HEAMOGLOBIN POLYMORPHISM FROM TWO POPULATIONS OF WEST AFRICAN DWARF GOAT IN WESTERN NIGERIA

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Abstract
Haemoglobin Polymorphism in West African Dwarf (WAD) goat populations from Oyo and Ogun State was investigated using cellulose acetate electrophoresis system. Five milliliters (5ml) of blood sample were collected through jugular vein into a heparinized bottle from 60 WAD goat, comprising of 30 goats from each population. The result showed different band of alleles of haemoglobin which were counted and score accordingly, Data generated were analysed using Tool For Population Genetic Analysis (TFPGA) software. The result of the analysis revealed two alleles Hb^A and Hb^B controlling three genotypes Hb^{AA}, Hb^{AB} and Hb^{BB}. Frequency ranged between 0.217 and 0.500 for Hb^A with an average of 0.359 while it ranged from 0.500 to 0.783 for Hb^B allele with an average of 0.41. Test for Hardy-Weinberg' Equilibrium was not significant ($p < 0.05$) for the two populations. Observed and Expected heterozygosity (H_O and H_E) ranged from 0.333 to 0.435 with an average of 0.384 and from 0.348 to 509 with an average of 0.428. The gene diversity (D) ranged between 0.340 and 5.00 with a mean of 0.470. The Wright F statistic analysis revealed F_{IT}, F_{ST} and F_{IS} value as 0.261, 0.138 and 0.148 respectively indicating and inbreeding population with moderate genetic diversity.

Keywords: *Genetic diversity; Heamoglobin; West African Dwarf goat; Heterozygosity; Electrophoresis; Polymorphism.*

Introduction

Genetic variations among ruminants including WAD goat in Nigeria have been a point of concern to geneticists (Salako et al, 2010). This is because the amount of genetic diversity detectable in an animal population is related to the magnitude of genetic improvement achievable within the species (Salako et al, 2010). Thus genetic variation has become the objective tool traditionally used for improving animal species. The WAD goat is sparsely distributed across the Northern and Central part of Nigeria where it makes significant contributions to the livelihoods of impoverished families (Musa et al, 2016). They serve an important role in rural village economy of West Africa, especially among Small-holder livestock owners (Samuel and Jerzy, 2011). It is mostly found in the Southern parts of the country where it is able to thrive and survive due to its hardy nature and its tolerance to the common pest inhibiting ruminant production (Odubote, 2011). The WAD goat is markedly dwarf and has atypical height of 30 to 50 cm. The breed has large range of qualitative variations in coat colour. Most types have short stiff hair, and the colour varies; dark brown with black points is probably the most common, but black, red, white, pied and muilticoloured goats exist (Odubote, 2011: Ozoje and Mgbere, 2010). Food and Agricultural Organization (FAO, 2008) estimated goat population in Nigeria to be 53.8 million, which ranked Nigeria fifth among selected countries like China (149.37 million), India (125.7 million), Pakistan (60 million) and Bangladesh (56.4 million). Haemoglobin (Hb) is an important protein that has attracted attention because of its biochemical, biophysical and physiological properties, having relevance to the selection phenomenon of animals (Raushenbach and Kamenek, 2011). Terill (2013) reported that after electrophoresis, the haemoglobin genotype that migrated faster from the point of application at pH of 8.5 - 9.0 was labeled Hb ^{AA} while the slow moving fraction was identified as Hb ^{BB} and the heterozygote as Hb ^{AB}. Bindu and Raghavan (2010) reported the predominance of Hb^{A} over Hb^{B} in goat. According to Agaviezor et al. (2013), Haemoglobins are historically important for their part in the demonstration of the relationship between genetic information and protein structure. They also reported that haemoglobin have variants and these variants have been reported to be associated with environmental adaptability as well as being clinically important as causes of a variety of genetic disorders of blood. Consequently, the genetic background of the haemoglobin merits examination in some detail (Peters et al., 2012). Blood protein polymorphisms have been used by several researchers as markers to study evolutionary relationships in mammals. For example, evolutionary relationships between different sheep breeds, deer species, goat breeds and chicken genotypes have been examined (Awobajo, et al., 2020; Awobajo, et al., 2016; Guney, 2013). Different haemoglobin types may have selective advantages in different geographical regions (Ndamukong, 2013). For example, FAO (2008) reported that carriers of haemoglobin A have both demonstrated significant resistance against helminthes infections. Distasio (2007) suggested that this could be due to the better functional properties such as greater affinity for oxygen and higher haemoglobin concentration and Packed Cell Volume (PCV). Since genetic diversity is a basic requirement for animal improvement, it needs to be preserved through characterization and management. Blood groups and blood proteins have been widely used to characterize animal populations because of their polymorphism and their simple mode of inheritance (Dally et al., 2010). Moreover, they are supposed to be linked to productivity traits and environmental adaptation (Charon et al., 2013; Vicovan and Rascu, 2009). The characterization of West African Dwarf Goat diversity is essential to meet future needs in Africa and Nigeria in particular. Although there are reported works on haemoglobin polymorphism in selected Nigeria goat breeds (Akinyemi and Salako, 2012), there is paucity of information on work relating to the haemoglobin polymorphism in WAD goat especially in Oyo and Ogun state, Nigeria. Thus, this study intends to provide some relevant information on these aspects. The main objective of this study is to investigate haemoglobin (Hb) Polymorphism in WAD goat in Oyo and Ogun states with the view of finding out the Hardy-Weinberg equilibrium for the two populations, the gene diversity through the observed and expected heterozygosity and by evaluating haemoglobin (Hb) alleles and genotypes and their frequencies using cellulose acetate gel electrophoresis.

Materials and Methods

Sixty WAD goats were randomly selected across the two States, (Ogun and Oyo State) and 5ml blood samples were collected from each goat. Thirty WAD goats were sampled from each State. Blood was collected from the jugular vein into vacutainer tubes (heparinized bottle) and stored at 4ºC in a container and transported to the laboratory. The blood samples were centrifuged at 3500 rpm for ten minutes, the plasma supernatant was eliminated and then the erythrocyte obtained was washed thrice in saline (0.155M NaCl) and after each centrifugation the washing solution was removed. The erythrocyte obtained after the third washing was haemolysed using equal volume of distilled water to produce erythrocyte lyses. The haemoglobin solutions which resulted from the haemolysed red cells were ready for electrophoresis to separate the globin fractions of the β-chains of haemoglobin. The electrolytic solution contained Tris EDTA and Boric acid. The pH of the electrolytic solution was stabilized at 8.4. The electrophoretic migration time was four hours till the bands migrated to about 4cm from the start line. The cellulose acetate paper was stained with Ponceau Stain. The strips were destained with 5% acetic acid. The bands were scored visually based on their migratory pattern as described by (RIKEN, 2006). Tools for Population Genetic Analyses (TFPGA) software (add reference here) was used to generate the genetic distance according to (Nei's

Original, 1972), the allele frequency, observed and expected heterozygosity, Hardy-Weinberg Equilibrium coefficient, Wright's F_{IS}, F_{IT} and F_{ST} estimates, and drawing Unpaired Group Method of Algorithm (UPGMA) dendrograms. All computations were performed using Popgen (Yeh et al., 1997) and Tools for population genetic analysis (TFPGA; Miller, 1997).

Results

Table 1: Allele frequency

Allele	Ibadan	Ijebu-Ode	Total	Average
Hb^A	0.500	0.217	0.377	0.359
Hb^B	0.500	0.783	0.623	0.641

Table 1 showed that the frequency of heamoglobin for the two variants is 0.50 (in Hb^A and Hb^B in Ibadan) and the frequency and average frequency in Ijebu-Ode is 0.217 and 0.377 respectively. Hb^A and Hb^B for both populations were 0.359 and 0.641 respectively.

Table 2: Genotype frequency

Genotype	Ibadan	Ijebu-Ode	Overall
Hb^{AA}	0.333	N/A	0.189
Hb^{AB}	0.333	0.435	0.377
Hb^{BB}	0.333	0.565	0.434

It revealed that genotype frequency at Haemoglobin locus is uniform and is 0.333 for all the genotypes while that for Ijebu-Ode sample were 0.435 and 0.565 (Hb^{AB} and Hb^{BB}), Hb^{AA} was genotype does not exist in Ijebu-Ode population. The genotype frequencies for both populations were 0.189, 0.377 and 0.435 for the three genotype i.e Hb^{AA}, Hb^{AB} and Hb^{BB} respectively

Table 3: Test of Hardy-Weinberg's equilibrium

Population	Sample size	Population value	Status
Ibadan	60	0.072	NS
Ijebu-Ode	46	0.538	NS
Overall	106	0.006	SE 0.002 **

Table 3 showed no significant difference (P > 0.05) in the values of the Hardy-Weinberg's equilibrium for population sampled while the overall population revealed significant (p < 0.05) deviation from Hardy-Weinberg's Equilibrium.

Table4: F-Statistic Analysis

Allele	F (F_{IT})	ϴ (F_{ST})	f (F_{IS})	Nm
Hb^A	0.261	0.138	0.143	
Hb^B	0.261	0.138	0.143	
Overall	0.165	0.087	0.086	2.630

Table 4 revealed the Wright's F-Statistic and the values at the haemoglobin locus are the same for the populations and the alleles, and the value for gene flow is low.

Table 5: F_{IS} for WAD goat populations

Population	Hb^A	Hb^B
Ibadan	0.333	0.333
Ijebu-Ode	-0.278	-0.278
Overall	0.197	0.197

Table 5 revealed that F_{IS} for allele Hb^A and Hb^B for each of the population in Ibadan is 0.333 while the value for Ijebu - Ode population is -0.278 and the over value is 0.197 and the overall is 0.197 and all this values showed no significant difference (P > 0.05).

Table 6: Measure of genetic variation

Population	Sample size	Observed no of allele	Effective no of allele	Shannon info. Index	H_O	H_E	Nei's
Ibadan	60	2.000	2.000	0.693	0.333	0.509	0.500
Ijebu-Ode	46	2.000	1.516	0.524	0.435	0.348	0.340
Total	106	2.000	1.887	0.663	0.377	0.474	0.470
Mean	53	2.000	1.758	0.608	0.384	0.428	0.420
St.Dev	0	0.000	0.000	0.000	0.000	0.000	0.000

Table 6 showed that two alleles were detected at the haemoglobin locus in both populations sampled. Heamoglobin polymorphism was highly informative (Shannon information index > 0.60) which makes it useful in genetic diversity studies with mean of 60.84%. The observed number of alleles across both populations and effective number of alleles are same (2.000), the expected and effective number of alleles ranged from 1.516 to 2.000 from Ibadan and Ijebu-Ode population respectively. The average gene diversity, (Nei, 1973) the population ranged from 0.340 (Ijebu-Ode) to 0.500 (Ibadan) with an overall mean of 0.420.

Table 7: Heterozygosity (H_E and H_O)

	Ibadan	Ijebu-Ode	Average	St. Dev
H_O	0.333	0.435	0.384	0.740
H_E	0.509	0.348	0.428	0.733

Table 7 showed the value of heterozygosity, expected is higher than observed in Ibadan. While observed is higher than expected in Ijebu-Ode, Ho ranged between 0.333 to 0.435 (for Ibadan and Ijebu-Ode) with mean of 0.384 ± 0.740 from population sampled. H_E value also follow different trend from 0.348 to 0.509 for Ijebu-Ode and Ibadan with the mean of 0.428 ± 0.733 from population sampled.

Discussion

The result in Table 1 showed two alleles observed with an average frequency of 0.359 and 0.641 for allele Hb^A and Hb^B respectively across both populations. This aligned with the findings of Salako et al (2007) who reported 0.44±0.55 across the studied locus in the populations. In Table 2, three genotypes Hb^{AA}, Hb^{AB} and Hb^{BB} were revealed with an average frequency of 0.189, 0.377 and 0.434. The frequency of the genotypes is uniform for population from Ibadan (0.333) where as Hb^{AA} genotype does not exist in population from Ijebu Ode. This implied that the haemoglobin is polymorphic in the two populations studied this is further established by the Shannon Information Index value of 60.80%, this finding is contrary to the report of Nafti et al., (2016) who reported genotype frequency of WAD goat for Hb^{AA}, Hb^{AB} and Hb^{BB} as 0.779, 0.226 and 0.017 respectively. Hardy-Weinberg's Equilibrium exact test on Table 3 revealed no significant difference ($p < 0.0.5$) in each population studied but the overall value showed high significant difference ($p < 0.01$). This indicated a departure from random mating suggesting a homozygote population as confirmed by the heterozygosities and the F-Statistic analysis report. (Bruno-de-Sousa *et al.,* 2011; Dixit *et al.,* 2008). The result of F-Statistic in

Table 4 showed that F_{IT}, F_{ST} and F_{IS} are all in positive value, F_{IT} of 0.165 and F_{IS} of 0.086 indicated a deficient in heterozygote implying inbreeding population which may be due to factors like, population subdivision owing to genetic drift, null alleles and selection against heterozygote or inbreeding (Hoarau *et al.,* 2005). F_{ST} of 0.087 indicated a small genetic diversity among the populations and a low gene flow value (2.630) confirmed this. Mujibi (2005) reported a low F_{ST} of 5.8% for WAD goats in Kenya. According to Laval *et al.* (2000), migration may exert a greater effect than mutation or drift on the reduction in genetic differentiation between populations. In Table 5, F_{IS} value was the same for both alleles in the two populations though F_{IS} was negative for population from Ijebu Ode, indicating excess heterozygote and an outbred population, but the mean value was positive. Table 6 revealed the measures of genetic variation. The value of observed heterozygogosity (H_O) is lower than the value of expected heterozygosity (H_E) in population from Ibadan indicating deviation from random mating and an inbreeding population whereas the Ijebu Ode population H_O is higher than the H_E also indicating deviation from random mating and an outbred population but the mean value indication lower value of H_O than H_E suggesting a homozygote populations and may indicate on-going selection or may be linked to other loci affecting morphological, productive or adaptive traits undergoing selection (Bruno-de-Sousa *et al.,* 2011; Dixit *et al.,* 2008) or result from mating between relatives and consequent genetic drift, similar to what has been observed in many other goat populations (Dixit *et al.,* 2009; Agha *et al.,* 2008; Rout *et al.,* 2008). Gene diversity (D) ranged from 0.340 to 0.500 with a mean of 0.470, this finding was lower than 0.54 reported by Muema *et al.* (2009), 0.51 reported by Adebambo *et al.* (2011) in Nigerian goats, and 0.61–0.78 in Indian goats (Dixit *et al.,* 2010; Rout *et al.,* 2008). H_O and H_E were further emphasis in Table 7 and it is as earlier discussed in Table 6.

Conclusion

The study revealed that haemoglobin is polymorphic in the populations studied with two alleles and three genotypes. Allele Hb^B and genotype Hb^{BB} are more common than others in the populations. Population from Ibadan is homozygote or experiencing inbreeding while Ijebu Ode population is heterozygote or outbred population, there is moderate genetic differentiation among the two populations of WAD goat studied. This mean there is room for improvement in the two populations studied, this is confirmed by the average value of gene diversity of 0.470. The WAD goat populations in this study are in Hady-Weinberg genetic Equilirium at the haemoglobin locus.

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