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GENETIC DIVERSITY OF INDIGENOUS CATTLE BREEDS USING BLOOD BIOCHEMICAL POLYMORPHISM

[a]SANNI M.A.; [a]ADEKOYA A.O.; & [b]ADEYINKA, M. S AND AKINNAGBE T.L

[a]Department of Agricultural Science Education, Tai Solarin University of Education, Ijagun, Ogun State, Nigeria. [b]The Department of Agricultural Science Education, Lagos State University of Education, Otto-Ijanikin, Lagos Nigeria.

Abstract

This study was conducted to assess the genetic diversities of some indigenous cattle (Sokoto Gudali Red Bororo and White Fulani) using blood biochemicals. Blood samples were collected from 300 cattle comprising of 100 Sokoto Gudali, 100 Red Bororo and 100 White Fulani into heparinized bottles and the protein polymorphism were investigated using cellulose acetate electrophoresis. The observed allele frequencies and genotypes (%) were tested with Hardy-Weinberg's Equilibrium (χ^2), from the three breeds, only White Fulani was observed to be significant ($p<0.05$) while Sokoto Gudali and Red Bororo were not significant ($p>0.05$) Four alleles Tf^A, Tf^B, Tf^C and Tf^D controlling nine genotypes were observed at the Transferrin locus, three carbonic anhydrase alleles CA^A, CA^B, and CA^C controlling five genotypes and two alleles Hb^A and Hb^B controlling three genotypes were observed. The values for the observed heterozygosity in Sokoto Gudali, Red Bororo and White Fulani were 0.543, 0.563 and 0.543 respectively. The value of the expected heterozygosity for Sokoto Gudali, Red Bororo and White Fulani were 0.565, 0.536 and 0.546 respectively, while the gene diversity (D) for Sokoto Gudali, Red Bororo and White Fulani were 0.563, 0.532 and 0.543 respectively. The values of the examined fixation indices for F_{IS}, F_{IT} and F_{ST} were 0.041, 0.017 and 0.024respectively. The Genetic distance estimate in this study ranged from 0.006 to 0.047. Using protein loci, the genetic distance indicated that the

Key words: Cattle, Haemoglobin, Tranferrin, Carbonic anhydrase and Genetic diversity

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Red Bororo and White Fulani were the most genetically similar while Sokoto Gudali was farther from the two breeds indicating that Sokoto Gudali may have had different historical origins or prehistoric migration route.

Introduction

Genetic resources of livestock are threatened by erosion (Ajmone-Marson, 2010). The situation is most alarming for developing countries, where industrial breeds often replace local breeds that have unique disease resistance and adaptations to the local environment (Ajmone-Marson, 2010). In the absence of information about the genetic attributes of each breed for a breeding programme, development of local breeds is often ignored in favour of the introduction of germplasm from exotic breeds, about which more information is generally available (Ajmone-Marson, 2010).

Characterization of breeds both at the level of animal phenotypes and their interaction with production systems and at the genetic level is essential (FAO, 2011). DNA-based technologies are now the methods of choice for genetic characterization of livestock (Arora et al., 2011), however several alternative assays, such as protein/allozyme polymorphism, remains tremendously useful, especially in developing countries, because of its utility, ease, cost, amount of genetic information accessed and simplicity of data interpretation (Rege and Okeyo, 2006).

Additionally, the use of blood protein polymorphism in animal genetic diversity studies should not be underplayed, more so that genetic research in Africa is less fully developed as in Europe (Gifford-Gonzalez and Hanotte, 2011) and other developed countries. Mwacharo et al. (2002) reported that for populations whose genetic status is unknown, protein polymorphism may be used first to verify the degree of genetic relationship and then prioritize breeds to be analysed using sophisticated tools like microsatellites. Analysis of genetic markers based on protein variants detected by electrophoretic method has been a tool for studying genetic differentiation among breeds and in phylogenetic studies (Nyasamba et al., 2003; Ibeagha-Awemu and Erhardt, 2004a; Camoglu and Elmaci, 2005). Information on blood proteins has also been used to study the genetic relationships among farm animals (Ibeagha-Awemu and Erhardt, 2004b; Esharatkhah et al., 2007; Sun et al., 2009; Shahrbabak et al., 2010). Morphological traits are however complex in their mode of transmission and are influenced by the environment thereby underestimating the true levels of genetic variation (Tsunoda et al., 2010). Additionally, morphological characteristics do not necessarily correspond to the genetic characteristics of blood protein and non-protein polymorphisms (Tsunoda et al., 2010).

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The future development of livestock production in Sub-Saharan Africa is hindered by limited knowledge of the genetic potential of the local genetic resources and ways to best utilize these resources in a sustainable manner. The genetic makeup of the indigenous animal is often associated with low production potential in indigenous animals. However, according to him, indigenous animals have not been adequately characterized for their production potential and this have led to the growing recognition of the need to characterize, utilize and conserve indigenous Animal Genetic Resources (AnGR) (Rege et al., 2002). Because of this lack of information, decisions on genetic improvement of indigenous animals are often made which have negative consequences on indigenous AnGR (Rege et al., 2002). It is important to know the diversity and relationship between the African breeds and strains of livestock (Rege et al., 2002). Moreover, it is important because, quite often, the same breed may be known by different names or two breeds may be known by the same name, based on geographical locations of such populations (FAO, 2011). The need to curb the threats of dilution and extinction of the African animal genetic resources, by strategic development and conservation has never been more justifiable and is now, with the available technology, timely (FAO, 2011). Efficient strategies for conservation require sets of genetic markers, which characterize distinct populations. Genetic improvement of indigenous breeds of livestock is very valuable because of high adaptability to harsh environmental conditions of nutrition, climate and disease compared with exotic breeds. According to Groeneveld et al. (2010) many breeds of livestock may become lost germplasm in many third world countries due to crossing with exotics, which in addition to uncontrolled breeding in extensive management systemspose a great risk for the loss of valuable genes.

Literature Review

Cattle Haemoglobin polymorphism was first actually reported in the Algerian hill cattle (Pal and Mummed, 2014). Haemoglobin polymorphism in cattle seems to be breed influenced as some breeds have been shown to show a clear polymorphism with two alleles (AA, BB) and their possible phenotypes (AA, AB and BB), while others present only one allele type-AA (Osterhoff, 1964). Most studies have however reported on the occurrence of the two co-dominant alleles Hb^A and Hb^B (Pal and Mummed, 2014). Apart from the co-dominant Hb^A and Hb^B types, other variants have also been reported such as Hb^X, with a motility that is intermediate between those of Hb^A and Hb^B in Indian cattle (Naik et al., 1965). Another variant Hb^C whose electrophoretic motility is similar to that of Hb^X was also reported in the American Brahmin cattle by Crockett et al. (1963). Other variants reported include Hb Khillari with motility slower than that of Hb A in Indian cattle (Naik and Sangvi, 1964), and still

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another Hb variant which was slower than Hb A in Muturu cattle of West Africa (Efremov and Braend, 1965). Majid et al. (1994) observed a single type of Hb pattern; Hb AB in Tamaraw (Bubalus mindorensis) cattle in the Philippines. Schwellnus and Guerin (1977) also observed the presence of Hb^C variant in Brahman and seven indigenous cattle breeds of Southern Africa. Ahmed et al. (2010) however, reported four Hb variants in their study with cows and buffaloes with allelic frequencies of 0.51, 0.33, 0.014 and 0.01 for Hb^A, Hb^B, Hb^C and Hb^D, respectively. Essien et al., (2011) suggested that Hb type may have been influenced by environmental factors. The mechanism for such polymorphism has however not been made clear (Al-Samarrae et al., 2010) and needs to be further studied. Equally, no direct evidence exists of differences among the three Hb phenotypes (AA, AB and BB) for fitness has been found in cattle (De Vito et al., 2002).

Taraphder *et al.* (2011) reported that ewes with Tf^{AD} phenovariant showed best performance with respect to lamb production (2.71 ± 0.28 lambs per lambing) in the analysed flock, in the first lambing, the highest (2.50 ± 0.52 lamb per lambing) and lowest (0.25 ± 0.25 lambs per lambing) number of lambs were born by ewes with genotype Tf^{BC} and Tf^{AA} respectively. They further stated that in the second lambing the highest number of lambs (3.00 ± 0.49) was born by ewes with both genotype Tf^{AD} and Tf^{BC}, and the lowest number (1.50 ± 0.49) by ewes with genotype Tf^{DE}. In the third lambing the highest number of lambs produced was observed for ewes with genotype both Tf^{AD} and Tf^{BD} which produced 3.00 ± 40 and 3.00 ± 0.33 lambs per lambing while lowest number by ewes with Tf^{BC} genotype viz. 1.50 ± 0.49 lambs per lambing; it was then observed that ewes with Tf^{AD} genotype showed best performance with respect to prolificacy; and type Tf^{DE} is least productive in the analysed flock (Taraphder *et al.* 2011).

Osman (1967) studied serum transferrin polymorphism in desert sheep of Sudan and also suggested that some phenotypic combination may have some selective advantage for production traits in certain environments. Vanti and Bas (1994) observed that transferrin polymorphisms affected the number of lambs born in Awassis sheep. Rasmusen *et al.* (2009) reported that Finish landrace ewes of transferrin type BD had smaller litter than ewes of other types. Taraphder *et al.* (2011) observed that the heterozygous ewes at the Tf locus were more prolific than the homozygous ones at the same locus. They also observed that most prolific homozygous ewes were those with Tf^{CC} phenotype, they however concluded that transferrin heterozygous ewes have an obvious superior genotype comparatively with that of the Tf homozygous ewes' genotype.

Methodology

White Fulani, Red Bororo and Sokoto Gudali were sampled in Ijebu, Remo, Yew and Egba zones in Nigeria. 300 blood samples were collected, comprising 100 samples from each breed. 5ml of blood samples were collected from each animal into

heparinised tubes by vein puncture. Based on Riken (2006), Red blood cell (RBC) was prepared from the erythrocyte fraction of heparinized blood by centrifuging at 2500 – 3000rpm for 10mins at 4°C. The RBCs were lysed with a fourfold volume of distilled water to release the carbonic anhydrase. The plasma fraction was separated from the erythrocyte fraction of heparinized blood. Cellulose acetate plates were soaked in the same buffer as the electrode buffer. Samples were applied on to the plate using the applicator and once loaded; plates are rested on the wicks in the tank. The loaded zone on the plate was positioned at the cathodal end of the tank for haemoglobin (Hb) which migrate anodally, while carbonic anhydrase (CA), which migrates cathodally, the loading zone was placed on the anodal end. After the completion of the gel run, the plates were removed from the tank and placed in an empty Petri dish. The plates were stained with Ponceau stain before they dry out. After 20 min the plates were sufficiently stained, they were destained several times until clear and sharp bands appear, the bands were scored visually based on their migratory pattern as described by RIKEN (2006) and direct counting was used for calculating gene frequencies.

Electrophoretic Conditions

The method used was described by RIKEN (2006) as shown in Table 3.1

Table 1: Summary of the electrophoresis Procedure

Marker	Buffer	Time (mins)	ph	Stain
Trasferrin	Tris glycine	15	8.5	Ponceau
Hemoglobin	Tris EDTA borate	40	8.4	Ponceau
Carbonic anhydrase	EDTA sodium	45	5.6	Ponceau

Statistical Analysis: The allele and genotype frequency, observed and expected heterozygosity, Hardyweinberg's Equilibrium coefficient, Wright F_{IS}, F_{IT} and F_{ST} estimated and drawing Unpaired Group Method of Algorithm (UPGMA) dendogram as well as the genetic distance according to (Nei's, 1972) were generated using Tools for Population Genetics Analyses (TFPGA) software.

RESULTS AND DISCUSSIONS

Allele Frequency

Allele frequency in Table 2 ranged from 0.005 for CA^C in Red Bororo breed to 0.620 for CA^A in Red Bororo breed. CA^C was observed to be absent in White Fulani population sample. CA^A was observed to have the highest frequency all the populations except in Sokoto Gudali, which has Hb^A as its highest, while Tf^D has the

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lowest frequency in all the populations except in Red Bororo, which had CA^C as the lowest.

Table 2: Allele frequencies of blood protein variants in cattle population

Locus	Allele	Sokoto Gudali	Red Bororo	White Fulani
CA	**CA^A**	**0.469**	**0.620**	**0.576**
	CA^B	**0.515**	**0.376**	**0.424**
	CA^C	**0.015**	**0.005**	**0.000**
Hb	**Hb^A**	**0.587**	**0.491**	**0.466**
	Hb^B	**0.413**	**0.509**	**0.534**
Tf	**Tf^A**	**0.374**	**0.235**	**0.322**
	Tf^B	**0.313**	**0.524**	**0.467**
	Tf^C	**0.275**	**0.235**	**0.195**
	Tf^D	**0.039**	**0.006**	**0.017**

Hb — Haemoglobin, Tf — Transferr, CA — Carbonic anhydrase.

Genotype Frequencies of Blood Proteins

From Table 3, the genotype frequency ranged from 0.010 for genotype CA^{BC} in Sokoto Gudali and CA^{AC} in Red Bororo breed to 0.527 for genotype Hb^{AB} in White Fulani. Hb^{AB} was observed to have the highest genotype frequency in all the cattle population, while CA^{BC}, CA^{AC} in Sokoto Gudali and Red Bororo respectively, had the same values (0.010) for their lowest frequencies and Tf^{AA} had lowest inWhite Fulani. The measure of Hardy Weinberg's Equilibrium was only observed to be significant ($P< 0.05$) in CA locus of White Fulani population.

Table 3: Genotype Frequencies for the Loci Across the Breeds

Locus	Genotypes	Sokoto Gudali	Red Bororo	White Fulani
Carbonic anhydrase	AA	0.255	0.380	0.439
	AB	0.408	0.446	0.273
	BB	0.300	0.163	0.288
	AC	0.020	0.010	0.000
	BC	0.010	0.000	0.000

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X^2				2.914(NS)	0.953(NS)	
13.338(***)						
Significance				0.405	0.813	
0.00026						
Haemoglobin		AA		0.349	0.264	
0.203						
		AB		0.476	0.453	
		0.527				
		BB		0.175	0.283	
		0.270				
X^2				0.041	0.568	
0.200						
Significance				0.838(NS)	0.450(NS)	
0.654(NS)	Transferrin			AA	0.109	
0.306		0.011				
		AB		0.319	0.000	
		0.402				
		BB		0.066	0.306	
		0.161				
		AC		0.209	0.153	
0.184						
		BC		0.176	0.129	
0.207						
		CC		0.077	0.094	
0.000						
		DC		0.011	0.000	
		0.000				
	DD		0.033	0.000		0.000
	AD		0.000	0.012		0.034
X^2			81.106	17.951	23.179	
Significance			N.S	N.S		N.S

X^2: chi-square and Sig values are significant when P<0.05

Heterozygosity and Gene Diversity

In table 4, observed and expected heterozygosity (H_O and H_E) ranged from 0.273 in CA for White Fulani to 0.828 in Tf for White Fulani and 0.483 in CA for Red Bororo to 0.689 in Tf for Sokoto Gudali respectively. The transferrin locus was observed to have the highest observed and expected heterozygosity in all the populations, while the CA locus had lowest observed heterozygosity in Sokoto Gudali and White Fulani and the

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HB locus for Red Bororo and the lowest expected heterozygosity was seen in the CA locus of Red Bororo and White Fulani and the HB locus for Sokoto Gudali. Both in the carbonic anhydrase and haemoglobin locus, the observed hetgerozygosity, was observed to be lower than the expected heterozygosity in all the populations except in the haemoglobin locus of White Fulani breed, where the observed heterozygosity is higher than the expected. While in the transferrin locus the observed hetgerozygosity, was observed to be than than the expected heterozygosity in all the populations except in the Red Bororo population.

The nei gene diversity (D) ranged between 0.481 in CA for Red Bororo to 0.685 in Tf for Sokoto Gudali, while the highest values of the gene diversity (D) were observed in the Tf locus in all the population and the lowest values of gene diversity (D), were observed in the CA locus, for all the populations, except in Sokoto Gudali which had HB as the lowest. The average ranged from 0.494 for CA and HB to 0.627 for Tf for all the populations.

Table 4: Heterozygosity and polymorphism by population for codominant data

Population	Locus	n	H_O	H_E*	Nei**(D)	Av. H
Sokoto Gudali	CA	196	0.439	0.517	0.514	0.494
	Hb	126	0.476	0.489	0.489	0.494
	Tf	182	0.714	0.689	0.685	0.627
Mean			0.543	0.565	0.563	0.538
Red Bororo	CA	184	0.457	0.483	0.481	0.494
	HB	106	0.453	0.505	0.499	0.494
	Tf	170	0.600	0.619	0.615	0.627
Mean			0.503	0.536	0.532	0.538
White Fulani	CA	132	0.273	0.492	0.489	0.494
	HB	148	0.527	0.501	0.498	0.494
	Tf	174	0.828	0.645	0.641	0.627
Mean			0.543	0.546	0.543	0.538
Overall	CA	512	0.402	0.504	0.503	0.494
	HB	380	0.489	0.501	0.499	0.494
	Tf	526	0.723	0.645	0.647	0.627
Mean			0.538	0.550	0.549	0.538

n= sample size

H_O= Observed heterozygosity

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H_E= Expected heterozygosity
H= Heterozygosity
***Expected homozygosity and heterozygosity were computed using Levene (1949)**
****Nei's (1973) expected heterozygosity.**

Genetic Identity and Genetic Distance

Genetic distancing estimates of the Nei's (1972/1978) were calculated using alleles frequency and presented in Table 5. The distance between populations ranged from 0.006 to 0.047. The smallest genetic distance (0.006) was observed between White Fulani and Red Bororo populations while the farthest (0.047) was between Red Bororo and Sokoto Gudali populations. The farthest genetic identity (0.954) was observed between Red Bororo and Sokoto Gudali while the closest in genetic identity (0.994) was observed between White Fulani and Red Bororo.

Table 5: Nei's Original Measures of Genetic Identity and Genetic distance

POPULATION	SOKOTO GUDALI	RED BORORO	WHITE FULANI
SOKOTO GUDALI	****	0.954	0.970
RED BORORO	0.047	****	0.994
WHITE FULANI	0.031	0.006	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among Nigerian three cattle populations.

F-Statistics and Gene Flow

F_{IS} ranged between -0.072 in Tf to 0.205 in CA with a mean of 0.041, F_{IT} ranged between 0.009 in Hb to 0.023 Tf with a mean of 0.017 while the F_{ST} ranged from – 0.097 in Tf to 0.191 in CA with a mean of 0.024. The gene flow ranged from -2.827 in Tf to 0.983 in Hb with a mean of 9.417.

Table 6: F-Statistics and Gene Flow for all population in each locus

Locus	F_{IS}	F_{IT}	F_{ST}	Nm*
CA	0.205	0.018	0.191	0.809
Hb	0.026	0.009	0.017	0.983
Tf	-0.072	0.023	-0.097	-2.827
Mean	0.041	0.017	0.024	9.417

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

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Hardy – Weinberg Equilibrium

The measure of Handy Weinberg Equilibrium on table 6 ranged from 0.0003 in the CA locus for White Fulani to 0.8885 in the Hb locus of Sokoto Gudali. There was not significant deviation ($p<0.05$) in all the populations except for the population of White Fulani which was highly significant ($p<0.001$) at CA locus and significant ($p<0.05$) at Tf locus.

Table 7: Summary of Chi– Square Tests for Hardy Weinberg Equilibrium

Population	Locus	ChiSq	Prob
Sokoto Gudali	CA	2.5872	0.1080(NS)
	Tf	1.4662	0.2259(NS)
	Hb	0.0197	0.8885(NS)
Red Bororo	CA	0.0917	0.7620(NS)
	Tf	1.3813	0.2399(NS)
	Hb	0.4685	0.3937(NS)
White FulaniCA		12.8782	0.0003(***)
	Tf	4.3736	0.0365(***)
	Hb	0.2566	0.6125 (NS)

Dendogram of Three Nigerian Indigeneous Cattle

The tree below showed the relationship among the three Nigerian cattle breeds. The dendogram showed Red Bororo and White Fulani branched from the same root while Sokoto Gudali branched from another root.

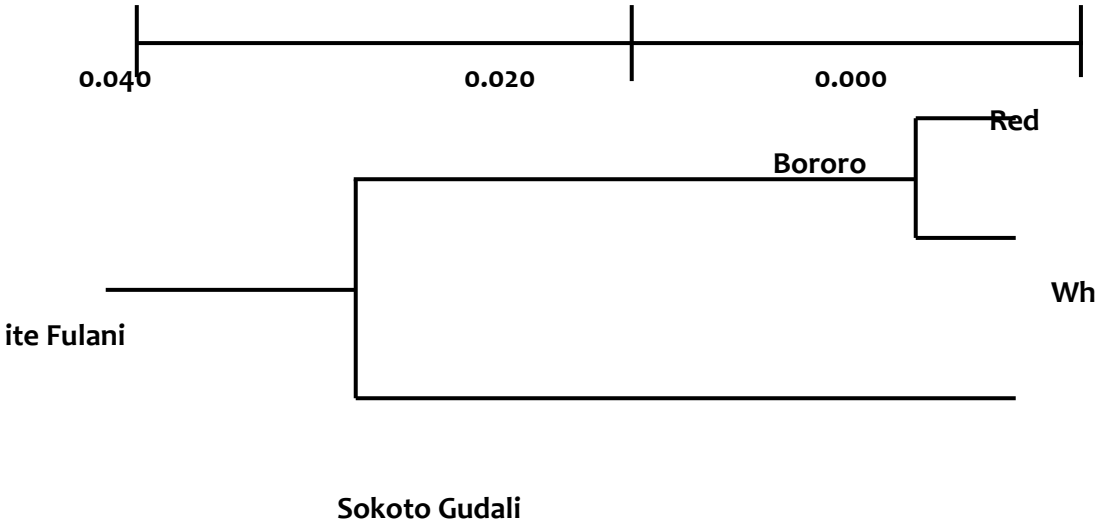


Figure 1: Dendogram of Nigerian Indigenous Cattle

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Discussions

Genotype and Gene Frequency: The populations of the Nigerian cattle that were studied, were polymorphic, as the carbonic anhydrase (CA) locus had five genotypes (CA^{AA}, CA^{AB}, CA^{BB}, CA^{AC} and CA^{BC}), that are controlled by three co-dominant alleles (CA^{A}, CA^{B}, and CA^{C}), the transferrin locus had four alleles (Tf^{A} Tf^{B} Tf^{C} and Tf^{D}) controlling nine genotypes (Tf^{AA}, Tf^{AB}, Tf^{BB}, Tf^{AC}, Tf^{BC}, Tf^{CC}, Tf^{DC}, Tf^{DD} and Tf^{AD}) and the haemoglobin locus had two alleles (Hb^{A} and Hb^{B}) that are controlling three genotypes (Hb^{AA}, Hb^{BB} and Hb^{AB}). The transferrin locus was more polymorphic than the two other loci, as it had more alleles and genotypes. Similar results have been obtained by Mwacharo et al. (2002), Boujenane et al. (2008), and Shahrbabak et al. (2010) who reported the occureence of polymorphism in most sheep breeds while Abdussamad *et al.,* (2004), Bunjah (2016) and Essien *et al., (*2011) on Bunaji, Friesian X Bunaji cross and Gudali cattle. Bunjah (2016) reported a similar result on his work on Bunaji, Gudali and Friesian X Bunaji crossbreed as he observed two alleles (Hb^{A} and Hb^{B}) and three genotypes (Hb^{AA} Hb^{AB} and Hb^{BB}) in the three breeds while he reported two alleles (Tf^{A} and Tf^{B}) and three genotypes (Tf^{AA} Tf^{AB} and Tf^{BB}).

The different alleles and genotypes observed in study are in agreement with earlier research works, as Bunjah (2016), reported the presence of Tf^{AA}, Tf^{AB} and Tf^{BB} genotypes and the alleles Tf^{A} and Tf^{B}, on his work on Bunaji, Friesian X Bunaji cross and Gudali. Osaiyuwu and Salako, (2018) in their work on Genetic structure of indigenous sheep breeds in Nigeria based on electrophoretic polymorphous systems, observed Tf^{A}, Tf^{B}, Tf^{C}, and Tf^{D} as the alleles in transferring and the presence of Tf^{B}, as well as Tf^{D} alleles in this study was also reported by Ibeagha-Awemu and Erhardt (2004). Awobajo *et al.,*(2018) also reported the presence of Tf^{AA}, Tf^{AB} and Tf^{BB} genotypes and the alleles Tf^{A} and Tf^{B}, in Nigerian West African Dwarf Goat. Camoglu (2005), reported the presence of CA^{F}, CA^{S}, CA^{FS} and CA^{SS} in Turkish sheep breeds which agrees with the result in this study as CA^{A} and CA^{B} observed in this study is the same as CA^{F} and CA^{S} respectively observed in prior studies. Osaiyuwu *et al.*, (2018), also reported the presence alleles observed in this study in Nigerian Indigeneous Sheep (Balami, Uda, Yankassa and West African Dwarf) which are apparently the same as those reported earlier. Hb^{AA}, Hb^{AB} and Hb^{BB} in this study was similar to the report of Essien *et al., (*2011) on Bunaji cattle in Zaria, Nigeria, with frequencies of heterozygote Hb^{AB} considerably higher than either of the homozygote Hb^{AA} and Hb^{BB}. Gwaza et al., (2019) also observed the genotypes Hb^{AA}, Hb^{AB} and Hb^{BB} in his work on West Africa dwarf goat, and among the three genotypes of the haemoglobin reported, the genotype Hb^{AB} has the highest frequency. This increased frequency may be due to an improved advantage of the heterozygote (Hb^{AB}) genotype that favoured it survival,

fitness and adaptation to enviromental challenges compared to the homozygotes (Hb^{AA} and Hb^{BB}) genotypes.

All of the alleles were observed to be present in all of the populations except for CA^{C} in White Fulani. Sokoto Gudali population was observed to have more genotypes when compared to other populations, as all the genotypes were present in it except Tf^{AD}, while Red Bororo had all the genotypes except CA^{BC}, TF^{AB}, TF^{DC} and TF^{DD}. And the White Fulani population does not have CA^{AC}, CA^{BC}, TF^{CC}, TF^{DC} and TF^{DD}. In the carbonic anhydrase locus, genotype CA^{AB} has the highest frequency in all the breeds except for White Fulani which has genotype AA as it's highest while in the transferrin locus Tf^{AB} has the highest frequency in all the breed except in Red Bororo where Tf^{AA} and Tf^{BB} had the highest values. CA^{A} was the highest in Red Bororo and White Fulani as opposed to report on Indian Zebu cattle by Shanker *et al*., (1983), where CA^{S} was the highest in Sahiwal, Red Sindhi, Holstein Friesian, Jersey, Karan Swiss, Karan Fries, Jersey x Tharparkar and only CA^{S} was observed in Tharparkar. CA^{S} has been noticed to be the predominant allele in previous studies, it was also observed in Han and Suzuki (1976) for the Korean cattle studied but in this study, CA^{A} was observed to be the most prevalent in all the breeds except for Sokoto Gudali where CA^{B} was the most occurring. It can be said that this variation in White Fulani and Red Bororo is as a result of selective advantages of CA in some geographical zones. Some alleles where observed by other researchers which were not present in this study, Ibeagha-Awemu and Erhardt (2004) posited that the absence of these alleles may not totally exclude their occurrence in the breeds but may have exposed the limitation of the method of starch gel electrophoresis in separating Transerrin variants.

CA^{A} was the highest in Red Bororo and White Fulani as opposed to report on Indian Zebu cattle by Shanker *et al*., (1983), where CA^{S} was the highest in Sahiwal, Red Sindhi, Holstein Friesian, Jersey, Karan Swiss, Karan Fries, Jersey x Tharparkar and only CA^{S} was observed in Tharparkar. CA^{S} has been noticed to be the predominant allele in previous studies, it was also observed in Han and Suzuki (1976) for the Korean cattle studied but in this study, CA^{A} was observed to be the most prevalent in all the breeds except for Sokoto Gudali where CA^{B} was the most occurring. It can be said that this variation in White Fulani and Red Bororo is as a result of selective advantages of CA in some geographical zones.

Heterozygosity and Gene Diversity: The observed heterozygosity (H_O) was lower than the expected heterozygosty in Sokoto Gudali and Red Bororo while observed heterozygosity was higher than the expected heterozygosty in White Fulani. This is similar to the report of Awobajo *et al.,* (2016) that the average H_O was more than the H_E in West African Dwarf goats by allozyme markers, and the average expected gene diversity (Nei, 1973) within the population ranged from 0.34 (Hb) to 0.50 (Tf) with an

overall mean of 0.44 ± 0.09. This means that there is high variability as well as a lot of in-breeding in Sokoto Gudali and Red Bororo, while the White Fulani breed has a lot of out-breeding in its population. The values of the gene diversity (Nei's expected heterozygosity) were higher than the observed heterozygosity in Sokoto Gudali and Red Bororo populations while the White Fulani populations the gene diversity (Nei's expected heterozygosity) were lower than the observed heterozygosity. This means that the gene diversity is high in Sokoto Gudali and Red Bororo populations indicating or implying inbreeding is taking place within the Sokoto Gudali and Red Bororo breeds. Ajayi *et al.,* (2016) reported that Sokoto Gudali recorded the highest average heterozygosity of 0.583 which is in agreement with the result gotten in this work, as the observed and expected heterozygosity (H_O and H_E) ranged from 0.273 in CA for White Fulani to 0.828 in Tf for White Fulani and 0.483 in CA for Red Bororo to 0.689 in Tf for Sokoto Gudali respectively. Pandey *et al.,* (2006) reported the mean observed heterozygosity of 0.574 and 0.540 for Khergarh and Kenkatha cattle which is similar to that of this study 0.538. Demir and Balcioglu (2019) reported observed heterozygosity ranged from 0.300 to 0.980 with a mean of 0.63, while the expected heterozygosity ranged from 0.510 to 0.880 with a mean of 0.74. Generally the observed heterozygosity was lower than expected heterozygosity, which may be due to the presence of more homozygous individuals in the analysed samples.

Hardy-Weinberg's Equilibrium: The significant deviations from HWE ($P<0.05$) observed for White Fulani could be attributed to migration, high mutation rate and artificial selection in the breeds. There was no significant deviation from HWE ($P>0.05$) for Sokoto Gudali and Red Bororo. Thus, genotypes of Sokoto Gudali and Red Bororo are in Hardy-Weinberg's equilibrium while that of White Fulani is not in Hardy-Weinberg's equilibrium. Significant ($P < 0.05$) deviations of allele frequencies may occur owing to crossing and linking, sample error, population bottle neck and random genetic drift. Ideal Hardy-Weinberg population do not actually occur in nature owing to various factors, which can shift the equilibrium and disrupt the stability of a population, giving rise to change in the genetic structure (Sargent *et al.,* 1999). Deviations from HWE suggest that there was selective mating, migration, mutation and small population. Deviation from HWE at protein loci have also been reported in various cases such as in Southern African sheep (Sargent et al., 1999). Aminafshar et al. (2008) also that Significant deviations HWE ($P< 0.05$) observed for loci of White Fulani breed could be attributed to unobserved null alleles, excess of heterozygote individuals than homozygote individuals, migration, high mutation rate and artificial selection in the breeds (Aminafshar et al., 2008).

Gene flow and F- statistics: F-Statistic values of F_{ST} and F_{IT} are measures of deviation from Hardy-Weinberg's proportions and total populations respectively (Cormack *et*

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al., 1990). Positive values indicate a deficiency in heterozygotes and negative values indicate an excess of heterozygotes. F_{IS} can be interpreted as a measure of inbreeding (the measure of allelic fixation of individuals relative to the subpopulations) (Cormack *et al*., 1990). F_{IS} in this study was positive (0.041), indicating deficiency of heterozygotes in the populations and that mates were more related in comparison with the average relationship of the population. The global heterozygosity deficit (F_{IT}) was estimated at 0.017, which indicates there excess of homozygotes in the populations and that mate were less related in comparison with the average relationship of the population. According to Hartle (1980), F_{ST} values up to 0.05 indicate moderate genetic variations while values greater than 0.25 indicate large genetic differentiation amongst populations. The current study reported large level of genetic differentiation among the three populations of cattle studied as the F_{ST} value is 0.024. The degree of differentiation observed between the Nigerian cattle breeds could be due to geographic proximity, similarities in environment and breeding practices, but most likely due to past genetic exchange among them since mean gene flow over all loci was 0.626. The Mean estimate of F-statistics; F_{IS} (0.193, within population inbreeding estimate), F_{ST} (0.123, estimate of population differentiation) and F_{IT} (-0.047, overall global heterozygote deficit across population) reported by Osaiyuwu and Salako (2016) and 0.44, 0.03 and 0.46 for F_{IS}, F_{ST} and F_{IT} respectively reported by Awobajo *et al.,* (2016) were different from the values in the current study.

Genetic Distance, Genetic Identity and Dendogram: The Nei (1972) standard genetic distance was used to determine the genetic relationship between populations that is genetic distance and identity between the populations. The genetic distance obtained in this study (0.006-0.047) indicates the level of genetic differentiation between the breeds, and this was similar to 0.001 - 0.055 reported for Spanish sheep breeds (Ordas and Primitivo, 1986). 0.01 - 0.22 reported by Nigussie et al. (2016) for eastern Ethiopia sheep breeds, was higher than 0.006 – 0.047 of this study. Zanotti et al. (1990) reported genetic distance ranging between 0.012 and 0.060 in five Italian sheep breeds. Boujenane et al. (2008) reported a genetic distance range of 0.006 to 0.026. Distances obtained in this study between breeds were also higher to that of Akinyemi and Salako (2012) who reported a range of 0.003 to 0.015. The distance estimate in this current study revealed a closer relationship between Red Bororo and White Fulani. From the dendogram constructed, Sokoto Gudali branched from another root aside from the one Red Bororo and White Fulani branched from. Signifying prehistoric separation of origin in the Gudali breed. The genetic relationship between the Red Bororo and White Fulani breed may be attributed to possible interbreeding between these two populations which are predominantly Northern breeds to form a

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homogenous population separated by administrative boundaries. According to Mwacharo et al., (2002) the close genetic relationship between the breeds may also be ascribed to similarity in ecological zones and production systems as well as the incidents of cross border livestock rustling contributing to the migration and movement of livestock and subsequent interbreeding between such livestock.

Conclusion: Allozyme studies can integrate genetic and ecological information to strengthen inferences about specific aspects of population structure, especially breeding structure and effective gene flow. Furthermore, the results of the cattle breeds are important for future monitoring of gene flow in populations, in order to determine level of inbreeding and crossbreeding in each breed, and to improve the global information on domestic animal diversity. The Genetic distance estimate in this study using protein loci indicated that the Red Bororo and White Fulani were the most genetically similar while Sokoto Gudali was farther from the two breeds indicating that Sokoto Gudali may have had different historical origins or prehistoric migration route.

Recommendation:

Based on the results of this study, it is recommended that distant breeds are a good fit for improved animal population. As established by the statistical studies that has indicated significant differences, breeding involving the crossing of the Gudali with either White Fulani or Red Bororo breeds will produce a better improved population of animals, since the breeds are heterogeneous and distinctly different in their genetic and origin relationship is expected to produce vigor (positive heterosis) in the hybrid.

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