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Haemoglobin Polymorphism and Genetic Diversity of Indigenous Nigerian Cattle Breeds: Insights from Gudali, Red Bororo, and White Fulani Populations

[1]Osaiyuwu, O. H.; [1]Hamzat O. Y.; [2]Igbatigbi, L. O.

[1]Department of Animal Science, University of Ibadan, Ibadan, Nigeria.

[2]Department of Zoology, Federal University Lokoja, Nigeria.

Corresponding Author: cosamede@yahoo.com

Abstract

Polymorphism is the occurrence of varieties attributed to biochemical differences which are under genetic control. It has provided a medium for genetic improvement of farm animals, because it is a useful tool for characterization of livestock breeds and population. The degree of resemblance or differences within and between breeds can be ascertained, and they are important raw materials for genetic improvement of animals. This study was carried out to analyse the haemoglobin (Hb) polymorphism in 380 cattle comprising of three different breeds; 126 Gudali (G), 106 Red Bororo (RB), and 148 White Fulani (WF), at the general abattoir, Ibadan. The samples were subjected to Cellulose Acetate Electrophoresis (CAE) to determine the genetic variants of Haemoglobin using Tris EDTA borate buffer. The result showed that Haemoglobin (Hb) exhibited two alleles A and B, with three genotypes (HbAA, HbAB, and HbBB). In the G, the allele frequencies were A (0.588) and B (0.412) respectively, while A (0.491) and B (0.509) were observed in the RB, A (0.466) and B (0.534) were observed in WF, and in the overall population A (0.514) and B (0.486). The genotype frequencies obtained in all populations showed that genotype HbAB had the highest frequency in all the breeds, and highest individually in Gudali (0.476), White Fulani (0.527), and Red Bororo (0.453), while HbBB was lowest in Gudali (0.175), and also lowest in the overall population (0.243). All the breeds studied were found to be in Hardy-Weinberg Equilibrium.

Keywords: Alleles, Breeds, Genotype, Haemoglobin HWE- Hardy, Polymorphism

INTRODUCTION

The pressure on selection has increased in the last few decades, leading to the introduction of commercial breeds of livestock across different countries of the world. Often times at the detriment of local breeds, resulting in the loss of highly valuable genetic resources. Thus, priority should be given to indigenous breeds to preserve their genetic resources (Teletchea, 2019). Cattle are a large group of ruminant animals belonging to the family Bovidae, in the order artiodactyl. The cattle population in Nigeria is believed to have stemmed from two types, which are the taurine (humpless) type and the Zebu (humped). Presently the cattle breeds that are found to be indigenous to this region are humpless, short-horned types: Muturu, Keteku, Sokoto Gudali and Adamawa Gudali. The humped and long horned- White Fulani, Red Bororo, and Kuri. The humped cattle are all traditionally associated with the parts of Nigeria (mostly the North part) and neighbouring countries (Korger, 2011). Understanding the genetic diversity of these indigenous cattle breeds is therefore essential, particularly at the molecular level, where traits such as haemoglobin variation can provide valuable insights into their adaptability and productivity.

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Haemoglobin is a blood protein responsible for the transport of oxygen and carbon (IV) oxide in the blood of vertebrates. Haemoglobin polymorphism has been considered in a number of species as it shows variation in its globin partition (Pieragostini and Allogio, 2010). Haemoglobin polymorphism in cattle has been reported to be breed-influenced, as some breeds show a clear polymorphism with two alleles while others present only one allele (Salzano et al., 2015).

Many indigenous cattle breeds in Nigeria are yet to be fully explored and utilized for their genetic potentials using molecular and biochemical approach, because polymorphism at the protein level could be used for preliminary studies of these local breeds in developing countries like Nigeria, which is challenged with insufficient facilities for other comprehensive research (Ukwu et al., 2017). The African indigenous population of cattle are not only in danger of complete dilution by exotic breeds, but are also poorly characterized and are not systemically classified. A comprehensive knowledge of their physical, biological and adaptive characteristics, is needed to facilitate the development of strategies for conservation and improvement of indigenous breeds with high genetic potentials (Odeku et al., 2017).

Genetic research in recent times has been directed towards the investigation of the relationship between the physiological, biochemical and metabolic products, to the productive efficiency of farm animals. Data gotten from such studies, could become useful as genetic markers for important economic traits, and could significantly contribute to selection of superior animals for breeding purposes (Okonkwo and Clinton, 2016). This study examines the genetic diversity at the Haemoglobin locus in Gudali, Red Sokoto and White Fulani Cattle breeds in Nigeria.

MATERIALS AND METHODS

Location of the study

This study was carried out at the central abattoir, Amosun Village, Ibadan, Oyo State, Nigeria.

Experimental animals and sample collection

Three hundred and eighty (380) cattle, comprising of 126 Gudali (G), 106 Red Bororo (RB), and 148 White Fulani (WF), were used for this experiment. Blood samples (5mls) were collected via the jugular vein into labelled (for ease of identification) heparinized bottles, and transported in ice park to the laboratory for analysis

Laboratory analysis and Experimental procedure

Laboratory analysis of the blood sample was carried out at the Breeding and Genetics laboratory, Department of Animal Science, University of Ibadan.

Red blood cells (RBCs) were prepared from the heparinized blood samples by centrifugation at 3000 rpm for 10 mins at 4°C; the plasma was decanted. The red blood fraction was washed two times in saline by repeating the centrifugation at 3000 rpm for 5 minutes at 4°C. After the second wash, distilled water was added to the erythrocyte fraction to release the haemoglobin through lysis. The cellulose acetate plate electrophoresis conditions followed for the haemoglobin locus were as described by RIKEN (2006). Cellulose acetate electrophoresis was carried out using the Mini-PROTEIN 3 Gel Electrophoresis System (BioRad#165-3301) with Tris EDTA Borate at pH8.6 as the buffer system.

Cellulose acetate strips and wicks were soaked, very slowly, in Tris EDTA borate buffer for about five minutes. The cellulose acetate strips were blotted lightly with filter paper to remove excess buffer. The hemolysate was applied in the applicator plate with a pipette and then stamped with the applicator on the strip and electrophoresis was run for 35 minutes.

The haemoglobin pattern in the order of mobility was detected based on the molecular weight of the haemoglobin molecules HbAA, HbAB and HbBB by staining with ponceau-S and then de-staining with 5% acetic acid. The bands were scored visually as: fastest band = HbAA, slowest = HbBB, intermediate = HbAB.

Statistical analysis

Data obtained were subjected to PopGene 32 version 1.32 for allele and genotype frequency counts, HWE, and genetic distance analysis.

RESULTS AND DISCUSSION

Results

The distribution of haemoglobin alleles and genotypes across the Nigerian indigenous cattle populations is presented in Table 1. Two alleles (A and B) were observed in all populations. Allele A occurred at a higher frequency in Gudali (0.588), while allele B was slightly more frequent in Red Bororo (0.509) and White Fulani (0.534). Overall, allele A (0.514) was marginally more prevalent than allele B (0.486) across the combined populations.

Table 1. Allele and Genotype frequencies for Haemoglobin locus in Nigerian indigenous cattle.

Population	Allele	Frequency	Genotype	Frequency	Chi square	Significance
Gudali	A	0.588	AA	0.349	0.041	0.838
	B	0.412	AB	0.476		
			BB	0.175		
Red Bororo	A	0.491	AA	0.264	0.568	0.450
	B	0.509	AB	0.453		
			BB	0.283		
White Fulani	A	0.466	AA	0.203	0.200	0.654
	B	0.534	AB	0.527		
			BB	0.270	0.100	0.751
Overall	A	0.514	AA	0.268		
	B	0.486	AB	0.489		
			BB	0.243		

Genotypic frequencies showed that the heterozygous genotype (AB) was the most common across all populations, with values of 0.476, 0.453, and 0.527 in Gudali, Red Bororo, and White Fulani, respectively. The homozygous genotypes AA and BB occurred at relatively lower frequencies. Chi-square analysis indicated no significant deviation from Hardy-Weinberg equilibrium in Gudali (χ^2 = 0.041, $p > 0.05$), Red Bororo (χ^2 = 0.568, $p > 0.05$), and White Fulani (χ^2 = 0.200, $p > 0.05$), suggesting that the populations are in genetic equilibrium at the haemoglobin locus (Table 1).

Table 2. Heterozygosity statistics for Haemoglobin in Nigerian indigenous Cattle

Population	Sample size	Obs_{hom}	Obs_{het}	Exp_{hom}	Exp_{het}	Nei's	Ave_{het}
Gudali	126	0.524	0.476	0.511	0.489	0.484	0.494
Red Bororo	106	0.547	0.452	0.495	0.504	0.499	0.494
White Fulani	148	0.473	0.527	0.498	0.501	0.497	0.494
Overall	380	0.510	0.489	0.499	0.501	0.499	0.494

Note: Obs_{hom}= observed homozygosity, Obs_{het} = observed heterozygosity, Exp_{hom} = expected homozygosity, Exp_{het} = expected heterozygosity, Nei's = Nei's expected heterozygosity, Ave_{het} = average heterozygosity.

Heterozygosity statistics for the haemoglobin locus are summarized in Table 2. Observed heterozygosity (Obshet) ranged from 0.452 in Red Bororo to 0.527 in White Fulani, while Gudali showed a value of 0.476. These values were comparable to the expected heterozygosity (Exphet), which ranged from 0.489 to 0.504 across the populations. The overall observed (0.489) and expected (0.501) heterozygosity values were also similar, indicating a moderate level of genetic variability. Nei's expected heterozygosity values were consistent across populations (0.484–0.499), with an overall value of 0.499, while the average heterozygosity (Avehet) was uniform (0.494) across all populations (Table 2).

Genetic variation indices are presented in Table 3. The observed number of alleles (Na) was constant (2.00) across all populations, indicating no allelic loss at the haemoglobin locus. The effective number of alleles (Ne) ranged from 1.94 in Gudali to 1.99 in Red Bororo and White Fulani, reflecting relatively balanced allele frequencies. Shannon's information index (I) showed similar values across the populations (0.68–0.69), suggesting comparable levels of genetic diversity among the breeds.

Table 3. Genetic variation statistics of Haemoglobin between Nigerian indigenous cattle breeds.

Population	Sample size	Na*	Ne*	I*
Gudali	126	2.00	1.94	0.68
Red Bororo	106	2.00	1.99	0.69
White Fulani	148	2.00	1.99	0.69
Overall	380	2.00	1.99	0.69

Note: Na = observed number of alleles, Ne = Effective number of alleles, I = Shannon's

The genetic relationships among the cattle populations are shown in Table 4. Nei's genetic identity values were high, ranging from 0.971 (Gudali vs. White Fulani) to 0.999 (Red Bororo vs. White Fulani), indicating close genetic relatedness among the populations. Correspondingly, genetic distance values were low, with the highest distance observed between Gudali and White Fulani (0.029) and the lowest between Red Bororo and White Fulani (0.001). These results suggest minimal genetic differentiation among the indigenous cattle breeds studied.

Table 4. Nei's measure of Genetic identity and Genetic distance among indigenous cattle.

	Gudali	Red Bororo	White Fulani
Gudali		0.982	0.971
Red Bororo	0.018		0.999
White Fulani	0.029	0.001	

Genetic identity (upper diagonal) and Genetic distance (lower diagonal

DISCUSSION

Allele and genotype frequency

The results of this study confirm the presence of two haemoglobin alleles (HbA and HbB) across all three Nigerian indigenous cattle breeds, indicating polymorphism at the haemoglobin locus. The predominance of HbA in Gudali and the relatively higher frequency of HbB in Red Bororo and White Fulani are consistent with earlier reports in Zebu cattle (Tawah and Rege, 1996). The higher frequency of the heterozygous genotype (HbAB) observed across all populations suggests substantial genetic variability within these breeds. Such polymorphism is important, as it provides a genetic basis for adaptability and potential improvement through selection.

Hardy-Weinberg's Equilibrium (HWE)

Hardy-Weinberg Equilibrium (HWE) is a very essential theory in population genetics, as it infers the independence of alleles within genotypes, in addition to constant allele frequencies across generations (Mora, 2015). The Chi-square test (X2) is a computational method used to verify the conformation to or deviation of a population from the assumptions of Hardy-Weinberg equilibrium.

The test for Hardy–Weinberg equilibrium showed no significant deviation ($p > 0.05$) in all populations, indicating that allele frequencies are stable and that random mating is likely occurring within these populations. This suggests minimal influence of evolutionary forces such as selection, migration, or genetic drift at the haemoglobin locus. The implication is that the observed genetic structure is being maintained across generations, which is desirable for conservation and breeding programs (Mora, 2015; Sargent et al., 1999).

In the overall population the Chi-square test was 0.100, implying that the population has not

deviated from Hardy-Weinberg equilibrium and is at equilibrium.

Heterozygosity estimate

Heterozygosity estimates revealed moderate genetic diversity across the populations. The observed heterozygosity was slightly lower than the expected values in Gudali and Red Bororo, which may suggest some level of inbreeding or population substructuring. In contrast, the White Fulani population exhibited higher observed heterozygosity than expected, indicating greater genetic mixing and reduced inbreeding. The overall similarity between observed and expected heterozygosity values supports the earlier inference of Hardy–Weinberg equilibrium and reflects a relatively balanced genetic structure.

The effective number of alleles (Ne) and Shannon's information index (I) further support the presence of moderate genetic diversity within the populations. Although two alleles were consistently observed (Na = 2.00), the effective number of alleles was slightly lower, reflecting unequal allele frequencies. The Shannon's index values (0.68–0.69) indicate moderate diversity, suggesting that while variation exists, it is not highly extensive. These findings may be influenced by population size, breeding practices, or sampling effects.

Analysis of genetic identity and distance revealed a high degree of genetic similarity among the breeds. The closest relationship was observed between Red Bororo and White Fulani, while Gudali showed slightly greater genetic distance from the other two breeds. Nevertheless, the overall low genetic distances indicate that the breeds share a common genetic background, likely due to geographical proximity, gene flow, or shared ancestry.

CONCLUSION

This study demonstrates that Nigerian indigenous cattle breeds (Gudali, Red Bororo, and White Fulani) are polymorphic at the haemoglobin locus, possessing two alleles (HbA and HbB) and three genotypes (HbAA, HbAB, and HbBB). The predominance of the heterozygous genotype and the moderate heterozygosity values indicate appreciable genetic variability within the populations.

Furthermore, the populations were found to be in Hardy–Weinberg equilibrium, suggesting stable allele frequencies and limited influence of evolutionary forces at this locus. Genetic distance analysis showed close relationships among the breeds, with Red Bororo and White Fulani being the most genetically similar, while Gudali was relatively more distinct.

Overall, the findings highlight the presence of useful genetic variation within Nigerian indigenous cattle, which can be harnessed for improvement and conservation strategies.

RECOMMENDATION

Based on the findings of this study, it is recommended that:

1. Crossbreeding programs should be encouraged, particularly between genetically distinct breeds such as Gudali and White Fulani or Red Bororo, to exploit heterosis and improve productivity.
2. Conservation efforts should be strengthened to preserve the existing genetic diversity within indigenous cattle breeds.
3. Further molecular studies involving additional genetic markers should be conducted to provide a more comprehensive understanding of genetic diversity and population structure.
4. Controlled breeding strategies should be implemented to minimize inbreeding, especially in populations where observed heterozygosity is lower than expected.

These measures will help ensure sustainable utilization and long-term genetic improvement of Nigerian indigenous cattle breeds without compromising their unique genetic resources.

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