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Genetic Similarity of Yoruba Ecotype Indigenous Chickens Using Polyacrylamide Gel Electrophoresis.

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Abstract
The study investigated genetic similarity of Yoruba Ecotype Indigenous chicken at four protein Loci: Globulin (95SkDa), Transferrin (66kDa), Albumin (36kDa) and post albumin (29kDa) using Sodium Do decyl Sulphate Polyacrylamide Gel electrophoresis (SDS PAGE) following the procedure of Bio rad resource centre. Dendogram was plotted from data generated using PAST (Palenthological statistics) soft ware for each of the protein. Similarity indices were; Transferrin (58%), Albumin (19%), Globulin (18%) and post albumin (40%). The population is genetically similar at Transferrin locus and varied widely at Albumin, Globulin and Post Albumin loci. Conclusively, the population is still under the control of natural selection. Further research should be extended to more protein loci and DNA characterisation

Introduction

There is a great concern by international organizations over the loss of biodiversity in domestic Animals. Part of African heritage lies in the genetic diversity of native breeds which have adapted to harsh African Environment. There is little or no information on genetic diversity of these populations and that they have not been adequately characterized.

There is a major global thrust on genetic preservation and biodiversity which is reflected on the development of the genome data bank. This will thus safe guard the continued crossbreeding and in breeding practices in indigenous chickens which conventionally not consider gene preservation aspects and will consequently lead to the erosion of the native germplasm FAO (2003).

Little has been done on the studies leading to the conservation of chicken resources genetic pool within Nigeria, however, attention is directed to commercialization using improved breeds and once animal genetic diversity is lost, it cannot be replaced, the economic implications of maintaining existing indigenous chicken genetic resources in their natural environment will equally be affected.

The study of the structure and function of genes at the molecular level in a breeding population can help to determine the similarity of the genetic material carried by populations and the genetic variation they possess Lee *et al* (2000). Several techniques have been developed to estimate the genetic variation or polymorphism in populations and hence the genetic relationship amongst the population; these methods include biochemical polymorphisms, DNA hybridization, RFLP, mtDNA, microsatellite and SNP.

The study was therefore designed to investigate the genetic diversity of Yoruba ecotype indigenous chickens at four protein Loci; Globulin, Transferin; Albumin and post albumin using SDS-PAGE electrophoresis. This method is rapid, affordable and reliable, but requires fresh blood samples based on the difference of their molecular weight followed by histochemical recognition of difference in banding patterns for particular protein (Baker et al 1992)

Materials and Method

One hundred Yoruba Ecotype indigenous chickens were sampled from major market in villages around Ogbomoso. Geographically Ogbomoso lies within the derived savannah region of Nigeria. Ogbomoso is 104km North East of Ibadan, 57km South west of Ilorin and 58km North west of Osogbo. It lies approximately 4^0 15^1 of the longitude and latitude 8^07^1 North of the equator. The mean annual temperature of Ogbomoso is about 26.2^0c and the mean annual rainfall of 1200mm. the relative humidity ranges within 75-95% (Oladuntan Olademeji 1999).

About 5ml of blood was individually collect from each bird with the aid of sterile hypodermic syringe and needle and it was transferred into serological tube furnished with a wooden spill around.

Clot was removed and centrifugation was carried out at 2,510rpm for 10min. the supernatant which contain protein was carefully transferred into a clean 2ml Eppendorf microtable and stored at 25^0c which latter subjected to sodium do decylsulphate polyacrylamide Gel Electrophoreses (SDS-PAGE)

The separation of protein was carried out using Bio-rad mini protein II cell (10ml capacity) Electrophoresis kit. 7.5% B-mecaptoethanol

Sample buffer was used for the preparation of the sample. To each 10µl of protein samples in well labelled Eppendarf microtubes, 10µl of mixture sample buffer was added. In addition 10ul of high and low molecular weight protein marker for Abumin, Transferrin, globulin and post albumin was treated in the same way as reference markers (polymerase chain reaction). Using the run TOM-BAC, programme

The samples were heated at 95^{0}c for 5min with PCR and the samples were loaded in each of the well including the marker sample and its buffer. The separation of protein was carried out with the aid of Bio rad Electrophoresis power supply model 200/2.0 in Bio rad mini protein II cell at 150v for 45min.

Coomassie-Blue Gel-staining for SDS PAGE

After the electrophoresis, the gels were carefully removed and placed in a staining solution which is done for about 1hour and thereafter the staining solution was removed and destaining solution was then added and allowed to distain for 2hours.

Statistical Analysis.

Individual gels were placed under a light beam which allowed the band to be seen clearly and were scored visually. Presence or absence of protein bands classification was used (Betiku, 2006). The position of the molecular weight marker assisted in scoring the protein bands on each gel. Data generated were subjected to statistical analysis by means of PAST (Palentholgical statistics) to generate dendogram that measure genetic similarity.

Results and Discussion

Plate 1 showed the representative gels obtained from the sodium do decyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis for each of the samples collected from Yoruba Ecotype Nigeria Indigenous chicken population. The results displayed in each of plate contain ten lanes (1-10) of different sample. The first lane is the molecular marker corresponding to each of the protein studied (Globulin, Transferrin, Albumin and Post Albumin). The gels were visually scored using a glass illuminator which allowed the bands to be seen clearly. The results of the band counting were subjected to statistical analysis using PAST package (Palenthological Statistics) to draw dendogram (Figs 1-4) which measures genetic similarity within the population.

The dendogram obtained for the population indicated low genetic similarity at Albumin (19%), Globulin (18%), and Post Albumin (40%) fig 1, 2, 3 respectively while high genetic similarity was observed at Transferrin (58%) locus. This is agreement with Barker (1994), and FAO (1980). This high genetic variation indicated that the population is still under the influence of natural selection. The importance of this can not be overemphasized because high genetic variation which is sometimes referred to as heterozygousity is a measure of the populations' ability to adapt to environmental changes and stress and thereby enable them to survive in the same condition (FAO, 1980).

In several Animals, including chickens, possessing high genetic variation have shown to have better survival rates and thus serves as reservoir of genetic materials for future improvement.

It can be concluded that there is still opportunity for conservation and improvement of Yoruba Ecotype Indigenous Chickens before their gene become diluted as a result of uncontrolled breeding with other ecotypes and exotic chickens. More protein loci should be further investigated and in addition future study should be extended to DNA characterization level to document complete characterization of Nigeria indigenous chicken. Also initiation of conservation programme to prevent loss of biodiversity in the indigenous chicken population for future genetic use is also recommended.

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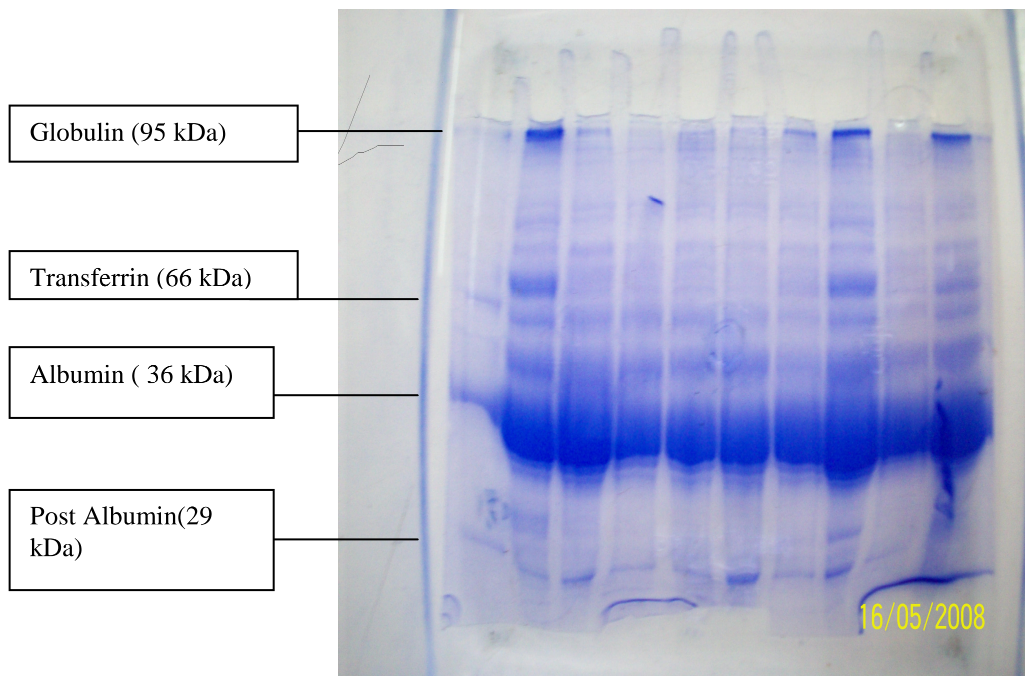

Plate 1: Migration of Globulin, Transferrin, Albumin and Post-albumin on the polyacrylamide gel.

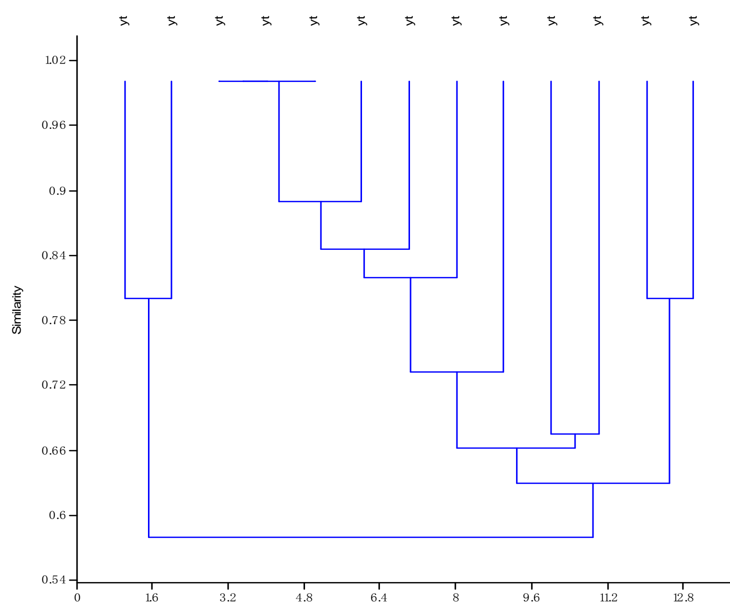

Fig 1: Dendogram showing genetic similarity at Transferrin locus

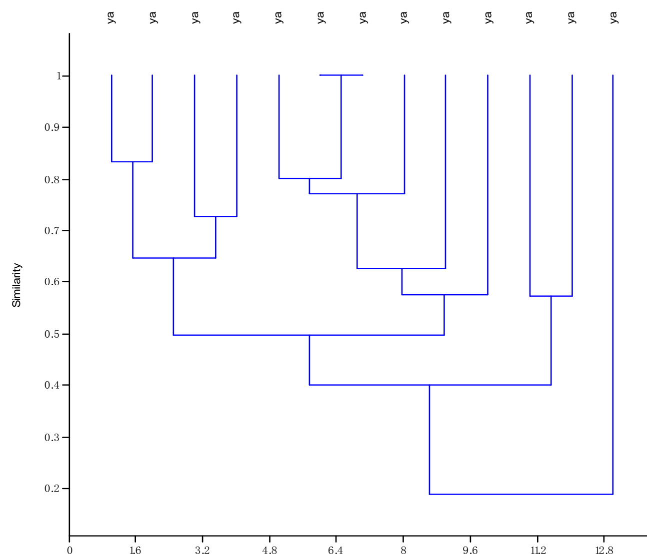

Fig 2: Dendogram showing genetic similarity at Albumin locus

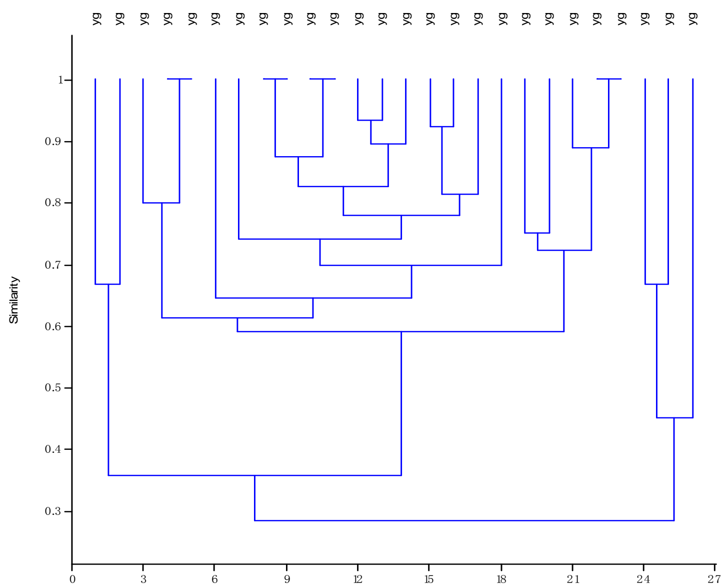

Fig 3: Dendogram showing genetic similarity at Globulin locus

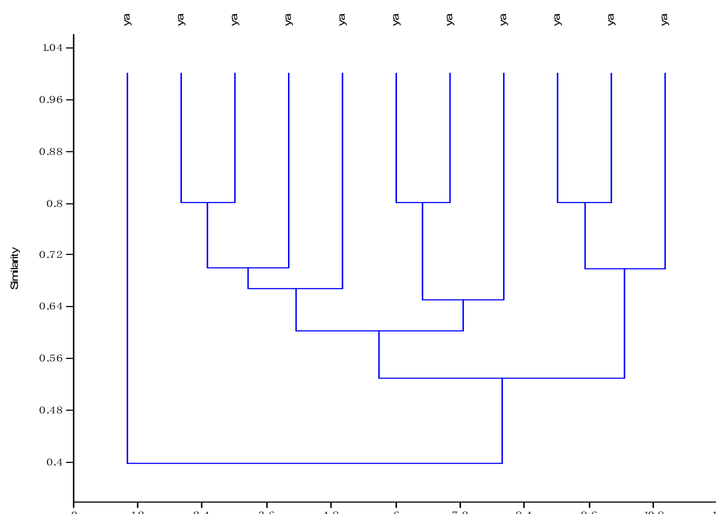

Fig 4: Dendogram showing genetic similarity at Post Albumin locus

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