

## Research Article

## Biochemical Characterization of Two Nigerian Indigenous Chicken Ecotypes

Gambo<sup>1</sup>, D., Momoh<sup>2</sup>, O. M., Gwaza<sup>2</sup>, D. S., Ogah<sup>1</sup>, D. M., Ubu<sup>2</sup>, I., Agbu<sup>1</sup>, C. S. and Abdullahi<sup>1</sup>, J.<sup>1</sup>Department of Animal Science, Faculty of Agriculture, Nasarawa State University Keffi, Nasarawa State, Nigeria<sup>2</sup>Department of Animal Breeding and Physiology, College of Animal Science, University of Agriculture, Makurdi, Benue State, Nigeria**Article History**

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**Abstract:** The study was undertaken to investigate biochemical diversity within and between Tiv and Fulani local chicken ecotypes of Nigeria. The experimental birds were randomly sourced from ten locations. The locations (1-5) for the Tiv ecotype were Uikpan, Daudu, Kadarko, Yelwata and Cohor (in Benue and Nasarawa States) while that of the Fulani ecotype were Lafia, Akurba, Adogi, Asakio and Namu (in Nasarawa and Plateau States). At maturity, four (4) male and four (4) female birds were randomly selected from each location per ecotype to give a total of eighty (80) adult birds (40 birds each for Tiv and Fulani ecotype) and used for blood protein characterization study. Blood protein loci, namely haemoglobin, albumen, transferrin and carbonic anhydrase were analyzed using electrophoresis. Data collected from the biochemical analysis were analyzed using popgene version 1.31. The results indicate that, Allele A was prevalence in both the Tiv (0.563) and the Fulani (0.769) ecotypes for haemoglobin while Allele C for albumen was most prevalent in both ecotypes. In transferrin however, allele D (0.419) and allele A (0.689) were most prevalent in the Tiv and the Fulani ecotype, respectively. Carbonic anhydrase showed that Allele A dominated in both ecotypes with frequencies of 0.550 and 0.632 for the Tiv and the Fulani ecotypes respectively. The genetic variability (heterozygosity) value of the two ecotypes ranged from 0.360 to 0.654. The Tiv ecotype had higher heterozygosity value at all loci than the Fulani ecotype. All the protein loci for the two ecotypes had 100 percent polymorphic loci except in the Fulani ecotype where 75 percent polymorphic loci were observed for locations 2 and 3. The effective number of alleles ( $n_e$ ) was 2.273 and 1.779 for the Tiv and the Fulani ecotype, respectively with an average of 2.026. From the findings of this study, it was concluded that the Tiv and the Fulani chicken ecotypes are distinct genetic groups with sufficient genetic variability within and between them to justify the use of selection tool to bring about genetic improvement in both ecotypes. Cross breeding between the two groups should be exploited to take advantage of heterosis.

**Keywords:** allelic frequency, blood protein, electrophoresis, heterozygosity, polymorphism.

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### INTRODUCTION

Nigeria has a high human population which is continuously on the increase. This increase has led to high demand for the available but insufficient animal and poultry products in the country. Poultry, particularly chickens are very important and have been recognized as important genetic resources among the avian species (Olowofeso *et al.*, 2005). Poultry products are one of the cheapest and easily affordable animal protein sources for the teeming population. Chickens are the most widely distributed of all poultry types in Nigeria with a population of 166 million birds (FAOSTAT, 2007). Thus Chickens play very significant socio-cultural and economic roles in most African societies. Genetic diversities in the indigenous livestock species in developing countries are valuable attributes or assets for production, adaptation and

resistance of the indigenous animals to endemic diseases. Many technologies including DNA based technology are employed in diversity studies.

DNA-based technologies are now the methods of choice for genetic characterization of livestock (Arora *et al.*, 2011); but its applications in developing countries are limited due to its complex nature, facilities and high cost. Nevertheless, other biotechnological techniques has opened up molecular techniques, such as routine electrophoresis being employed for the detection of polymorphism at protein and enzyme loci as well as other serological and immuno-genetic procedures for measurement of genetic variations (Salako *et al.*, 2007).

Proteins or allozyme polymorphs remain

tremendously useful in developing countries because of their lower complexities, cost, simplicity of data interpretation and amount of genetic information accessed (Rege and Okeyo, 2006). A major advantage of biochemical characterization is that they do not depend on environmental factors, stable throughout ontogenesis and have a simple type of inheritance (Lee *et al.*, 1995). Though investigation on the genetic diversity of farm animals using microsatellite markers (molecular markers) and other high technology procedures have been carried out in developing world; nevertheless, blood protein markers of farm animals have also been widely used for genetic structure and phylogenetic studies among breeds of animals (Boujenane *et al.*, 2008; Zahrane *et al.*, 2011; Akinyemi and Salako, 2012) as well as for characterization and estimation of genetic diversity within and between breeds and species (Akinyemi and Salako, 2012; Yakubu and Aya, 2012). The objective of the study was to determine the genotype and allele frequencies of haemoglobin, transferrin, carbonic anhydrase and albumin in the populations under study.

## MATERIALS AND METHODS

Two local chicken ecotypes comprising of the Tiv (long legged) and the Fulani birds from five locations each were used for this experiment. The locations (1-5) for the Tiv ecotype were Uikpan, Daudu, Kadarko, Yelwata and Cohor (in Benue and Nasarawa States) while that of the Fulani ecotype were Lafia, Akurba, Adogi, Asakio and Namu (in Nasarawa and Plateau States). At maturity, four (4) males and four (4) females were randomly selected from each location per ecotype to give a total of forty (40) adult birds (20 males and 20 females) per ecotype and used for the blood protein characterization study.

Whole blood (3-5 ml) was collected from the wing vein of each of the selected healthy birds into correspondingly labelled heparinized tube. Heparin acted as anti-coagulant and blood contamination was prevented by using separate syringes and needles for individual birds. These samples were kept refrigerated in ice packs and transported to Animal Science Laboratory of the University of Ibadan, Ibadan, Oyo State for electrophoresis analysis.

The blood samples were centrifuged at 4 °C for 20 mins at 3 000 rpm in order to separate plasma and erythrocyte. Erythrocyte was washed with 9 percent NaCl to free them from plasma proteins and was then lysed with four fold of cold distilled water in order to release the haemoglobin. Then plasma and

haemolysates aliquots were stored at 4°C prior to electrophoresis analysis. The lysed red blood cells were used to determine Haemoglobin (Hb). However, the blood plasma was used to determine albumin (Alb), carbonic anhydrase (Ca) and transferrin (Tf) genotypes. Electrophoresis was performed using cellulose acetate strips as described by Akinyemi and Salako (2012). After electrophoresis, strips of bands were stained for 25 – 30 minutes. The stained bands were thereafter washed with 1 percent HCl for Ca and 5 percent HCl for Hb, Alb and Tf (Akinyemi and Salako, 2012). After washing, the bands were covered by destaining solution (1% and 5% HCl) until the electrophoresis bands became visible, and then air dried and scored.

### Parameters Measured

The blood protein parameters such as haemoglobin, albumin, carbonic anhydrase and transferrin were determined using electrophoresis.

### Experimental Design and Data Analysis

The design of the experiment was Completely Randomized Design (CRD). Stratified random sampling technique was employed in assembling the experimental bird's population. Four female and male birds of each of the Tiv and the Fulani ecotypes were randomly sampled from each of the five localities for blood protein analysis. Biochemical variability for blood protein (haemoglobin, transferrin, carbonic anhydrase and plasma albumin) within and between the ecotype and location was estimated using popgene statistical software version 1.31.

## RESULT

### Allelic frequency

The allelic frequencies at the haemoglobin, albumin, transferrin and carbonic anhydrase loci in the two Nigerian local chicken ecotypes are presented in Table 1. Two haemoglobin alleles were expressed in both the Tiv and the Fulani ecotypes. Haemoglobin allele A was prevalence in both the Tiv (0.563) and the Fulani (0.769) ecotypes. Allele A for albumen was equally most prevalent in both ecotypes. However, albumin allele B was least in the Tiv ecotype and non-existence in the Fulani ecotype. In transferrin, allele D (0.419) and allele A (0.689) was most prevalent in the Tiv and the Fulani ecotypes, respectively. Allele C was not noted in both ecotypes while allele B was only found in the Tiv ecotype. Allele A in carbonic anhydrase was prevalent in both ecotypes with frequencies of 0.550 and 0.632 for the Tiv and the Fulani ecotypes, respectively.

**Table 1:** Allelic Frequencies at Haemoglobin, Albumin, Transferrin and Carbonic Anhydrase Loci in two Ecotypes of Nigerian Local Chickens

Locus	Alleles	Tiv ecotype (N = 40)	Fulani ecotype (N = 40)
Haemoglobin	A	0.563	0.769
	B	0.437	0.231
Albumin	A	0.500	0.539
	B	0.013	-
	C	0.487	0.461
Transferrin	A	0.216	0.689
	B	0.365	-
	C	-	-
	D	0.419	0.311
Carbonic anhydrase	A	0.550	0.632
	B	0.188	0.013
	C	0.262	0.355
Total no. of alleles	12	11	9

N = Sample size, A, B, C, D = haemoglobin, albumin, transferrin and carbonic anhydrase allele respectively

Table 2 presents the effect of location on allelic frequencies at blood protein loci of the two ecotypes of Nigerian local chicken. The allelic frequencies vary from location to location in both the Tiv and the Fulani ecotypes. For haemoglobin locus in the Tiv ecotype, alleles A and B had the same frequencies (0.500 each) in birds from Uikpan, Daudu, Kadarko and Yelwata (locations 1, 2, 3 and 4) while allele A overwhelmingly dominated allele B (0.813 vs 0.188) in birds from Cohor (location 5). However, in the Fulani ecotype, apart from birds in Asakio (location 4) where alleles A and B had equal frequency (0.500), allele A had higher frequencies at other four locations with 1.00 frequency in birds from Akurba (location 2). In the albumin locus, allele A and C had equal frequencies in birds from Daudu, Kadarko, Yelwata and Cohor (locations 2, 3, 4 and 5) for the Tiv ecotype while in the Fulani ecotype, equal frequencies of allele A and C were observed in birds from Lafia, Asakio and Namu (location 1, 4 and 5). Allele B was only found with a very negligible frequency (0.063) in the Tiv birds

from Uikpan (location 1). Transferrin locus indicated mostly allele A, B and D in the Tiv ecotype and only allele A and D in the Fulani ecotype. In the Tiv ecotype, allele B dominated in birds from Uikpan and Daudu (locations 1 and 2) while allele D was prevalent in birds from Kadarko and Yelwata (locations 3 and 4). Allele A however, was prevalent in birds from Cohor (location 5). In the Fulani ecotype, allele A dominated in all the locations except in birds from Namu (location 5) where allele D was prevalent. Carbonic anhydrase showed 3 alleles (A, B and C) in both ecotypes. In the Tiv ecotype, allele B dominated in birds from Uikpan (location 1) while allele A was prevalent in birds from Daudu, Yelwata and Cohor (locations 2, 4 and 5). Birds from Kadarko (location 3) had equal frequencies of allele A and C. In a similar fashion, allele A was the most prevalent in birds from Lafia, Akurba and Adogi (locations 1, 2 and 3) while birds in location 4 and 5 (Asakio and Namu) had equal frequencies of allele A and C.

**Table 2:** Effect of Location on Allelic Frequencies at some blood protein Loci in two Ecotypes of Nigerian Local Chickens

Locus	Allele	L1 (N = 8)	L2 (N = 8)	L3 (N = 8)	L4 (N = 8)	L5 (N = 8)
<b>Tiv ecotype</b>						
Haemoglobin	A	0.500	0.500	0.500	0.500	0.812
	B	0.500	0.500	0.500	0.500	0.188
Albumin	A	0.500	0.500	0.500	0.500	0.500
	B	0.062	-	-	-	-
	C	0.438	0.500	0.500	0.500	0.500
Transferrin	A	-	0.063	0.143	0.375	0.583
	B	0.688	0.688	0.357	-	-
	C	-	-	-	-	-
	D	0.313	0.250	0.500	0.625	0.417
Carbonic anhydrase	A	0.250	0.500	0.500	0.750	0.750
	B	0.563	0.375	-	-	-
	C	0.188	0.125	0.500	0.250	0.250

Total no. of allele		12	10	10	9	8	8
<b>Fulani ecotype</b>							
Haemoglobin	A	0.875	1.00	0.563	0.500	0.936	
	B	0.125	0.00	0.438	0.500	0.063	
Albumin	A	0.500	0.563	0.625	0.500	0.500	
	B	-	-	-	-	-	
	C	0.500	0.438	0.375	0.500	0.500	
Transferrin	A	0.700	0.813	1.00	0.813	0.125	
	B	-	-	-	-	-	
	C	-	-	-	-	-	
Carbonic anhydrase	D	0.300	0.187	-	0.188	0.875	
	A	0.625	0.813	0.714	0.500	0.500	
	B	-	0.063	-	-	-	
	C	0.375	0.125	0.286	0.500	0.500	
Total no. of allele		12	8	9	7	8	8

N = Sample size, L = location, A, B, C, D = haemoglobin, albumin, transferrin and carbonic anhydrase allele respectively

### Genetic variability (heterozygosity)

Genetic variability (heterozygosity) in the four loci of the two ecotypes populations of Nigerian local chicken is presented in Table 3. The heterozygosity values of the two ecotypes ranges from 0.360 to 0.654. The Tiv ecotype had higher heterozygosity value at all loci than the Fulani ecotype. All the protein loci for the two ecotypes were 100 percent polymorphic. The effective numbers of allele ( $n_e$ ) were 2.273 and 1.779 for the Tiv and the Fulani ecotypes, respectively with an average value of 2.026.

Genetic variability (heterozygosity) in the four loci at the five locations in the two ecotypes of Nigerian local chicken is presented in Table 4. In the Tiv ecotype, variability at the haemoglobin locus was the same (0.533) in birds from Uikpan, Daudu, Kadarko and Yelwata (locations 1, 2, 3 and 4). It ranges from 0.325 to 0.533 with an average of 0.491. However, in the Fulani ecotype the haemoglobin variability ranges from 0.00 in birds from Akurba (location 2) to 0.533 in birds from Asakio (location 4). Variability at albumin locus in the Tiv ecotype ranges from 0.533 (in locations 4 and 5) to 0.592 (in location 1) with a mean of 0.547

while that of the Fulani ecotype ranges from 0.500 (in location 3) to 0.539 (in location 4) with an average of 0.526. Transferrin variability in the Tiv ecotype ranges from 0.458 in location 1 to 0.648 in location 3 with an average of 0.527 while that of the Fulani ecotype was 0.00 in location 3 to 0.467 in location 1 with a mean of 0.270. Carbonic anhydrase in the Tiv ecotype showed a variability of 0.400 in location 4 and 5 to 0.633 in location 2 with an average of 0.521. That of Fulani ecotype ranges from 0.342 to 0.539 with an average of 0.471.

All the protein loci for the five locations in each ecotype had 100 percent polymorphic loci except location 2 and 3 in Fulani ecotype which had 75 percent polymorphic loci. The effective number of allele ( $n_e$ ) ranges from 1.788 in location 5 to 2.253 in location 3 with an average of 2.082 in the Tiv ecotype. That of the Fulani ecotype ranges from 1.192 in location 2 to 1.936 in location 4 with an average of 1.550. Effective numbers of allele ( $n_e$ ) in each location were consistently higher in the Tiv ecotype compared to the Fulani ecotype.

**Table 3:** Genetic Variability (Heterozygosity) at some blood protein Loci in two Ecotypes of Nigerian Local Chickens

Loci	Tiv ecotype (N = 40)	Fulani ecotype (N = 40)	Mean
Haemoglobin	0.498	0.360	0.429
Albumin	0.520	0.504	0.512
Transferrin	0.654	0.434	0.544
Carbonic anhydrase	0.601	0.481	0.541
Mean H	0.568	0.445	0.507
2 $n_e$	2.273	1.779	2.026
%P	100	100	100

N = Sample size, H = heterozygosity, 2 $n_e$  = effective number of allele, %P = percentage of polymorphism

**Table 4:** Effect of Location on Genetic Variability (Heterozygosity) at some blood protein Loci in two Ecotypes of Nigerian Local Chickens

Loci	L1 (N = 8)	L2 (N = 8)	L3 (N = 8)	L4 (N = 8)	L5 (N = 8)	Mean
<b>Tiv ecotype</b>						
Haemoglobin	0.533	0.533	0.533	0.533	0.325	0.491
Albumin	0.592	0.539	0.539	0.533	0.533	0.547
Transferrin	0.458	0.492	0.648	0.500	0.530	0.527
Carbonic anhydrase	0.625	0.633	0.533	0.400	0.400	0.518
Mean H	0.552	0.549	0.563	0.492	0.447	0.521
2ne	2.208	2.197	2.253	1.966	1.788	2.082
%P	100	100	100	100	100	100
<b>Fulani ecotype</b>						
Haemoglobin	0.233	0.00	0.525	0.533	0.125	0.283
Albumin	0.533	0.525	0.500	0.539	0.533	0.526
Transferrin	0.467	0.325	0.00	0.325	0.233	0.270
Carbonic anhydrase	0.500	0.342	0.440	0.539	0.533	0.471
Mean H	0.433	0.298	0.366	0.484	0.356	0.387
2ne	1.733	1.192	1.465	1.936	1.424	1.550
%P	100	75	75	100	100	90

N = Sample size, L = location, H = heterozygosity,  $2_{ne}$  = effective number of allele, %P = percentage of polymorphism

## DISCUSSION

### Allelic Frequency Distribution

The effect of location on allelic frequencies at haemoglobin, albumin, transferrin and carbonic anhydrase loci in two ecotypes of Nigerian local chicken obtained in this study indicated that allelic frequencies mostly vary from location to location in both the Tiv and the Fulani ecotypes.

The Two alleles (A and B) expressed at the haemoglobin locus in both the Tiv and the Fulani ecotype across the five locations agreed with the reports of other workers on Nigerian poultry types (Ugur *et al.*, 2006; Salako and Ige, 2006; Yakubu and Aya, 2012; Oguntuji and Ayorinde, 2015). The prevalence of Allele A in both the Tiv (0.563) and the Fulani (0.769) chicken ecotypes across locations compared to allele B in this study also strongly agreed with the report for Nigerian local chicken (Salako and Ige, 2006; Ige *et al.*, 2013; Yakubu and Aya, 2012); Nigerian indigenous Muscovy duck from derived savannah (Oguntuji and Ayorinde, 2015) and Chuckers and Pheasant (Ugur *et al.*, 2006). The observe differences in prevalence of alleles in the Tiv and the Fulani ecotypes is suggestive of possible adaptive role of these alleles in different ecotypes.

Three alleles (A, B and C) were observed for albumen in this study as similarly reported for Dabbling ducks (Paulauskas *et al.*, 2009) and for Muscovy duck in guinea savannah and rain forest zones of Nigeria (Oguntuji and Ayorinde, 2015). However, Oguntuji and Ayorinde (2015) reported a fourth allele (D) in Muscovy duck in derived savannah. There are no literatures for Nigerian local chicken to compare this

result with. The prevalence of allele A across locations in both ecotypes strongly agreed with the report of Oguntuji and Ayorinde (2015) who reported the prevalence of allele A for Muscovy duck in guinea savannah and rain forest zones of Nigeria.

Four alleles were found in transferrin as similarly reported by Oguntuji and Ayorinde (2015) for Nigerian indigenous Muscovy ducks. The two Nigerian chicken ecotypes in this study had more alleles at the transferrin locus when compared to Kerinci ducks with three alleles (Nur *et al.*, 2012) but lower than Dabbling ducks with five alleles (Paulauskas *et al.*, 2009). While Oguntuji and Ayorinde (2015) reported that allele B dominated at the transferrin locus in Muscovy duck from derived savannah, guinea savannah and rain forest zones of Nigeria. Allele D (0.419) and allele A (0.689) were most prevalent in the Tiv and the Fulani ecotypes, respectively. The prevalence of any allele may suggest importance of these alleles to the survival and adaptation of the birds in the study area.

The three allele expressed at the carbonic anhydrase locus are similar to the report of Oguntuji and Ayorinde (2015) who reported allele A, B and C for Muscovy duck from derived savannah, guinea savannah and rain forest zone of Nigeria. Similarly, allele A dominated in both ecotypes with frequencies of 0.550 and 0.632 for Tiv and Fulani ecotypes, respectively as reported by Oguntuji and Ayorinde (2015) for the Nigerian Muscovy ducks. This suggests the important role of allele A at the carbonic anhydrase locus.

### Genetic Variability (Heterozygosity)

The mean heterozygosity values for haemoglobin, albumin and transferrin loci in both the Tiv and the Fulani ecotypes are within the range of 0.308-0.420 reported for similar loci in Nigerian Muscovy ducks by Oguntuji and Ayorinde (2015). However, the mean heterozygous value for carbonic anhydrase in this study is higher than the range of reported for carbonic anhydrase locus in the Muscovy ducks by Oguntuji and Ayorinde (2015).

Similarly, the mean heterozygosity values in the present study for the four loci are comparable to the range of 0.43 - 0.41 reported for Pekin (Ahmadi *et al.*, 2007). On the contrary, the mean heterozygosity obtained in this study is higher than the range of 0.094-0.119 and 0.185-0.366 for Lesser Snow Geese and dabbling ducks, respectively as reported by Kuznetsov *et al.*, (1998) and Paulauskas *et al.*, (2009) using blood protein markers. Also, this result is slightly lower than 0.514, 0.527, 0.808 and 0.86 reported for Beijing and Cherry Valley duck lines (Wu *et al.*, 2009), Muscovy duck in China (Wu *et al.*, 2008), and ten Chinese indigenous egg-type duck breeds (Hui-Fang *et al.*, 2010), respectively using microsatellite marker. Heterozygosity values are usually higher in microsatellite markers due to the ability of the technique to detect higher level of polymorphism than those obtained with protein markers (Ibeagba-Awemu and Erhardt, 2004; Akinyemi and Salako, 2012). Higher values obtained in those studies could be linked to higher numbers of allele's segregation at different loci. A major possible factor responsible for moderate to high heterozygosity value cum diversity in the present study within and between the two ecotypes might be attributed to the unrestricted 'gene flow' between chicken of different ecotypes through inter-regional trade and human movement; thus promoting intermingling and exchange of genetic materials among chickens of different ecotypes. Also, these local birds have not been selected for genetic improvement. Therefore, the comparable values of heterozygosity estimated are suggestive that these populations are under the influence of similar evolutionary forces. Also, the average heterozygosity (0.360 to 0.654) of the two ecotypes using blood protein markers in this study were within the recommended range (0.03 to 0.8) for markers to be useful for measuring genetic variation in a population (Takezaki and Nei, 1996) and also indicates allelic richness and diversity of this population. Heterozygosity is one of the indices used to assay genetic variation of each population and the value indicates the diversity level of the marker (Wu *et al.*, 2008).

### Percent of polymorphic loci (Percent P)

The complete (100 percent) polymorphism at the protein-coding loci investigated in this study corroborated the report of Nur *et al.*, (2012) and Oguntuji and Ayorinde (2015) for Indonesia Kerinci

duck and Nigerian Muscovy duck, respectively. Paulauskas *et al.*, (2009), however reported that only 7 out of 15 loci examined in dabbling ducks were polymorphic. The observed polymorphism is an indication of absence of selection with respect to the investigated loci and reinforces the widely reported diversity in genome of indigenous livestock species in developing countries.

### Effective number of alleles ( $n_e$ )

The value of 2.026 observed for effective number of alleles ( $n_e$ ) is slightly higher than 1.821 for Nigerian Muscovy ducks reported by Oguntuji and Ayorinde (2015) using blood protein biochemical markers but lower than 5.7351 for Chinese Muscovy ducks (Wu *et al.*, 2008) using microsatellite marker. The observed disparity between this study and the earlier report of Oguntuji and Ayorinde (2015) could be due to species difference (duck vs chicken) while the wide disparity between the present observed  $n_e$  value and the report of (Wu *et al.*, 2008) could be due to differences in techniques used, species and number of allele. High effective numbers of alleles ( $n_e$ ) values suggests that the sub-population under study have better abilities to keep the effective alleles when selection, mutation or genetic drift have occurred (Wu *et al.*, 2008). Effective number of alleles ( $n_e$ ) is an index used to reveal the genetic diversity of the population and also to assay the effect of alleles in each population (Wu *et al.*, 2008).

## CONCLUSION

The similarities in the allelic and genotypic frequencies at the four blood proteins loci of the two ecotypes indicated that the Tiv and the Fulani chicken populations are under similar evolutionary forces. The value of the estimated genetic diversity for the Tiv and the Fulani chicken ecotypes indicated that the two populations are variable in their genome and that there are chances of genetic improvement when crossed between themselves across location or with exotic breeds. The high heterozygosity values obtained is linked to higher numbers of alleles segregating at different loci. The genetic diversity within and between the Tiv and the Fulani chicken ecotypes observed in this study should be exploited through selection within each ecotype and subsequent crossing between birds from different locations/ecotype to take advantage of heterosis. The observed uniqueness/distinctness of the Tiv and the Fulani ecotypes should be preserved through such conservative measures as in-situ and ex-situ conservation techniques.

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