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Biochemical characterization of indigenous Fulani and Yoruba ecotypes chicken of Nigeria

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The study was carried out to characterize two indigenous chickens of Nigeria using protein markers; haemolglobin (HB) and carbonic anhydrase (CA). Separation of the two proteins was achieved by cellulose acetate electrophoresis and direct gene counting method was employed to interpret the result. Palentological statistics was used to generate dendrogram that measured genetic similarity within and between each of the population studied. HB was interpreted into three phenotypes: AA, AB and BB which were genetically controlled two codominant alleles HB^A and HB^B. Allele frequencies of HB^A and HB^B in Yoruba ecotype chicken were 0.34 and 0.66, respectively, while those of Fulani ecotype were 0.28 and 0.72, respectively. CA was also interpreted into three phenotypes (FF, FS and SS) which are genetically controlled by two codominant alleles CA^F and CA^S; their respective allele frequencies were 0.33 and 0.67 in Yoruba ecotype chicken and 0.24 and 0.76 in Fulani ecotype chicken. Genetic similarity within ecotype indicated 60% in Fulani, 80% in Yoruba and 40% between Yoruba and Fulani at HB locus while at CA locus, genetic similarity was 69% in Fulani ecotype, 50% in Yoruba ecotype and 42% between Yoruba and Fulani ecotype. Cavalli-Sforza genetic distance between the two Ecotypes was $2.1x10^{-2}$. Conclusively, the two populations were genetically related and further studies should focus on other protein markers and at molecular level.

Key words: Ecotype, protein marker, genetic distance and selection.

INTRODUCTION

There is a great concern globally over the loss of biodiversity in domestic animal and plants. Part of the Nigerian heritage lies in the genetic diversity of native breed. Very limited information on these populations concerning genetic diversity exists. There is a major global thrust on genetic preservation and biodiversity which is reflected in efforts on the development of the genome data banks (Crawford and Gavora, 1993). These initiatives have come at an opportune time, because of continued uncontrolled breeding practices among indigenous chicken which do not that consider gene preservation aspects would lead to the erosion of native germplasm (Bessei, 1989). However, little has been done to characterize and conserve the indigenous chicken genetic resources of Nigeria. Unfortunately, like in other developing countries, attention is directed to commercialization using improved breeds. However, not enough attempts have been made to evaluate the genetic characteristics and consequently improve the Nigerian indigenous chickens.

The conservation of domestic animal diversity is essential to meet future needs in Nigeria and Africa as a whole. In order to cope with an unpredictable future, genetic reserves capable of readily responding to directional forces

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imposed by a broad spectrum environment must be maintained. Maintaining genetic diversity is an insurance package against future adverse conditions (FAO, 2000b). Due to diversity among environments, nutritional standards and challenges from infectious agent, a variety of breeds and population are required. These will act as storehouses of genetic variation which will form the basis for selection and may be drawn upon in times of biological stress such as famine, drought or disease epidemics.

In addition to increasing global human population pressures, the quantity of food and other products must increase. Not only should diversity be maintained for practical purposes, but also for cultural reasons. A community's domestic animals can enhance the environment as a living system, thus also enhancing the human inhabitant's quality of life. The need for characterization comes from the potential rate of decrease of genetic variation. 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 (FAO, 2000b). Within breed, high rates of loss of genetic variation leads to reduced chances of breed survival due to decreased fitness through inbreeding depression. These breeds become subject to faster changes in gene frequencies, greater rate of loss of genes and genetic constitutions. These are all due to small effective population sizes, or, equivalently, high rates of inbreeding (Meuwissen et al., 2001).Once animal genetic diversity has been lost, it cannot be replaced. Advances in biotechnology offer possibilities of improving, utilizing and characterizing present domestic animal diversity. Characterization at the biochemical and morphological level offer the opportunity to explore genetic diversity within and between livestock populations and to determine genetic relationships among populations and that the method is rapid, relatively affordable and reliable. The present study is therefore designed to characterize two ecotype of indigenous chickens through biochemical markers.

MATERIALS AND METHODS

The experiment was conducted at the Animal Breeding and Genetics laboratory section of the Department of Animal Science, University of Ibadan. Ibadan is located on the latitude 7°20'N and longitude 3°51 E, 200 m above the sea level.

Blood samples were collected through wing veins from 100 chickens comprising 50 adult Yoruba ecotype and 50 Fulani ecotype, placed in heparinized tubes to prevent coagulation and were refrigerated. Samples were prepared and subjected to cellulose acetate electrophoresis following the procedure of Riken (2006).

Sample preparation

Red cells

Blood samples (5 ml) collected were transferred from wing veins of the individual chickens into plastic tubes containing anticoagulant. Red blood cells (RBC_S) were prepared from the erythrocyte fraction of heparinized blood by centrifuging at 2500-3000 rpm for 10 min at 4°C. The RBCs were washed in saline buffer three times by repeating centrifugation at 2,500-3000 rpm for 5 min at 4°C. The RBCs were lysed with eight fold volumes of water. The red cell lysates were stored for further analysis of haemolglobin (HB) and carbonic anhydrase (CA).

Cellulose acetate electrophoresis protocol

The cellulose acetate membrane was soaked very slowly in the buffer solution for over 5 min. Buffer corresponding to each of the protein was poured into the electrophoresis chamber. Wicks were folded and moistened with appropriate buffer and placed on each of the support arm of the electrophoresis chamber. The samples were poured into the slots of the applicator. The soaked cellulose acetate membrane plate was gently placed between the paper towels on each support arms of the electrophoresis chamber. The comb was stamped into the applicator and placed on the gel upside down on the paper rows inside the electrophoresis chamber. Coin was placed on the gel to keep plate flat and ensured an even current distribution through the plate. The electrophoresis was run as described below for each of the protein.

Haemoglobin (HB)

For haemoglobin the electrophoresis included: tissue sample, RBCs in 8 volumes of H_2O; buffer system, Tris EDTA borate; pH 8.4; supporting media, cellulose acetate membrane; electrophoresis, voltage of 350 V; time of 40 min; temperature of 4°C; migration cathode (-) to anode (+); stain procedure, staining with Ponceau S and destaining in 5% acetic acid.

Carbonic anhydrase (CA)

For carbonic anhydrase, the electrophoresis included: tissue sample, RBCs in 4 volumes of H_20; buffer system: -EDTA sodium acetate; pH 5.6; supporting media, cellulose acetate membrane; electrophoresis voltage of 200 V; time of 45 min; temperature of 4°C; migration, anode (+) to cathode (-); stain procedure, staining with ponceau S and destaining in 1% acetic acid.

Temperature of 4°C was achieved by placing the electrophoresis chamber inside the refrigerator.

After destaining, the bands were clearly separated and direct allele counting method was used for each of the protein as follows: A single faster band was designated as the AA for HB and FF for CA homozygous. The presence of a single slower band was designated as BB for HB and SS for CA homozygous. The presence of both bands was designated AB for HB and FS for CA heterozygous.

Statistical analysis

Gene frequency was calculated using the expression provided by Roghgarden (1977) as follows:
Let P= Gene frequency of allele x; Q= gene frequency of allele y.

$$P = \frac{2(Nxx) + Nxy}{2N}$$

$$Q = \frac{2\,(Nyy) + Nxy}{2\,N}$$

Where, N is the total number of individual sampled; N_{xx} is the observed genotype number for xx; N_{xy} is the observed genotype

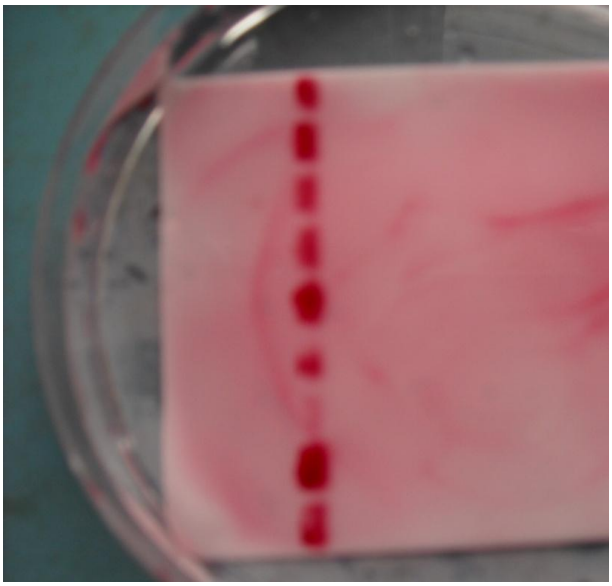

Plate 1. Electrophoretic separation of haemoglobin in fulani ecotype chickens.

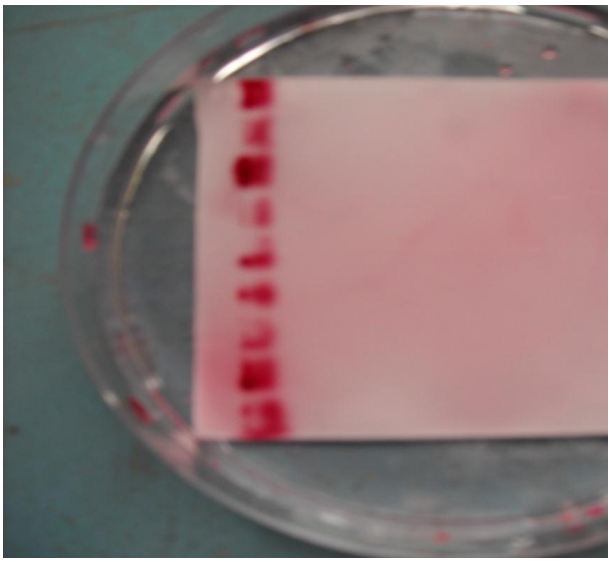

Plate 2. Electrophoretic separation of haemoglobin in yoruba ecotype chickens.

number for xy; N_{yy} is the observed genotype number for yy. Genotype frequency was calculated as follows: (Number of xx / Total individual) x 100; (Number of xy / Total individual) x 100; (Number of yy/ Total individual) x 100. Paleontological Statistical (PAST) package was used to generate dendogram that measure genetic similarity.

Estimation of genetic distance

The simplest measure of genetic distance as proposed by Nei (1972) and Cavalli-Sforza (1967) called minimum genetic distance (DM) was used.

Plate 3. Electrophoretic separation of carbonic anhydrase in fulani ecotype chickens.

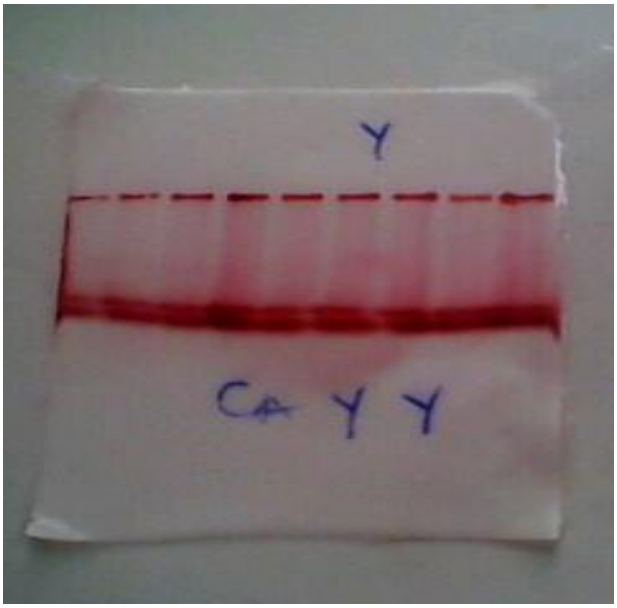

Plate 4. Electrophoretic separation of carbonic anhydrase in yoruba ecotype chickens.

RESULTS

Biochemical polymorphism

Electrophoretic separation of HB and CA are shown in Plates 1 to 4. HB was interpreted into three phenotypes (AA, AB and BB) which were genetically controlled by two codominant alleles (HB^A and HB^B). The allele frequencies (Table 1) of Hb A and Hb B in Yoruba ecotype chicken were 0.34 and 0.66 while those of Fulani ecotype chicken were 0.28 and 0.72, respectively. The genotype frequencies

Table 1. Allele and allelic frequencies at haemoglobin locus with respect to ecotype.

Ecotype	Number	Allelic frequency							
		AA	%	AB	%	BB	%	A	B
Yoruba	40	5	12.5	17	42.5	18	45	0.34	0.66
Fulani	36	6	16.67	8	22.22	22	61.11	0.277	0.722

Table 2. Allele and allelic frequencies at carbonic anhydrase locus with respect to ecotype.

Ecotype	Allele frequency								
	Number	FF	%	FS	%	SS	%	F	S
Yoruba	40	6	15	14	35	20	50	0.325	0.675
Fulani	36	3	8.33	11	30.55	22	61.11	0.236	0.763

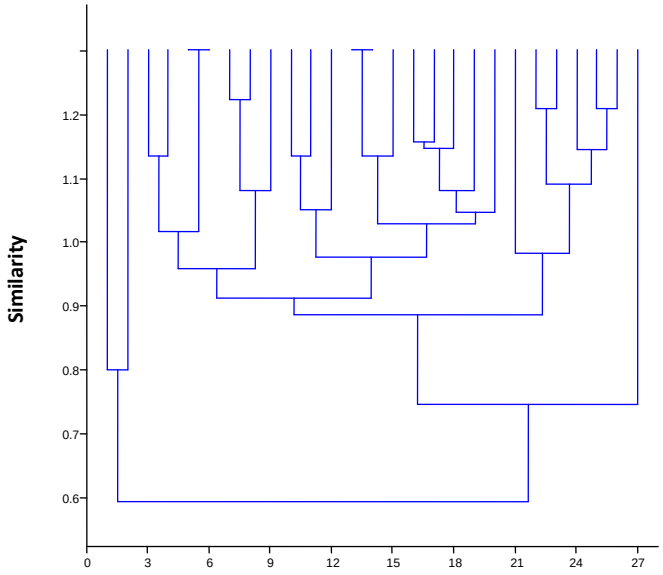

Figure 1. Dendogram of genetic similarity of fulani ecotype chicken at HB locus.

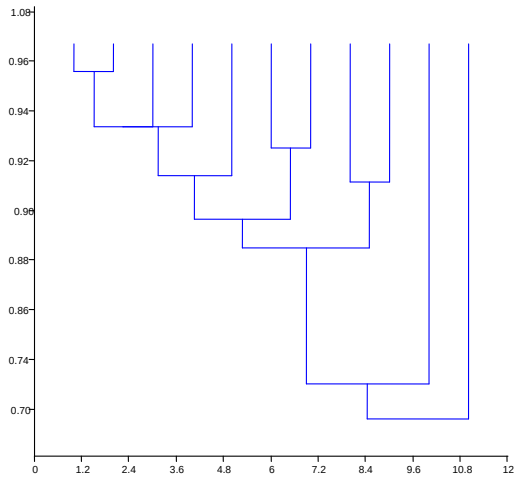

Figure 2. Dendogram of genetic similarity of Fulani ecotype chicken at CA locus.

of 12.5, 42.5 and 45% were recorded for AA, AB, BB, respectively, for Yoruba ecotype chickens while Fulani ecotype chickens had 16.67, 22.2 and 61.11%, respectively, for AA, AB and BB. HB^A was lower than HB^B in the two populations. The distribution of allele and allelic frequencies of CA (Table 2) is comparable to those obtained with HB and it was interpreted into three phenoltypes (FF, FS and SS) which are genetically controlled by two codominant allele (CA^F and CA^S). Their respective allele frequencies were 0.33 and 0.67 in Yoruba ecotype chicken and 0.24 and 0.76 in Fulani ecotype chicken, respectively. Genotype frequencies were 15, 35 and 50% in Yoruba ecotype and 8.33, 30.55 and 61.11% in Fulani ecotype for FF, FS and SS, respectively. Generally allele frequency of F allele was low in the two populations studied compared to S loci allele.

Figures 1-4 represent dendrograms that measured the genetic similarity within and between the two populations studied herein. In Fulani ecotype, three clusters were observed for HB (Figure 1); one main cluster (60%) and two sub clusters (75%) and two major clusters (69 and 69%) were observed at CA locus (Figure 2). Generally, the genetic similarity is high within the population. In Yoruba ecotype, two major clusters locus (80%, 62%) were observed at HB (Figure 3), while one major cluster (50%) was observed at CA locus (Figure 4) with two sub cluster (56 and 60%). High genetic similarity was also noticed at all the loci within the population. Dendrogram showing genetic similarity between the two populations studied (Yoruba and Fulani ecotype) at HB and CA loci are represented in Figures 5 and 6, respectively. One major cluster (40%) and two sub clusters were observed at HB locus. Similarly, one major cluster (42%) and two sub clusters were observed at CA locus. Also, genetic distance as indicated by Carvalli-Sforza (2.1×10^{-2}) is low which equally indicate high genetic similarity.

DISCUSSION

The biochemical markers have been extensively utilized

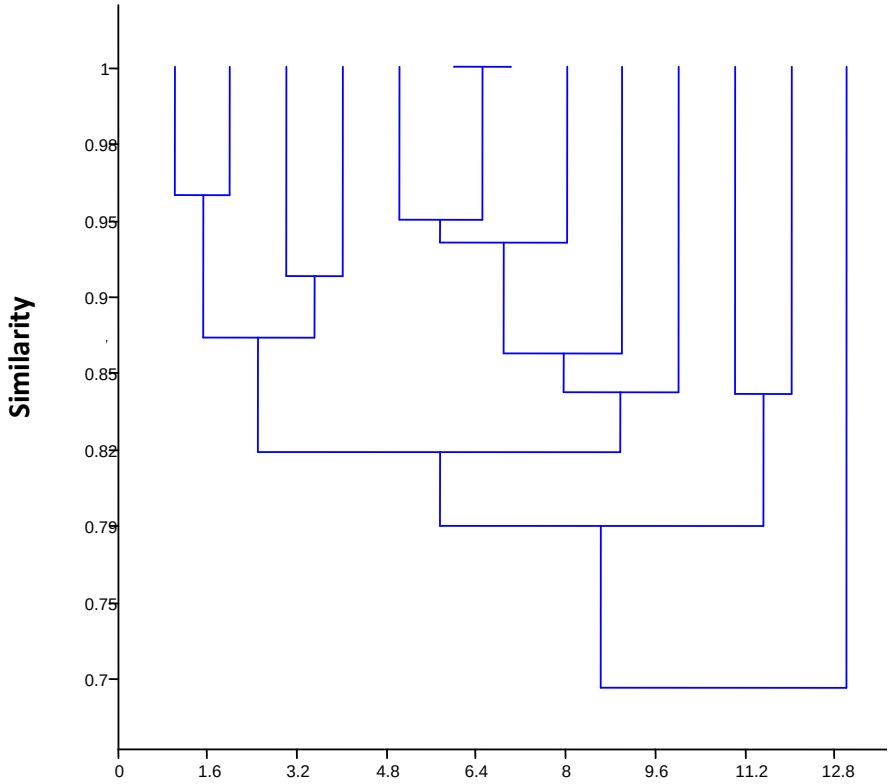

Figure 3. Dendogram of genetic similarity of Yoruba ecotype chicken at HB locus.

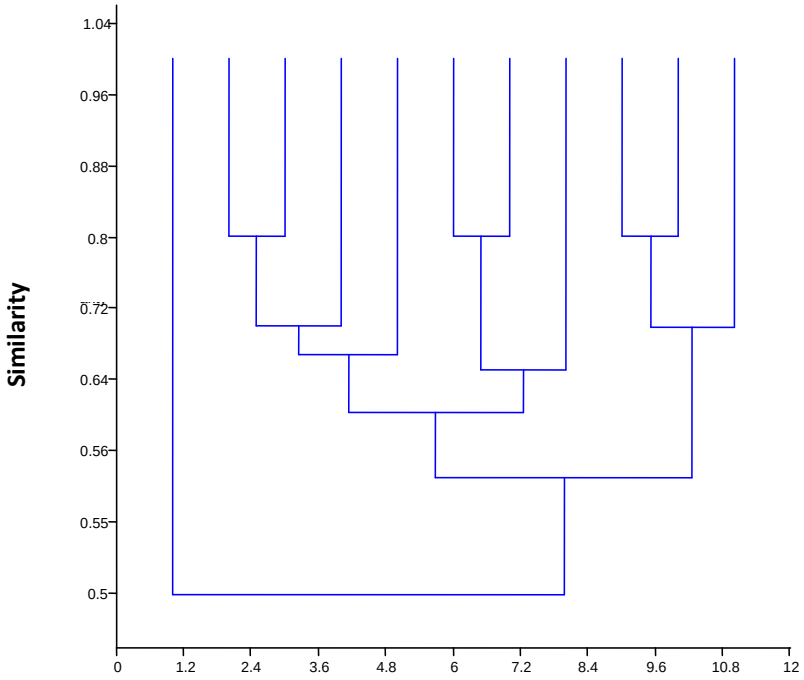

Figure 4. Dendogram of genetic similarity of Yoruba ecotype chicken at CA locus.

for documenting genetic similarities or diversities of different populations of livestock comprising a species, a strain or even closely related line (Lee et al., 2000; Esmaeilkhanian et al., 2000; Zhang et al., 2002 ; Salako

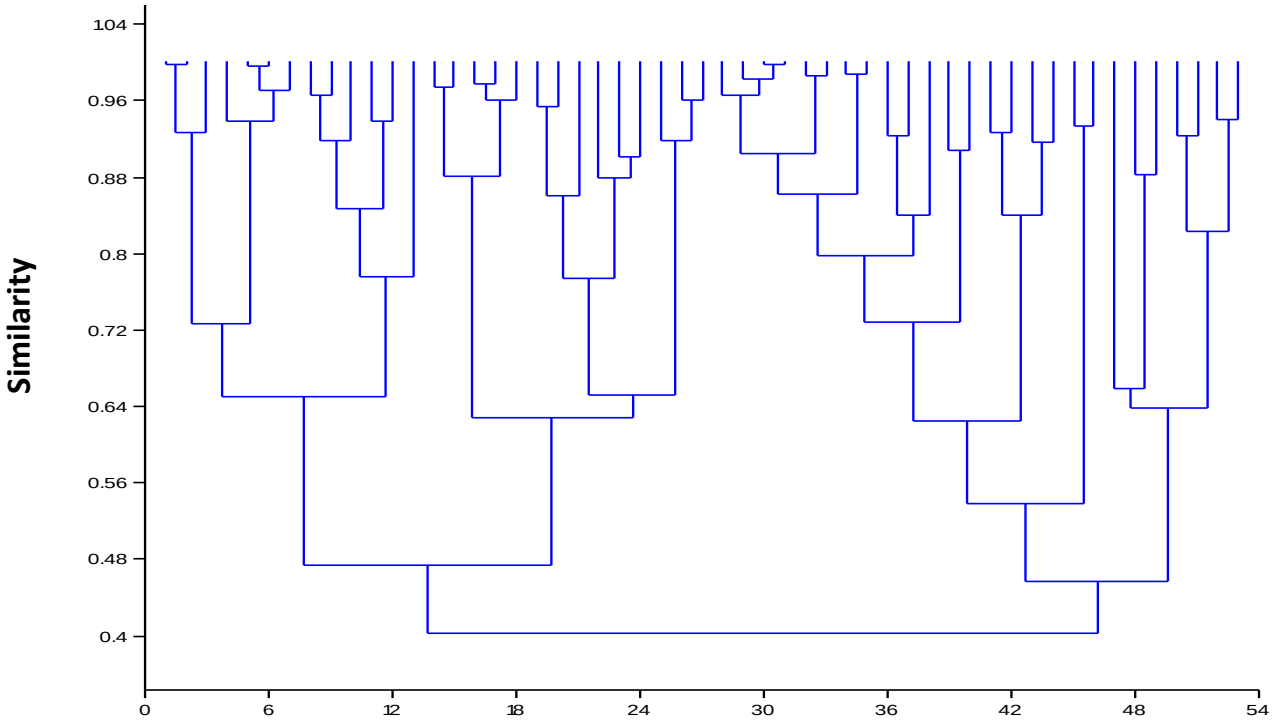

Figure 5. Dendogram of genetic similarity between Yoruba and Fulani Ecotype Chicken at HB locus.

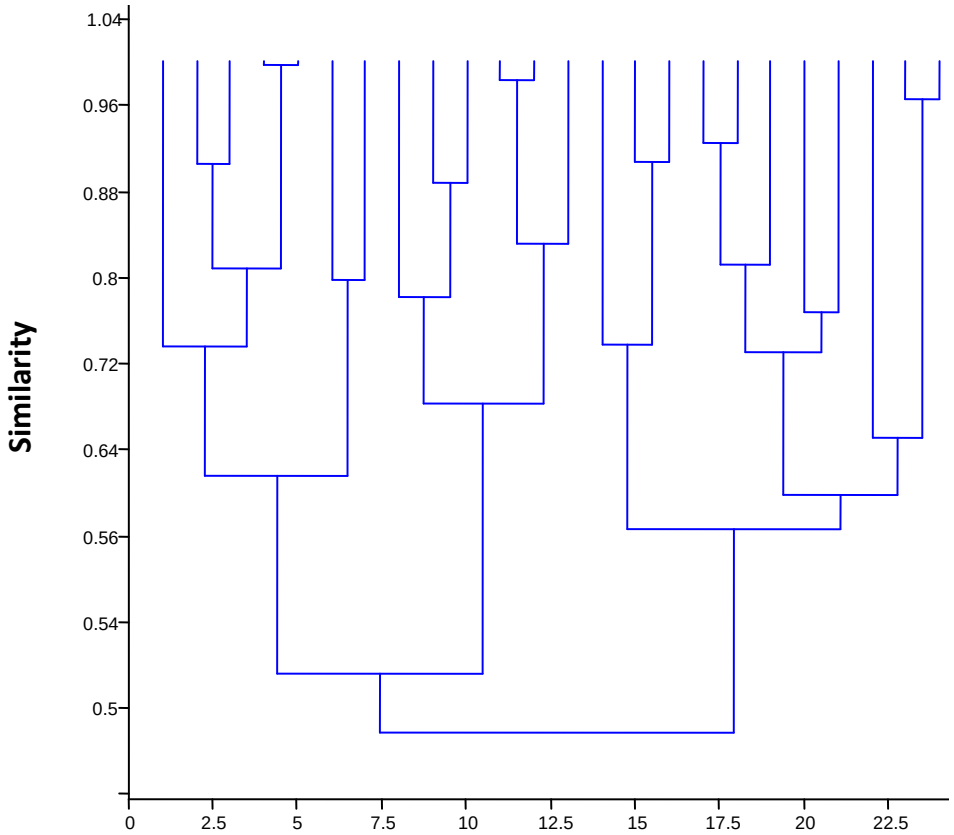

Figure 6. Dendogram of genetic similarity between Yoruba and Fulani Ecotype Chicken at CA locus.

and Ige, 2006 ; Dimri, 1981 ; Mazumder et al., 1989; Washburn et al., 1971 ; Yamamoto et al., 1996). According to Dimiri (1981), 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 studied. Mazumder et al. (1989) reported frequencies of 0.96 (HB^A) and 0.04 (HB^B) for white leghorn chickens, and 1.00 (HB^A) for broiler which contradicted the result of this work as frequency of HB^A were 0.34 and 0.28 in Yoruba and Fulani ecotype chickens, respectively, while frequency of HB^B were 0.66 and 0.72, respectively. Frequency of Hb^B was predominant in both populations. Mazumder et al. (1989) reported the presence of gene fixation as only genotype HB^{AA} was identified in their study. However, the discrepancy observed was primarily attributed to the specific genetic background of the breeds. Singh and Nordskog (1981) also found complete gene fixation for haemoglobin in inbred line chickens. Lee et al. (2000) also reported that Korea native chicken were monomorphic at haemoglobin locus. Salako and Ige (2006) reported frequencies of 0.68 (HB^A) and 0.33 (HB^B) in a mixed population of indigenous chickens of Nigeria. Washburn et al. (1971) related haemoglobin types with Marek disease and concluded that chickens with homozygous mutant haemoglobin genotypes were approximately 20% less susceptible to Marek disease. In the same way, Dimri (1981) reported that haemoglobin polymorphism affects growth rate and hatchability, with the highest in AA (62.20%) followed by HB AB (48.20%) and BB (31.50%). The transport of CO_2, haemoglobin utilization for controlling pH of body fluids and selection for the production of carbonate ions are facilitated by carbonic anhydrase. Frequency of Ca^F was higher than Ca^S in both Fulani and Yoruba ecotype population; this observation suggest a close relationship between the two populations. There is no available information in literature on carbonic anhydrase types in chicken, however, it has been reported extensively in other livestock animals. Also, activity of CA has been positively correlated with egg shell thickness.

Genetic differences between breeds, ecotypes and populations are controlled by mutation, genetic drift, selection and migration (Eding and Laval, 1999). Therefore, the evaluation of indigenous chicken population as genetic resources includes the determinations of genetic distance between the available populations (Hamnond, 1994). The genetic distance between the two ecotypes as measured by Cavalli-Sforza was 2.10×10^{-2} which is quite low indicating little genetic effect of drift or mutation. It also reflected that these populations are not genetically isolated from each other. Kaya and Yildiz (2008) reported similar findings among Turkish native chickens. They estimated genetic distance to be 6.5×10^{-2} between the populations. Hillel et al. (2003) reported higher value of 0.44 using Nei's mean genetic distance between given populations using microsatellite markers. These findings also imply high levels of genetic flow among the ecotypes resulting in admixed populations. Genetic similarity as measured by dendogram equally supported high genetic flow between two ecotypes.

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