^{S}					
Sagamu	CA^{F}	17	0.3939^{NS}	0.0909^{NS}	1.0000^{NS}	0.0330*
	CA^{S}					
Ijebu Imushin	CA^{F}	17	0.3939^{NS}	0.0909^{NS}	0.4286^{NS}	0.0026**
	CA^{S}					
Average	CA^{F}	70	0.0039**	0.0000***	0.1188^{NS}	0.0000**
	CA^{S}					*

*: $P \leq 0.05$; ***: $P \leq 0.001$; NS: not significant

The results of the statistics (F-statistics) of estimates of population structure are presented in Table 4. All estimates of F_{IS} and F_{IT} were positive value showing deficiency in heterozygote indicating the occurrence of inbred populations. Heterozygote deficiency may be due to factors like, population subdivision owing to genetic drift, null alleles and selection against heterozygotes or inbreeding (Hoarau *et al.,* 2005). All estimates of F_{IS}, F_{IT} and F_{ST} were not significant (P<0.05) except for F_{ST} value for population from Abeokuta North, Ado-odo otta and Ijebu Imushin (P<0.05). Average F_{ST} estimate for the whole population was 0.02, the value of which is considered to be an indication of small genetic differentiation among the populations (Weir and Cockerham, 2014).

According Table 5 the low observed heterozygosity, (H_O), for all the chicken types in all populations (0.21) tends to indicate decreasing genetic variability in the chicken populations at the Carbonic anhydrase locus. It also indicates departure from random mating which suggest that they are homozygous in these populations. This shows that they have been subjected to certain level of inbreeding as a result of some form of selection within the various populations. This also indicates on-going selection 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). This may be due to the population structure of the chicken sample (Rychlik et al., 2011). The observed is lower than expected and this maybe as a result of Non-random mating in the populations.

Table 4: Wright F-Statistic analysis of different chicken types.

Population	f(F_{IS})	F(F_{ST})	F(F_{IT})
Abeokuta North	0.7925^{NS}	0.0060**	0.8033^{NS}
Ado-odo otta	0.2899^{NS}	0.0327*	0.2477^{NS}
Sagamu	0.6808^{NS}	0.0667^{NS}	0.5686^{NS}
Ijebu Imushin	0.7664^{NS}	0.0091**	0.7825^{NS}
Total for all location	0.6471^{NS}	0.0214*	0.6118^{NS}

*: P ≤ 0.05; **: P ≤ 0.01; NS: not significant

Table 5: Effective sample size (N) and expected and observed heterozygosity (H_E and H_O).

Population	Observed and Expected Heterozygosity									Average		
	Layers			Broilers			Indigenous					
	N	H_E	H_O	N	H_E	H_O	N	H_E	H_O	N	H_E	H_O
Abeokuta North	7	0.86	0.14	7	1.00	0.00	5	0.80	0.20	19	0.90	0.10
Ado odo otta	6	0.67	0.33	6	0.67	0.33	5	0.40	0.60	17	0.59	0.41
Sagamu	6	0.83	0.17	6	1.00	0.00	5	0.40	0.60	17	0.77	0.23
Ijebu Imushin	6	0.83	0.17	6	1.00	0.00	5	0.80	0.20	17	0.89	0.11
Total	25	0.80	0.20	25	0.92	0.08	20	0.60	0.40	70	0.79	0.21

As shown in Table 6, the genetic distance among the population studied were small but within the range of 0.001 and 0.046 reported by Nei, (1976) for local breeds. The longest distance was observed between indigenous chicken and the broilers while the shortest distance was observed between broilers and layers. For identity, layers and broilers are 99.92% closer to each other while broiler and indigenous chicken were 95.51% closer to each other.

Table 6: Genetic distance and genetic identity of the Experimental chickens

Breed	Layers	Broilers	Indigenous
Layers	*****	0.0008	0.0345
Broilers	0.9992	*****	0.0459
Indigenous	0.9661	0.9551	*****

Nei's genetic identity above diagonal and genetic distance below diagonal

Figure 1 showed that the layer and broiler are genetically closer than the indigenous chickens. All the chicken type where together until a point where it branched and the layer and broiler cluster together while the indigenous on another node, later the layer and broiler separated to add different nodes.

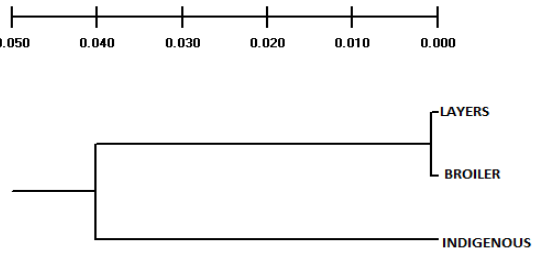

Figure 1: The Dendrogram of the three chicken types reared in Ogun State.

CONCLUSION

This result revealed that there is a high level of inbreeding within the various populations of the chicken types studied, with small genetic differentiation among the various chicken populations. It also revealed the closeness of broilers and layers compare to the indigenous chicken populations across the study state, with greater genetic distance occurring between the other two chicken types which have been selected for their specific production traits. This give room for improving the chicken types reared in Ogun State.

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