Iranian Journal of Applied Animal Science

# Transferrin Genetic Types in Fulani and Yoruba Ecotype of Nigeria Indigenous Chickens

Research Article

A.O. Ige[1*] and A.E. Salako[2]

[1] Department of Animal Nutrition and Biotechnology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria
[2] Department of Animal Science, Animal Breeding and Genetics Unit, University of Ibadan, Oyo State, Nigeria

Received on: 4 Mar 2013
Revised on: 13 May 2013
Accepted on: 1 Jun 2013
Online Published on: Mar 2014

*Correspondence E-mail: igeazeemng50@yahoo.com

Online version is available on: www.ijas.ir



## ABSTRACT

Fulani ecotype chickens (FEC) and Yoruba ecotype chickens (YEC) are the two major types of indigenous chickens in Nigeria. They subsist under traditional Animal husbandry and are thus liable to indiscriminate mating and consequently to loss of genetic diversity. Fulani ecotype chickens (FEC) and Yoruba ecotype chickens (YEC) were investigated to determine genetic variation at transferrin locus using cellulose acetate electrophoresis and to establish genetic relationship within and between the two ecotypes. Direct gene counting method was used to interpret the result after electrophoresis. Palenthological statistics (PAST) was used to generate dendogram which was used to measure genetic similarity. Transferrin was interpreted into six phenotypes (AA, AB, BB, AC and BC) whose inheritance is genetically controlled by three codominant alleles ($Tf^A$, $Tf^B$ and $Tf^C$). Gene frequencies of $Tf^A$, $Tf^B$ and $Tf^C$ were 0.35, 0.2 and 0.43 in Yoruba ecotype chickens (YEC) and 0.21, 0.32 and 0.44 in Fulani ecotype chickens (FEC). While Genotype frequencies were 12.5%, 10%, 75%, 35%, 17.5% and 15% for Yoruba ecotype chickens (YEC) and 11.19%, 2.5%, 16.6%, 22.2% and 27.7% for Fulani ecotype chickens (FEC). Main clusters observed from the dendogram indicated 72% genetic similarity within Fulani ecotype chickens (FEC), 58 % genetic similarity within Yoruba ecotype chickens (YEC) and 70 % genetic similarity between Fulani ecotype chickens (FEC) and Yoruba ecotype chickens (YEC). No genetic relationship existed between transferrin and phenotypic traits (sex, plumage colour and comb type). Conclusively, the two populations were closely related at tranferrin locus. Further study should be extended to other protein markers and DNA level and conservation methods should be initiated to prevent further dilution of these chickens and for future development of Nigeria chicken line breed.

KEY WORDS Fulani, Nigerian chickens, transferring, Yoruba.

## INTRODUCTION

The indigenous chicken breed is a general terminology given to animals kept in the extensive system, scavenging on free range, multipurpose and unimproved environment (Horst, 1989). The indigenous chickens are adapted to local environmental conditions and disease. Horst (1989) considered the indigenous chickens as gene reservoir, particularly, those genes that have adaptive values in the tropical and subtropical area and they contribute greatly to human supply of egg and meat.

However, little information on their detailed characterization is available. Also no effort to initiate conservation of indigenous chickens' genetic resources is on going. One of the important reasons to conserve indigenous chicken genetic resources is to keep genetic variation within and between ecotypes. The present and future improvement and sustainability of indigenous chicken production systems are dependent upon the availability of genetic variation (Banitez, 2002).

Biochemical characterization based on protein polymorphism has been reported to be useful in genetic characterization and diversity measures between and within livestock breeds (Lee *et al*. 2000; Yamamoto *et al*. 1996; Esmaeilkhanian *et al*. 2000). Das and Deb (2008) described the importance of biochemical polymorphism in the improvement of chicken. It is also noted by Rendel (1967) that some polymorphic alleles may be correlated with economic traits due to linkage pleotropy or general heterozygousity. Detection of biochemical polymorphism involves the use of electrophoresis which is the migration of ions in an electric field and widely used for analytical separation of biological molecules.

This study was therefore conducted to assess the genetic types of transferrin and establishes their gene and genotype frequencies in both Yoruba and Fulani ecotype indigenous chickens in the derived savannah zone of Nigeria.

## MATERIALS AND METHODS

Blood samples were collected from one hundred chickens comprising 50 adult Yoruba ecotype chickens (YEC) and 50 adult Fulani ecotype chickens (FEC). In addition qualitative traits such as sex comb type and plumage colour were recorded individually for each chicken. Samples were prepared and subjected to cellulose acetate electrophoresis following the procedure of Riken (2006).

### Sample preparation

Two ml of blood was collected from individual chickens through wing vein puncture and put into plastic tubes containing anticoagulant. The plasma fraction was separated from erythrocyte fraction of heparinized blood by centrifugation at 2500-3000 rpm for 10min at 10 ˚C. The supernatant was stored at 4 ˚C until the time of analysis.

### Cellulose acetate electrophoresis protocol

The cellulose acetate membrane was soaked very slowly in the buffer solution (Trisgylcine, pH 8.5) and the buffer was poured into the electrophoresis chamber. Wick was folded and moistened with the buffer and placed on each of the support arms of the electrophoresis chamber. The sample (supernatant) was poured into the slots of the applicator. The soaked cellulose acetate membrane plate was gently placed between the paper towels on each support arm 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 the plate flat and ensured an even current through the plate. The electrophoresis was run at 150 vol and 4 ˚C for 50min and the migration was from cathode to anode. After the electrophoresis, ponceaus was applied to stain the paper and destained with 5% acetic acid. The bands were clearly separated and direct gene counting was used for the interpretation of allele.

### Statistical analysis

Gene and genotype frequency were calculated using the expression provided by Roghgarden (1977) as follows:

P: gene frequency of allele x.
Q: gene frequency of allele y.

$P = (2\times(N_{xx})+N_{xy}) / (2\times N)$
$Q = (2\times(N_{yy})+N_{xy}) / (2\times N)$

Where:
N: the total number of individual sampled.
$N_{xx}$: observed genotype number for xx.
$N_{xy}$: observed genotype number for xy.
$N_{yy}$: observed genotype number for yy.

Genotype frequency was calculated as follows:

(number of xx/total individual) × 100
(number of xy/total individual) × 100
(number of yy/total individual) × 100

Palenthological statistical (PAST) package was used to generate a dendogram that measures the genetic similarity between chicken types.

## RESULTS AND DISCUSSION

Electrophoretic separation of transferrin is shown in plates 1 and 2 for Yoruba and Fulani Ecotype respectively. It was interpreted into six phenotypes (AA, AB, BB, AC, CC and BC) whose inheritance is genetically controlled by three co-dominant allele ($Tf^A$, $Tf^B$ and $Tf^C$). The distribution gene and genotype frequencies by ecotype were presented on Table 1.

Gene frequencies were 0.35, 0.2 and 0.43 for $Tf^A$, $Tf^B$ and $Tf^C$ respectively. However, the phenotypes AA, AB, BB, AC, CC and BC genotype frequencies of 12.5%, 10%, 7.5%, 35%, 17.5% and 15% in Yoruba ecotype chickens (YEC), respectively, while they were 11.19%, 2.8%, 16.6%, 16.6%, 22.2% and 27.7% in Fulani ecotype chickens (FEC) respectively. All types of transferrin were found in both sexes (Tables 2 and 3) where gene frequencies were $Tf^A$ (0.39), $Tf^B$ (0.25), $Tf^C$ (0.36) for male and $Tf^A$ (0.37), $Tf^B$ (0.17), $Tf^C$ (0.46) for female in Yoruba ecotype chickens (YEC), while in Fulani ecotype chickens (FEC) gene frequencies were $Tf^A$ (0.19), $Tf^B$ (0.44) $Tf^C$ (0.30) for male and $Tf^A$ (0.04), $Tf^B$ (0.38) $Tf^C$ (0.50) for female.

**Table 1** Genotypes and gene frequencies at transferrin locus based on Ecotype

| | | Genotype frequency | | | | | | | | | | | | Gene frequency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ecotype | No | AA | % | AC | % | BB | % | CC | % | BC | % | AB | % | A | B | C |
| Yoruba | 40 | 5 | 12.5 | 14 | 35 | 3 | 7.5 | 7 | 17.5 | 6 | 15 | 4 | 10 | 0.35 | 0.20 | 0.43 |
| Fulani | 36 | 4 | 11.1 | 6 | 16.66 | 6 | 16.66 | 8 | 22.2 | 10 | 27.77 | 1 | 2.77 | 0.21 | 0.32 | 0.44 |

**Table 2** Distribution of transferrin genotype and gene frequencies based on sex in Yoruba ecotype chickens (YEC)

| | | Genotype frequency | | | | | | | | | | | | Gene frequency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SEX | No | AA | % | AC | % | BB | % | CC | % | BC | % | AB | % | A | B | C |
| Male | 14 | 1 | 7.14 | 7 | 50.00 | 2 | 14.29 | 1 | 7.14 | 1 | 7.14 | 2 | 14.29 | 0.39 | 0.25 | 0.36 |
| Female | 26 | 7 | 15.37 | 9 | 34.62 | 2 | 7.69 | 6 | 23.08 | 3 | 11.54 | 2 | 7.69 | 0.37 | 0.17 | 0.46 |

**Table 3** Distribution of genotype and gene frequencies at transferrin locus with respect to sex in Fulani ecotype chickens (FEC)

| | | Genotype frequency | | | | | | | | | | | | Gene Frequency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | No | AA | % | AC | % | AB | % | BC | % | CC | % | BB | % | A | B | C |
| Male | 18 | 2 | 11.11 | 3 | 16.67 | 0 | 0 | 4 | 22.2 | 2 | 11.11 | 6 | 16.67 | 0.19 | 0.44 | 0.31 |
| Female | 18 | 2 | 11.11 | 4 | 22.22 | 1 | 5.55 | 3 | 16.67 | 7 | 38.88 | 5 | 27.77 | 0.04 | 0.38 | 0.58 |

The trend of the distribution of transferrin was not consistent with respect to sex in both ecotypes. Similar irregular pattern of the distribution was observed with respect to qualitative traits (plumage colour and comb type).

However, eight different patterns of plumage colour (Tables 6 and 7) were observed (white, black, brown, ash, multicoloured, red, yellow and whitish brown) and three distinct types of comb (single, rose and pea) (Tables 4 and 5) were found as well in the two populations. In both Fulani ecotype chickens (FEC) and Yoruba ecotype chickens (YEC), three main clusters were observed with 72%, 79% and 80% levels of similarity in Fulani ecotype chickens (FEC) and 58%, 65% and 66% levels of similarity Yoruba ecotype chickens (YEC) (Figures 1, 2 and 3).

On the contrary, four main clusters with similarities levels of 70%, 74%, 77% and 80% were observed between Fulani ecotype chickens (FEC) and Yoruba ecotype chickens (YEC). However, the variation within and between the ecotypes were generally low. Transferrins are the most heterogeneous of the blood proteins in poultry studies and therefore are well adapted to biochemical studies (Montag, 1992). Six phenotypes of transferrin (AA, AB, BB, AC, CC and BC) were observed in both Yoruba and Fulani ecotype chicken population using the nomenclature described by Ashton *et al.* (1966). Frequency of $Tf^{c}$ (0.43) was predominantly higher than frequencies of $Tf^{A}$ (0.35) and $Tf^{B}$ (0.2) in Yoruba ecotype chicken, similarly, in Fulani ecotype chicken the frequency of $Tf^{C}$ (0.44) was higher than frequencies of $Tf^{A}$ (0.21) and $Tf^{B}$ (0.32). This observation agrees with the work of Esmaeilkanian *et al.* (2000) on Iranian native poultry breeds. They reported a higher frequency of $Tf^{c}$ (0.531) than $Tf^{A}$ (0.031) and $Tf^{B}$ (0.43) in Dashtryary breed, a higher frequency of $Tf^{c}$ (0.574) than $Tf^{B}$ (0.407) and $Tf^{A}$ (0.019) in Lary breed, a higher frequency of $Tf^{c}$ (0.629) than $Tf^{B}$ (0.355) and $Tf^{A}$ (0.016) in Marandy breed, a higher freque ncy $Tf^{c}$ (0.523) than $Tf^{B}$ (0.432) and $Tf^{A}$ (0.045) in Naked Neck and higher $Tf^{c}$ (0.522) than $Tf^{B}$ (0.523) and Tf (0.075) in common breed. Similar observations are found by Montag (1992) in Pheasants where he scored high frequencies of transferrin C allele in all of the populations studied and he attributed it to the influence of environment which caused increased selection pressure toward birds with the $Tf^{c}$ allele.

However, Tanabe *et al.* (1991) and Okada *et al.* (1980) reported that the most frequent allele in their study was $Tf^{B}$. Stratil (1968) and Ogden *et al.* (1962) also stated that the frequency of $Tf^{B}$ allele is highest in strains such as Light Sussex, White Cornish, White Leghorn, Partridge Leghorn, White and Black Leghorn, Rhode Island Red and some of their crosses. Lee *et al.* (2000) similarly reported a high frequency of $Tf^{B}$ in red (0.982) and black (0.989) coloured lines of Korean native chickens. In addition, they recorded the presence of complete gene fixation for yellow line and all the foreign breeds investigated. Nevertheless, the present findings are concordant with that of Yamamoto *et al.* (1996) who found high frequency of $Tf^{B}$.

The difference may be due to differences in genetic background of the breeds examined herein and their purity. Vohs and Carr (1969) hypothesized that transferrin was controlled by four codominant alleles at a single locus in lowa Pheasants. Additionaly they found that one bird out of 869 sampled have the $Tf^{D}$ allele which was not found in this study. This may be due to variation in sample size as it is possible that if the two populations studied were sampled more extensively there is a possibility that $Tf^{D}$ allele would also be found. Transferrin polymorphisms have been shown to correlate with some production traits. Interestingly Stratil (1979) observed the superiority of chicken with $Tf^{B}$ over $Tf^{A}$ in terms of egg production. Lush (1966) noted that the effect of heterozygous transferrin ($Tf^{B}$ and $Tf^{C}$) appears to be significantly better in fertility, hatchability and egg production.

**Table 4** Distribution of genotype and gene frequencies at transferrin locus with respect to comb type in Yoruba ecotype chickens (YEC)

| | | Genotype frequency | | | | | | | | | | | | Gene frequency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Comb type | No | AA | % | AC | % | BB | % | CC | % | BC | % | AB | % | A | B | C |
| Single | 35 | 5 | 14.29 | 14 | 40.00 | 2 | 5.71 | 6 | 17.14 | 5 | 14.29 | 3 | 8.57 | 0.39 | 0.17 | 0.44 |
| Rose | 0 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 1 | 50.00 | 1 | 50.00 | 0.25 | 0.50 | 0.25 |
| Pea | 0 | 0 | 0.00 | 2 | 66.67 | 0 | 0.00 | 1 | 33.33 | 0 | 0.00 | 0 | 0.00 | 0.33 | 0.00 | 0.67 |

**Table 5** Distribution of genotype and gene frequencies at transferrin locus with respect to comb type in Fulani ecotype chickens (FEC)

| | | Genotype frequency | | | | | | | | | | | | Gene frequency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Comb type | No | AA | % | AC | % | BB | % | CC | % | BC | % | AB | % | A | B | C |
| Single | 31 | 5 | 16.12 | 4 | 12.90 | 4 | 12.90 | 8 | 25.80 | 8 | 25.80 | 1 | 3.22 | 0.24 | 0.27 | 0.45 |
| Rose | 3 | 0 | 0 | 2 | 66.66 | 1 | 33.33 | 0 | 0 | 0 | 0 | 0 | 0 | 0.33 | 0.33 | 0.33 |
| Pea | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 100 | 0 | 0 | 0 | 0.5 | 0.5 |

**Table 6** Distribution of genotype and gene frequency at transferrin locus based on Plumage colour in Fulani ecotype chickens (FEC)

| | | Genotype frequency | | | | | | | | | | | | Gene frequency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plumage colour | No | AA | % | AC | % | BB | % | CC | % | BC | % | AB | % | A | B | C |
| Brown | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 33.33 | 2 | 66.66 | 0 | 0 | 0 | 0.33 | 0.66 |
| Black | 6 | 0 | 0 | 1 | 16.66 | 2 | 33.33 | 0 | 0 | 3 | 50 | 0 | 0 | 0.08 | 0.58 | 0.33 |
| White | 6 | 1 | 16.66 | 2 | 33.33 | 1 | 16.66 | 0 | 0 | 2 | 33.33 | 0 | 0 | 0.33 | 0.33 | 0.33 |
| Yellow | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| Black + brown | 12 | 2 | 16.66 | 0 | 0 | 3 | 25 | 5 | 41.66 | 1 | 8.33 | 1 | 8.33 | 0.20 | 0.33 | 0.45 |
| Brown + white | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| White + black | 7 | 1 | 14.28 | 3 | 42.82 | 1 | 14.28 | 0 | 0 | 1 | 28.57 | 0 | 0 | 0.35 | 0.28 | 0.35 |

**Table 7** Distribution of genotype and gene frequencies at transferrin locus with respect to plumage colour in Yoruba ecotype chickens (YEC)

| | | Genotype frequency | | | | | | | | | | | | Gene frequency | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plumage colour | No | AA | % | AC | % | BB | % | CC | % | BC | % | AB | % | A | B | C |
| Brown | 12 | 1 | 8.33 | 7 | 58.33 | 0 | 0.00 | 2 | 16.67 | 1 | 8.33 | 1 | 8.33 | 0.42 | 0.08 | 0.50 |
| Black | 4 | 0 | 0.00 | 2 | 50.00 | 0 | 0.00 | 2 | 50.00 | 0 | 0.00 | 0 | 0.00 | 0.25 | 0.00 | 0.75 |
| White | 2 | 0 | 0.00 | 1 | 50.00 | 0 | 0.00 | 0 | 0.00 | 1 | 50.00 | 0 | 0.00 | 0.25 | 0.25 | 0.50 |
| Yellow | 1 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 1 | 100.00 | 0.50 | 0.50 | 0.00 |
| Black + brown | 8 | 2 | 25.00 | 1 | 12.50 | 1 | 12.50 | 1 | 12.50 | 2 | 25.00 | 1 | 12.50 | 0.38 | 0.31 | 0.31 |
| Brown+ white | 5 | 1 | 20.00 | 1 | 20.00 | 0 | 0.00 | 2 | 40.00 | 0 | 0.00 | 1 | 20.00 | 0.40 | 0.10 | 0.50 |
| White + black | 8 | 1 | 12.50 | 4 | 50.00 | 1 | 12.50 | 0 | 0.00 | 2 | 25.00 | 0 | 0.00 | 0.38 | 0.25 | 0.38 |

Accordingly he concluded that Chicken with $Tf^A$ appeared to have delayed sexual maturity while the chicken with $Tf^B$ reached their sexual maturity at earlier age.

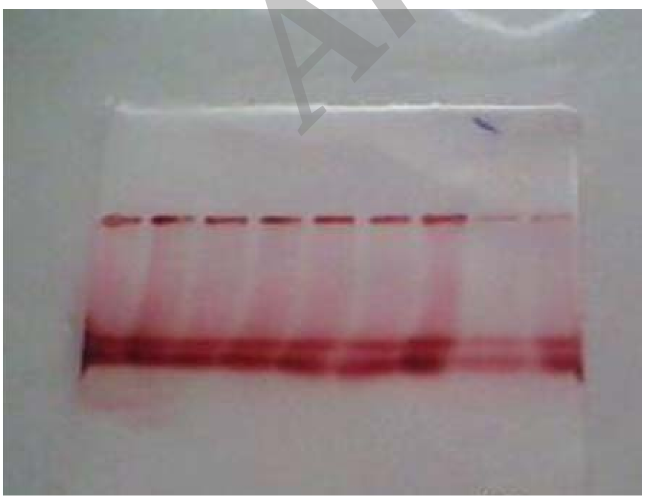

**Plate 1** Electrophoretic separation of transferrin in Yoruba ecotype chickens (YEC)

The ecotypes studied herein therefore appear to be mixture of populations as they have the potential to be both egg and meat producer if selection is based on the result of biochemical traits studies.

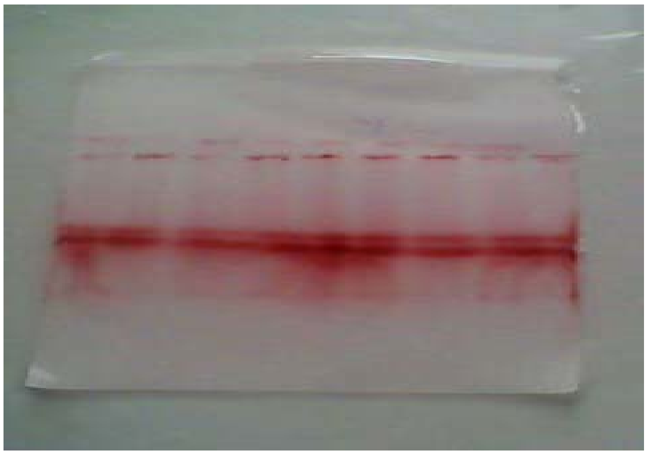

**Plate 2** Electrophoretic separation of transferrin in Fulani ecotype chickens (FEC)

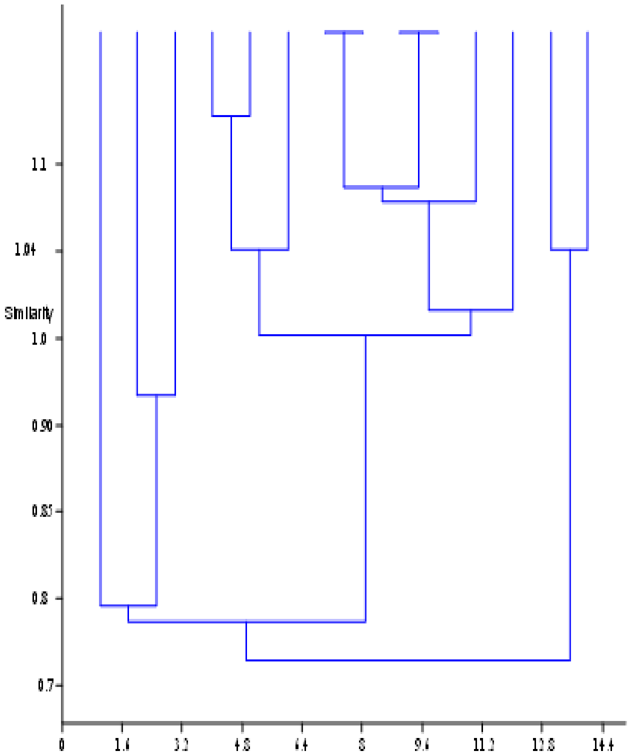

**Figure 1** Dendogram of genetic similarity of Fulani ecotype chickens (FEC) at transferrin locus

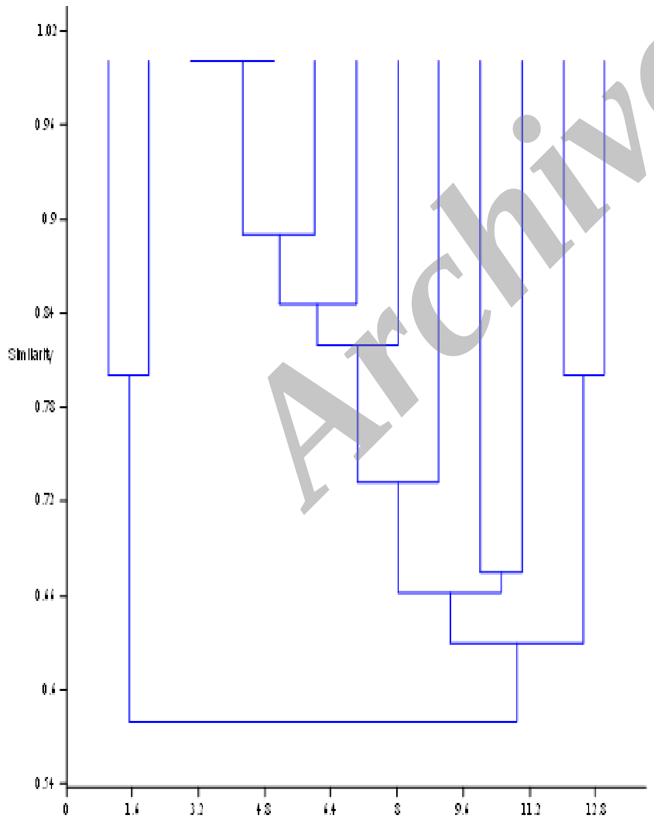

**Figure 2** Dendogram of genetic similarity of Yoruba ecotype chickens (YEC) at transferrin locus

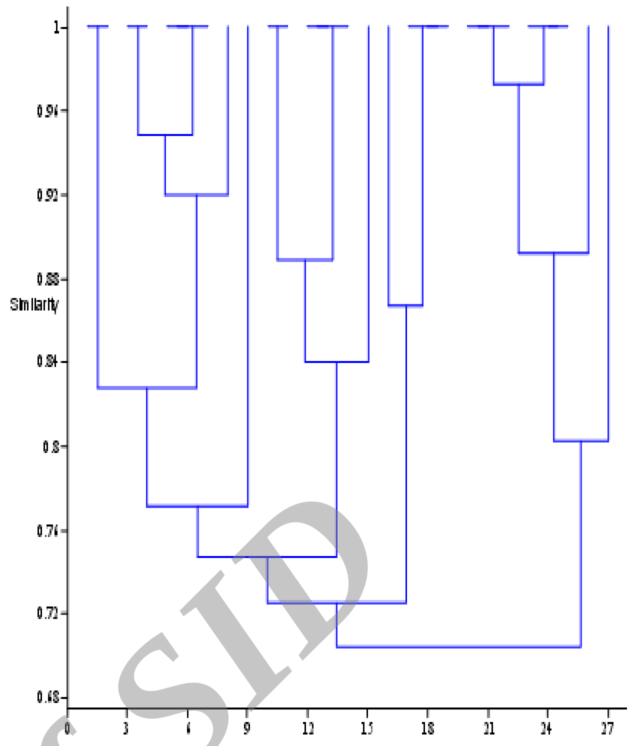

**Figure 3** Dendogram of genetic similarity between Yoruba and Fulani ecotype chickens (YEC and FEC) at transferrin locus

Salako and Ige (2006) found similar observation that in the strict sense of genetic homozygousity, indigenous chickens are heterogenous at the haemoglobin locus. In this present study, low genetic variation observed between the two ecotypes as shown by the dendogram indicated that the two ecotypes are genetically similar at transferrin locus and they are not genetically isolated from each other.

## CONCLUSION

Although no obvious genetic relationship is previously established between transferrin and qualitative traits, the current study showed that their inheritance is genetically controlled and that neither plumage colour nor comb type can be used to predict the type of transferrin of individual chicken examined. Plumage colour varied widely as eight different types (Brown, Black, White, Yellow, Black and Brown, Brown and White and White and Black) existed was scored Fulani ecotype chickens (FEC) and Yoruba ecotype chickens (YEC). In addition, three type of comb (Single, Rose and Pea) were also found in the two populations. The present study demonstrates the usefulness of protein marker to characterize the two ecotypes of indigenous chicken. The information got may be useful as an initial guide in defining objectives for designing future investigations of genetic integrity and developing conservation strategies for Nigeria indigenous chickens.

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