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Genetic Diversity between Exotic and Nigerian Indigenous Turkey at different Structural Loci

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Target Audience: *Animal breeding and genetics*

Abstract

Poultry genetic resources in general are considered to be the most endangered and under-conserved, detailed attention is therefore needed on the existing genetic resources to reduce or prevent the increasing genetic erosion of local livestock. This study was conducted to characterize and estimate genetic diversity in Nigerian indigenous turkey and exotic turkey using blood proteins (Haemoglobin, Transferrin and Albumin) and enzyme (Carbonic Anhydrase and Esterase 1) markers. A total of 110 turkeys comprising 50 Nigerian indigenous turkeys and 60 exotic turkeys were used for the analysis. Separation of blood protein genotypes was achieved using cellulose acetate electrophoresis. The populations were characterized for their genetic variability using allele frequencies, observed heterozygosity, F-statistics (F_{IT}, F_{IS}, F_{ST}), test for Hardy-Weinberg and Genetic distance. Eleven variants were found at the five loci studied, two co-dominant allele A and B controlling three genotypes AA, AB, and BB were observed at Haemoglobin, Carbonic Anhydrase, Albumin and Esterase1 loci for both indigenous and exotic turkey breeds, a third allele C was observed in Transferrin locus. Allele A was the most frequency at the Hb, CA, Alb and Es1 locus in Nigerian indigenous turkey with frequencies 0.541, 0.541, 0.520, and 0.520 respectively and exotic turkey at Hb, Alb, and Es1 with frequencies 0.508, 0.617, and 0.508 respectively. Chi Square result indicated deviations from Hardy Weinberg equilibrium in the two populations. The average heterozygosity values were 0.56 and 0.477 indicating high genetic variability, heterozygote excess F_{IT} was estimated at -0.050 while within breed excess as evaluated by F_{IS} ranged from 0.370 to -0.336. The fixation index F_{ST} revealed that genetic diversity within the studied population was slightly differentiated. Genetic distance among the populations quantified through calculation of Nei's Genetic distance was 0.008 while the identity was 0.992. Similarity in the estimated genetic variability parameters between the breeds indicates that the populations are closely related and there were no appreciable differences among them. This result obtained may be used as an initial guide in defining objectives for further investigations of genetic diversity and developing conservation strategies

Key Words: *Electrophesis; Genetic distance; Polymorphism; Structural loci.*

Description of Problem

Loss of genetic diversity within indigenous livestock populations has been a major global concern and realization of this has led to efforts to study genetic diversity in livestock species in order to provide a basis for conserving these potentially useful germplasms. The loss of genetic variation within and between breeds is detrimental not only from the perspectives of culture and conservation but also utility since lost genes may be of future economic importance (**1**).

Within breed, high rates of loss of genetic variation leads to reduced chances of breed survival due to decreased fitness through inbreeding depression. Poultry genetic resources in general are considered to be the most endangered and under-conserved; and strategic approaches to conservation at the National level need to be developed and maintained (**2**).

Therefore, characterization of breeds both at the level of animal phenotypes and their interaction with production at the genetic level is most essential (**1**). Detailed attention is needed on the existing genetic resources to reduce or prevent the increasing genetic erosion of local livestock (**3**). Maintaining genetic diversity is an insurance package against adverse conditions. Due to diversity among environments, nutritional standards and challenges from infectious agents, a variety of breeds and population are required. These acts as storehouse of genetic variation which form the basis for selection (**4**) to be drawn upon in times of stressful environmental conditions such as high disease incidence (**5**) and erosion (**6**)

The knowledge of the extent of this genetic diversity is essential for the genetic improvement of breeds and development of appropriate breeding programs (**4**). Protein polymorphisms were the first molecular markers used in livestock (**7**). The structures of proteins enable them to serve as the carriers of essential substances within the organisms, to serve as regulators, of physiological relationship and to serve as building block units for substances, cellular and organic structures (**8**). Biochemical variants of different proteins may present higher accuracy procedures for better measurement of genetic variation because of their polymorphism and simple mode of inheritance (**4**).

Poultry plays very important roles in supplying meat and egg to meet up the protein requirement of Nigeria. It has been manifested by manifold increase in demand during the recent past. But, unfortunately, the indigenous birds of Nigeria are poor producer of both meat and egg. It has also been observed that crossbreeding indigenous birds with exotic breeds is getting popularity in Nigeria. It is therefore, imperative to know the genetic make-up of the crossbred birds as well as their difference with exotic and indigenous turkeys. Studies on estimation of genetic diversity in Turkey using blood protein polymorphism are scarce **(9).**

Characterization and genetic diversities among members of a species are fundamental to their improvement and conservation, Diversity can provide insights into the genetic relationship among the population to be utilized. Keeping in view the above facts, the present experiment was conducted to study the genetic constitution with respect to Heamoglobin, Carbonic Anhydrase, Transferrin, Esterase 1, and Albumin in indigenous turkey and exotic breeds of turkey in Nigeria.

This present study is aimed at investigating genetic variability and phylogenetic relationship between exotic and Nigerian indigenous turkey at different structural loci, therefore, providing useful genetic information essential for developing effective management plans for the breeding and improvement of these genetic resources as well as conserving them.

Materials and Methods

Study Area

The study was carried out in Breeding and Genetics Laboratory, Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

Experimental animals

Birds were sampled from four different locations to ensure they are not related, exotic birds were sampled from LHYW (Lord Have

Your Way) Livestock services (30) and Divine Farms(30) in Benue state while indigenous turkey were sampled from Tarmondo Farms (20) in Benue State and Amazing Grace farms (30) in Oyo State.

Sample collection and processing

Blood samples were collected from one hundred and ten (110) unrelated turkey birds comprising 50 Nigeria indigenous turkeys and 60 exotic turkeys. Blood was collected from veins in their wings through standard brachial venipuncture. About 3–5 ml of whole blood was collected from the wing vein of each apparently healthy bird into correspondingly labeled heparinized sample bottle. Heparin was added as anti-coagulant and blood contamination was prevented using separate syringes and needles for individual birds. These samples were kept refrigerated in ice packs and transported to the Animal Breeding and Genetics Laboratory of the Department of Animal Science, University of Ibadan, Ibadan, Oyo State, Nigeria for electrophoresis analysis.

The blood samples were centrifuged at 4°C for 20 min at 3000 rpm in order to separate plasma and erythrocyte. Erythrocytes were washed with saline water to free them from plasma proteins and were then lysed with distilled water in order to release the haemoglobin, and plasma. The haemolysates aliquots were stored at 4°C prior to electrophoresis analysis. The lysed red blood cells were used to determine Haemoglobin (Hb) and Carbonic Anhydrase (CA) genotypes while plasma was used to detect Transferrin (Tf), Albumin and Esterase 1 genotypes. Electrophoresis polymorphisms were performed for the CA, Hb, Tf, Alb and Esterase 1 using cellulose acetate papers. The electrophoresis for CA, Hb, Tf, Alb and Esterase 1 was carried out following the procedure described by (**11**) with slight modifications by (**10**). After electrophoresis, each strip was stained for few minutes and thereafter covered by destaining solution until the electrophoresis bands were visible, and scored. The method used was described by RIKEN (**11**) and Akinyemi and Salako (**10**) with minor modification to suit samples used in this study.

Statistical analysis

Allozymes bands for each locus were marked in the order of increasing mobility Haemoglobin (Hb), Transferrin (Tf), Albumin (Al), Esterase 1 (EST 1) and Carbonic Anhydrase (CA) A was the fastest allele and B was the slowest allele. Allele frequency and genotypic frequencies for each locus in each sample were computed by direct counting and tested for fit to Hardy-Weinberg ratios using X^2 goodness of fit test. Allele frequencies were calculated by direct gene counting method for all the loci studied. The genetic variability within and between breeds, allele frequencies and test of Hardy- Weinberg equilibrium (HWE) were performed using GenAlEX 6.5 (**12**), Genetic variability within the population were quantified by measuring the average heterozygosity (Het) and genetic distance value as described by Nei (**13**).

Results

The five studied loci were polymorphic in the two breeds. Frequencies of observed alleles at the investigated loci are in Table 1. Eleven variants were found at five loci. The highest number of alleles occurred at the Transferrin (Tf) locus (three alleles) while two alleles were observed in the other loci. The most frequency alleles were Hb^A, Alb^A and $Es1^A$ for both breeds while Ca^F was most frequent for Nigerian indigenous turkey and Ca^S for exotic turkey. In all the investigated loci, the homozygote genotype AA represents the presence of a single fast band; single slower band was designated as BB homozygote, while the presence of both bands (fast and slow band) was designated as AB heterozygote.

In Table 2, three genotypes of Hb (Hb^{AA}, Hb^{AB} and Hb^{BB}) determine by two codominant alleles were observed in both breeds of turkey with the frequency of Hb^{AA} (0.40) being highest for indigenous turkey and Hb^{AB} (0.45) highest for exotic turkey. The Ca locus has three genotypes controlled by two codominant alleles (CA^{FF}, CA^{FS}, and CA^{SS}). The three genotypes occurred in both indigenous and exotic breed of turkey with genotype CA^{FF}(0.36) being more frequent in indigenous turkey while CA^{SS} (0.38) is more frequent in the exotic turkey. Three genotypes of Albumin (Alb^{AA}, Alb^{AB} and ALB^{BB}) determined by two codominant alleles were also observed. The genotype heterozygous Alb^{AB} (0.91, 0.41) had the highest value for the two breeds of turkey; the homozygous Al^{AA} (0.061) has the lowest frequency for indigenous turkey while the homozygous Al^{BB} (0.28) has the lowest value for exotic turkey. The Es1 locus was polymorphic, having three genotypes ($Es1^{AA}$, $Es1^{AB}$ and $Es1^{BB}$) controlled by two codominant alleles. The $Es1^{AB}$ (0.67, 0.63) occurred in both breeds of turkey as the most frequent while the $Es1^{BB}$ (0.14, 0.067) indigenous and exotic respectively has the lowest frequency for both breeds. The Transferrin locus was the most polymorphic with six genotypes (Tf^{AA}, Tf^{AB}, Tf^{AC}, Tf^{BB}, Tf^{BC} and Tf^{CC}) controlled by three codominant alleles.

Discussion

Allele Frequency

According to Dimri (**14**), three types of haemoglobin were observed (AA, AB and BB) and which were controlled by two autosomal alleles A and B. Similar result was observed in the two populations of turkeys in this study. The three heamoglobin genotypes observed are similar with those reported in Nigerian indigenous turkey by Fatai (**9**), chukars and pheasants by Ugur (**15**). Heamoglobin Hb^{A}has the highest allele frequency in the two studied population. Results in this study are similar to the report of (**16**, **17**, **18**, **and 19**) in chicken.

Biochemical markers have been extensively utilized for documenting genetic similarities or diversities of different populations of livestock comprising a species, a strain or even closely related line (**14**; **20**). In the indigenous turkey, the selective advantage of Hb^{A} is due to biophysical, biochemical and physiological peculiarities of the haemoglobin molecule type A, which has high saturation capacity with oxygen, dissociation curve of oxyhaemoglobin, erythrocyte load with haemoglobin and metabolic profile of the erythrocyte (**22**).

In this study CA^{FF} was the highest observed (0.34) genotype for both population studied. The frequency of CA^{FF} was higher than CA^{SS} in Nigerian indigenous turkey while in the exotic turkey it was reversed as CA^{SS} was the highest. Report of Fatai (**9**) in indigenous turkey agrees with the result of this study. Ige (**19**) reported three CA phenotypes (FF, FS, and SS) in two Nigerian indigenous chicken (Yoruba and Fulani ecotypes) which are genetically controlled by two codominant alleles CA^{F} and CA^{S} while Das and Deb (**8**) observed six CA phenotypes viz, AA, BB, CC, AB, AC and BC which were controlled by three co-dominant alleles (CA-1A, CA-1B and CA-1C) located at an autosomal locus CA-1 in poultry. No significant differences were detected between various biochemical types and economic traits in poultry; however the activity of CA has been positively correlated with egg shell thickness (**8**).

The observed three albumin genotype (AA, AB, and BB) which were controlled by two codominant alleles Alb^{A} and Alb^{B} in this study agrees with the report of Quinteros (**21**) where three albumin genotypes were observed in domestic turkey (*Meleagrisgallopavo*), the Ocellated turkey (*M. ocellata*) and in the descendants from a domestic turkey X Ocellated turkey cross. Three genotypes were

also reported in Nigerian indigenous turkey (**9**). Albumin polymorphisms have been reported to be present in several species. Esmaeilkhanian (**20**) observed three albumin phenotypes i.e. AA, AB and BB in Iranian Native poultry breed. Ismoyowati (**23**) reported Alb^{AA}, Alb^{AB}, Alb^{BB} and Alb^{BC} albumin genotypes in Kampung chicken. Johari (**24**) stated that albumin locus allele is controlled by two alleles of A and B in Kedu chickens. Other species such as the Muscovy and Peking ducks (**25** and **26**) have been reported to exhibit albumin polymorphism. Azmi (**25)** and Johari (**26)** reported high frequency of Alb^{A} for Magelang duck (0.719), Tegal duck (0.800) and Talangbenih ducks.

In this study, transferrin exhibited three different alleles (TfA, TfB and TfC) agrees with the findings of Ismoyowati (**23**). Fatai (**9)** reported two transferrin allele variations in Nigerian indigenous turkey. In this study both exotic and indigenous turkey breeds have the three alleles. The variation in this study may be due to sampling area and size of population used. The frequency of allele Tf^{B} for both the exotic and indigenous turkey breeds in this study was higher than the frequency of allele Tf^{A} and Tf^{C} gene.

Stratil **(28)** interestingly observed the chickens with a type 'TfB' to have the advantageous egg production over the chicken with TfA. The effect of heterozygous transferrin (TfBC) is significant in the fertility, hatchability and egg production. Chicken with TfA appears to have delayed sexual maturity while the chicken with the TfB attained sexual maturity earlier (**8**).

Esterase 1 is controlled by two co-dominant alleles as observed in this study, Alleles (A and B). Fatai (**9)** reported that Esterase1 was fixed for Nigerian indigenous turkey, however, Okamoto **(29)** reported the presence of four alleles (A, B, C and D) at this locus in Laos native chickens.

Heterozygosity

The observed heterozygosity in Nigerian indigenous turkey and exotic turkey based on the five loci investigated was 0.567 and 0.477 respectively (Table 3). This relatively high heterozygosity in turkey breed indicates high genetic variability. Observed heterozygosity is the proportion of heterozygotes observed at a locus while expected heterozygosity or gene diversity is the proportion of expected heterozygotes under random mating. This is an indication from the mean number of allele occurring in each breed.

Carbonic Anhydrase (CA) has the lowest observed heterozygosity (0.31) and this corresponds with the report of (**9**), the average heterozygosity coefficient for all the loci and population studied was 0.522. The higher heterozygosity in Nigerian indigenous turkey could be a contributing factor to better adaptability to the prevailing tropical conditions. High heterozygosity values in this study may be an indicator of higher reproductive characteristic, mixing of two previously isolated populations and the presence of store of genetic diversity irrespective of the low level of differentiation within close relatives.

Maeda (**30**) reported that increasing the number of loci studied is a way to help detect small differences of heterozygosity, and it can be achieved now more easily through using molecular markers. Mannen (**31**) examined DNA fingerprints of Japanese quail lines selected for large and small body weight, and on which Ardiningsas (**32**) has analyzed protein polymorphism.

Hardy-Weinberg Equilibrium

Chi-square analysis showed a significant difference in the observed and the expected frequencies of the Nigerian indigenous turkey and the Exotic turkey (Table 5). This indicates that the gene and genotype frequencies of the two populations were not in Hardy – Weinberg

proportions (P<0.05) this may be as a result of high mutation rate, genetic migration, reduced artificial selection and non-random breeding in the population. This result is not similar to the report of Maina (**33**) that the observed and expected genotypes for haptoglobin in Kenyan Indigenous chickens were in Hardy-Weinberg equilibrium. However, on the overall data set there were significant deviations from HWE in all the 5 studied loci. This could be attributed to migration occasioned by the introduction of birds from different sources into the respective populations and selection.

Gene Flow and F-Statistics

Negative F_{IT} was observed in three loci of the five loci studied for the two populations; this indicates a deficiency of homozygotes in the populations and that mate were less related in comparison with the average relationship of the population. F_{IS} observed three negative loci out of five; this indicates a deficiency of homozygotes in the population. The observed excess of heterozygotes could be due to mating and genetic exchange between populations. The degree of differentiation observed between the two turkey populations studied could be due to similarities in environment and breeding practices. The estimated F_{ST} values correspond to the amount of genetic variability in environment and breeding practices. Gene flow from one protein to another ranged from 53.91 (Transferrin) to 1712.99 (Albumin), this indicates high genetic migration within the population, which may be due to the geographical distance.

Genetic distance and genetic identity

The analysis of the genetic similarity was carried out according to Nei's (**13**) genetic Identity and distance calculations. Nei (**13**) standard genetic distance obtained in this study between Nigerian indigenous turkey and exotic turkey was 0.008 which indicates little genetic differentiation between the Nigerian indigenous turkey and exotic turkey. Vaida (**35)** reported D value of 0.022 between white and motley quail using data from 10 loci. Butkauskas **(36)** reported that the shortest genetic distance (0.013) was observed between turkey and quail populations from loci studied. The degree of closeness may be characterized by a common breeding system and genetic migration between both populations.

Conclusion and Applications

Based on the result of the electrophoresis analysis of exotic and Nigerian indigenous turkeys;

1. All the sampled populations had similar number of alleles at all the investigated loci except transferrin that has three alleles.
2. Genetic similarity as measured by dendrogram equally supported high genetic flow between two breeds of turkeys, the two populations were genetically related and further studies should focus on other protein markers at the molecular level.
3. The information from this study may be useful as a guide in defining objectives for designing investigations of the genetic integrity and developing conservation strategies for exotic turkeys and Nigerian indigenous turkeys.

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Table 1: Allele frequency for polymorphic loci of Nigerian indigenous and exotic turkey

Locus	N	Allele	Observed no of allele	Populations		Total
				Indigenous turkey	Exotic turkey	
HB	109	A	114	0.54	0.50	0.52
		B	104	0.45	0.49	0.47
CA	109	F	110	0.54	0.47	0.50
		S	108	0.45	0.52	0.49
ALB	109	A	112	0.52	0.61	0.57
		B	106	0.48	0.38	0.42
ES1	109	A	125	0.52	0.50	0.51
		B	93	0.48	0.49	0.48
TF	109	A	23	0.09	0.11	0.10
		B	142	0.62	0.67	0.65
		C	53	0.28	0.20	0.24

HB – Haemoglobin, CA – Carbonic Anhydrase, ES1 – Esterase 1, ALB – Albumin, TF – Transferrin, A, B, and C – alleles

Table 2: Genotype frequencies at Haemoglobin, Carbonic Anhydrase, Albumin, Esterase 1 and Transferrin in Nigerian indigenous and exotic turkey breeds

Locus	Genotype	Indigenous Turkey	Exotic Turkey	Total
Haemoglobin	AA	0.40	0.28	0.34
	AB	0.26	0.45	0.37
	BB	0.32	0.26	0.29
	Chi-Square	10.6	0.59	7.89
	Probability	0.001	0.44	0.005
Carbonic anhydrase	FF	0.36	0.33	0.34
	FS	0.34	0.28	0.37
	SS	0.28	0.38	0.33
	Chi-Square	4.45	11.1	15.7
	Probability	0.035	0.001	0.00
Albumin	AA	0.061	0.30	0.19
	AB	0.91	0.41	0.64
	BB	0.020	0.28	0.16
	Chi-Square	34.5	1.66	8.59
	Probability	0.00	0.19	0.003
Esterase 1	AA	0.18	0.30	0.24
	AB	0.67	0.63	0.65
	BB	0.14	0.067	0.10
	Chi-Square	5.97	6.92	11.6
	Probability	0.015	0.009	0.001
Transferrin	AA	0.020	0.017	0.018
	AB	0.14	0.18	0.16
	AC	0.00	0.017	0.009

Table 3: Mean number of allele, mean heterozygosity, and derivations from Hardy-Weinberg's equilibrium for the two population

Breed	N	Heterozygosity		DHWE
		Ho	He	
Indigenous turkey	49	0.56	0.50	5
Exotic turkey	60	0.47	0.49	3

Ho= observed heterozygosity, He= expected heterozygosity, Ave.H= average heterozygosity, DHWE = Deviations from Hardy-Weinberg's equilibrium

Table 4: Mean number of allele, mean heterozygosity, and derivations from Hardy-Weinberg's equilibrium per breed for the allozyme loci

Protein Loci	N	Heterozygosity		DHWE
		Ho	He	
HB	218	0.35	0.49	1
CA	218	0.31	0.49	2
ES1	218	0.65	0.48	1
ALB	218	0.66	0.50	2
TF	218	0.61	0.51	2

Hb= Hemoglobin, CA=Carbonic anhydrase, Alb=Albumin, Tf=Transferrin, Es-1=Esterase1, Ho= observed heterozygosity, He= expected heterozygosity, Ave.H= average heterozygosity, DHWE = Deviations from Hardy-Weinberg's equilibrium

Table 5: Chi–Squared probability for Hardy-Weinberg equilibrium for indigenous and exotic turkey

Blood proteins	Population		
	Indigenous turkey	Exotic turkey	Entire data set
HB	10.6**	0.59ns	7.89
CA	4.45*	11.1***	15.7
ALB	34.5***	1.66ns	8.59
ES 1	5.97*	6.92**	11.6
TF	8.06*	7.85*	14.4

HB – Haemoglobin, CA – Carbonic Anhydrase, ES1 – Esterase 1, ALB – Albumin, TF – Transferrin, A, B, and C – alleles

Ns – Not significant *P<0.05

Table 6: Genetic identity and genetic distance between Nigerian indigenous turkey and exotic turkey

Population	Indigenous Turkey	Exotic Turkey
Indigenous turkey	****	0.99
Exotic turkey	0.008	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)(**13**)

Table 7: F- Statistics and gene flow for 5 blood proteins in turkey

Locus	Sample Size	F_{IS}	F_{IT}	F_{ST}	Nm*
HB	218	0.28	0.28	0.001	236.1
CA	218	0.36	0.37	0.004	57.4
ES1	218	-0.34	-0.33	0.009	26.2
ALB	218	-0.33	-0.33	0.000	1712.9
TF	218	-0.22	-0.21	0.005	53.9
Mean	218	-0.050	-0.046	0.004	417.3

* Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst.

FIGURES

Dendrogram of genetic distance between Nigerian indigenous turkey and exotic turkey

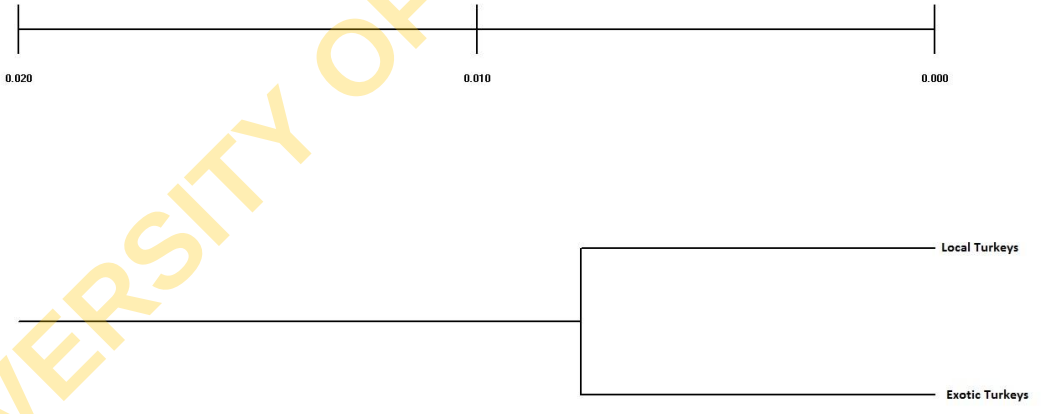

Figure1: Genetic distance between Nigerian indigenous turkey and exotic turkey